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Treatment of sickle cell anemia using CRISPR and prime editing

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Abstract. Sickle cell disease is a hereditary blood disease caused by an invisible genetic mutation on the autosome, caused by a transversion mutation in which the 6th amino acid of the β-peptide chain, glutamate, is replaced by valine, resulting in the formation of sickle hemoglobin, instead of normal hemoglobin. Due to genetic mutations leading to structural abnormalities in hemoglobin and morphological changes in red blood cells, red blood cells lose their deformability in hypoxic environments, leading to vascular blockage, causing severe pain and partial tissue ischemia in patients. Oxygen needs to be transported to various parts of the body through red blood cells, thereby affecting the transportation of oxygen in the body. In general, red blood cells are circular and elastic, making them easy to move in blood vessels. However, in sickle cells, these red blood cells are compressed and deformed, causing the transportation of oxygen in the blood to become stiff and viscous, potentially slowing or blocking blood flow. The most common complication of SCA is blockage of blood vessels, which leads to pain. Although this is a complex phenomenon, HbS aggregation is a fundamental pathological physiological phenomenon in SCA. HbS aggregation can lead to malignant changes in the shape and physical properties of red blood cells, leading to hemolytic anemia and preventing blood flow, especially in small blood vessels, which may damage some organs. Clinical manifestations include anemia, immunodeficiency, multiple organ damage, severe acute and chronic pain, and premature death. The quality of life of the patients was seriously affected.

Keywords: sickle-cell disease, CRISPR, prime editing, gene editing

1. Introduction

Sickle cell anemia (SCA) is an autosomal recessive inherited hemoglobin disease that mainly occurs in Africa, the Middle East, and the Mediterranean region [1, 2]. It causes people to develop SCD by altering the morphology of red blood cells [3-7].At present, traditional treatment methods mainly include blood transfusion, drug relief for pain crisis and antibiotics to prevent infection, but these methods can not cure the disease. After treatment, the patient's daily life is still troubled and the prognosis is still very limited. The average age of death for people with sickle cell anemia (homozygous sickle hemoglobin) is only over 40 years old [8].This shows that sickle cell disease is still a serious threat to human beings. Therefore, it is urgent to find a more effective treatment strategy.

Currently, multiple methods have been used in clinic to treat SCD, including pain management, blood transfusion, drug treatment, bone marrow treatment, warfarin and lifestyle changes [4, 9].

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Pain is the most frequent symptom of SCA. Treatment usually includes painkillers, such as aspirin, or more powerful drugs. Blood transfusion: This medical procedure can help provide normal red blood cells, thereby reducing symptoms and the risk of complications. The downside, however, is the risk of mismatched blood types or infectious disease, and long-term blood transfusions can lead to iron excess. Hydroxylurea (HU) is a therapeutic drug approved by the US Food and Drug Administration of sickle cell anemia, can reduce the crisis of the disease [10]. There are many beneficial aspects for patients with SCA by using Hu, such as: (I) added production of fetal hemoglobin (Hbf) , thereby prolonging the delayed impact of the erythrocyte sickling process; (II) a reduction in white blood cell (WBC) count and cell adhesion molecule expression patterns, and (III) a reduction in transfusion frequency. These advantages can reduce the harm of SCA to patients. However, through recent clinical studies, HU has been ineffective in many patients and the specific cause has not yet been identified. In addition, the above studies suggest that there is heterogeneity in the clinical expression of SCA, which leads to the unpredictability of VOC risk and poses a serious challenge to disease management. However, it can cause side effects such as myelosuppression, nausea and diarrhea. Endari is another drug that can reduce the number of painful crises and hospital admissions, but can also cause side effects such as gastrointestinal problems and headaches. Bone Marrow Transplant is the only treatment that has the potential to eradicate sickle cell anemia by transplanting healthy bone marrow to produce healthy red blood cells. However, finding the right donor is a challenge, and complications such as graft-versus-host disease can occur after surgery [4, 11]. Lifestyle changes include staying hydrated and eating a balanced diet to avoid excessive physical exertion and stress. While this does not cure the disease, it can help patients better manage their symptoms. However, this requires long-term self-management and can be challenging.

While traditional treatments can only relieve symptoms, CRISPR and prime have shown great potential as new gene-editing tools to treat genetic disorder [1, 12]. So we discuss recent advances and challenges through using CRISPR and prime editing technologies to cure sickle-cell disease.

2. CRISPR/Cas system

2.1. Introduction

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) is a tool that can edit specific genes, accurately identifying and repairing defects in gene sequences through the natural immune system of bacteria. CRISPR/Cas9 is currently a highly efficient tool commonly used by most scientists to edit genes [13]. The evolution of the CRISPR/Cas9 system in bacteria can defend against phage infection and plasmid transfer [14]. When bacteria in nature are first infiltrated by exogenous bacteriophages or plasmids, they can be inserted into the CRISPR spacer region to obtain DNA sequences [15, 16]. If infected with homologous DNA again, the bacteria will transcribe in the CRISPR region. Then, through a series of processing and maturation processes, a single guiding RNA (sgRNA) is generated, which can cleave and destroy the DNA strand of the homologous spacer region by guiding Cas9. The recognition process of sgRNA requires the prototype spacer to work together with adjacent motifs (PAMs), which are short sequences and contain a lot of guanine. The preferred PAMCas9 (SpCas9) for Streptococcus pyogenes is NGG, which exists in the genome of most organisms, thereby promoting the application of CRISPR technology in animal and plant science and biomedical fields [17]. By altering a small portion of the nucleotide sequence that guides RNA, CRISPR/Cas9 can accurately target almost any desired genomic site to correct pathogenic mutations or silence disease-related genes [18-22]. However, CRISPR/Cas9 may not be able to interview some high chromatin regions in the genome. This technology may be used in the future to treat sickle cell anemia, cardiovascular disease, and cancer.

2.2. Application of CRISPR technology in sickle-cell disease therapy

CRISPR technology provides a feasible therapeutic approach for sickle-cell disease disease caused by single gene mutation. By introducing the Cas9 protein and the corresponding RNA fragment in the CRISPR system, researchers can selectively target and cleave mutant sites in the hemoglobin gene,

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triggering gene repair and restoration of red blood cell morphology. The γ-globin gene can be increased by culling long non-coding RNAs to improve red blood cell oxygenation. The combination of a single guide RNA and Cas9 induces small insertions and deficits in the red enhancer region of the BCL11A gene. The protein product of this gene is responsible for inhibiting the first year of life of the fetus γ Hemoglobin induction in adults β production of hemoglobin [23]. Due to the downregulation of BCLA11 and the accompanying γ- Individuals with elevated hemoglobin levels carrying genetic variations in the red blood cell enhancer region are immune to the influence of SCD. Another method is to use endogenous homologous directed repair (HDR), and the CRISPR-Cas9 system insert therapeutic genes into the genome, using a combination of supramolecular nanoparticles (SMNP)/supramolecular nanomaterial mediated delivery (SNSMD) to promote hemoglobin delivery beta. CRISPR-Cas9 knockout involves gradually processing two SMNP vectors through HDR. The Cas9 · unidirectional RNA (sgRNA) compound and the HBB/green fluorescent protein (GFP) encoding plasmid are encapsulated through this vector [24].

2.3. Advances and challenges in CRISPR

Researches in recent years, there has been some progress in the use of CRISPR to treat sickle-cell disease. The researchers successfully used these techniques to restore the normal form and function of diseased red blood cells and observed significant improvements in treatment outcomes. However, CRISPR technology still faces many challenges in sickle-cell disease therapy, such as the difficulty to achieve efficient and accurate gene editing, genome editing and ethical issues that may lead to accidents; Such as improving the accuracy and effectiveness of CRISPR technology to address specific gene editing caused by unexpected mutations. Further studies are needed to assess the safety and long-term efficacy of treatment. However, compared to the past two generations of gene editing technology, the CRISPR/Cas9 system, as the third-generation targeted genome editing technology, has great advantages. The advantages of CRISPR Cas9 are very obvious. Firstly, the vector construction is easy and has the ability to accurately locate. It is possible to match DNA sequences by constructing a CRISPR sgRNA with dozens of bases, thereby mediating Cas9 protein cleavage of DNA sequences; Secondly, CRISPR-Cas9 has a higher distribution frequency of editable loci, which facilitates gene editing of suitable genes; The most important thing is that the CRISPR-Cas9 can perform multisite editing on the genome at the same time. The CRISPR/Cas9 system, due to its profit of uncomplicated system structure, convenient operation, high mutation efficiency, and low cost, has become the most rapidly developing and widely researched and applied targeted gene editing technology for various biological genomes by domestic and foreign scholars. Therefore, with the development of CRISPR technology, the solutions to the problems are emerging, providing new hope for gene therapy.

3. Prime editting

3.1. The introduction of prime editing

Unlike CRISPR, prime editing is a relatively new tool for gene editing. It utilizes a modified Cas9 protein and a self-repairing template to achieve highly accurate gene repair. Compared with traditional CRISPR/Cas9 systems, prime editing technology enables more precise gene editing, reducing unexpected mutations and cytotoxic effects. The principle of Prime editing technology is to combine modified guide RNA (pegRNA) with Prime editor protein. PegRNA has a dual function, which can guide the editing protein to the target site and contain the edited template sequence. The prime editor protein is formed by fusion of Cas9 cleavage enzyme and reverse transcriptase. Unlike ordinary gRNAs, this type of pegRNA can bind to specific DNA regions that need to be edited and can be modified through its own template. The binding protein formed by the N-terminal and/or C-terminal connection of thermostable reverse transcriptase to DNA in the Cas9 system will precisely cleave a DNA strand under the traction of pegRNA, and then synthesize DNA containing the correct sequence through a “modification template”. DNA within cells can integrate this newly synthesized sequence into the genome for repair. After Cas9 cleavage of the target site, pegRNA can be used as a template for reverse

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transcription by reverse transcriptase, and then the DNA is directly aggregated onto the DNA strand of the incision.

3.2. Application of Prime editing technology in sickle-cell disease

In sickle-cell disease therapy, Prime editing technology can more precisely repair gene mutation, so as to achieve the full restoration of red blood cell morphology and function [1].Due to the operability of this system, single gene editing that was previously impossible, such as transforming T to A, has also become probable. Compared to only 4 conversions for single base editing, this lead editing system can perform 12 conversions for single bases. That is to say, they can convert any base into any other base, including all 12 possibilities.

3.3. The challenge and prospect of Prime editing technology

Prime editing technology is one of the important technologies in gene editing field. Compared with traditional gene editing technology, Prime editing technology has higher accuracy and lower non-specific editing risk, enabling precise and efficient gene mutation repair. First, through gene sequencing technology, we can determine the specific location and type of mutations in patients, and then use Prime editing technology to precisely repair these mutations, so that red blood cells can return to normal shape and function, and then achieve treatment. Although the Prime-editing technique is still in its early stages in the medical care of sickle-cell anemia, preliminary experimental results have shown the great potential of the technique. As the technology is further developed and refined, there is reason to believe that Prime editing will revolutionize the therapy of sickle cell anemia and a range of other genetic disorder. Research into the application of Prime editing technology in sickle cell processing is still at an early stage and requires more empirical studies and addressing possible ethical and safety issues, this is an important task for future research.

As new gene editing tools, CRISPR and prime not only offer new strategies and hope for the treatment of sickle-cell disease diseases, but also offer feasible methods for other genetic diseases. Diseases such as chorea, Kendine's disease, and SCA1 can be cured by precisely cutting the repetitive sequences on the affected genes, thereby restoring the normal form and function of the affected genes. However, the related research is still in the early stage, and further clinical research and technical improvement are still needed. As the technology continues to evolve, it is believed that in the near future, CRISPR technology and prime editing technology will bring more hope and rehabilitation opportunities to more patients.

According to the above analysis, CRISPR technology and Prime editing technology have their own advantages in the treatment of sickle cell anemia. CRISPR technology can solve the problem of sickle cell anemia by precise gene cutting. Prime editing, with its precise gene insertion, replacement and other operations, can more safely and effectively repair genetic mutations at the DNA level. Using these two techniques, we hope to achieve more effective treatment of sickle cell anemia.

4. Conclusion

As new gene editing tools, CRISPR and prime not only offer new strategies and hope for the treatment of sickle-cell disease diseases, but also offer feasible methods for other genetic diseases. Diseases such as chorea, Kendine's disease, and SCA1 can be cured by precisely cutting the repetitive sequences on the affected genes, thereby restoring the normal form and function of the affected genes. However, the related research is still in the early stage, and further clinical research and technical improvement are still needed. As the technology continues to evolve, it is believed that in the near future, CRISPR technology and prime editing technology will bring more hope and rehabilitation opportunities to more patients.

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techniques, we hope to achieve more effective treatment of sickle cell anemia. More novel therapies, such as hemoglobin and gene writing, may be used in the future[25].

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