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Applications of CRISPR-Cas9 Gene Editing Technology in Treatment of Sickle Cell Disease

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ABSTRACT
CRISPR-Cas9 gene editing technology has emerged as a breakthrough tool in the 21st century for biomedical research and clinical treatment. This approach allows the precise editing of only certain gene fragments, which can potentially correct or replace a mutation that causes disease. Its applications span personalized medicine, regenerative medicine, and the prevention and control of genetic diseases. Among many such diseases, sickle cell disease is one of the interesting targets amenable to CRISPR-Cas9 treatment. A point mutation in the gene coding for β-globin is responsible for the aberrant hemoglobin synthesis that leads to a myriad of severe symptoms in sickle cell disease. The main two approaches in treating sickle cell disease using CRISPR-Cas9 are correcting the mutation in the β-globin gene or reactivating the expression of fetal hemoglobin to blunt disease manifestations. But this technology is riddled with huge challenges: it has off-target effects and it raises ethical issues regarding gene editing in human embryos. This paper will discuss the use of CRISPR-Cas9 in the treatment of sickle cell disease, its application mechanisms, and the challenges that need to be overcome to realize its full therapeutic potential.

KEYWORDS
CRISPR-Cas9; Gene editing; Sickle cell disease, β-globin gene

1 Introduction

In the medical field of the 21st century, the emergence of CRISPR-Cas9 gene editing technology marks the beginning of a completely new era. It has become a focal point in biomedical research and clinical treatment. Unlike a century ago, patients would not need to suffer from physical pains like before, and would even gain a treatment plan just designed for themselves now with CIRSPR. The fact that this technology made gene fragments editable means that we can directly target and repair or replace a specific genetic variants fragment that cause a certain disease. The significant breakthrough of CRISPR has contributed to the development of personalized medicine, regenerative medicine, and the prevention and control of genetic diseases. From laboratory to clinical trials, the potential disease treatment application field of CRISPR-Cas9 technology is expanding and gradually becoming reality. Among all the diseases that could be treated using the method of CRISPR-Cas9, Sickle Cell Disease (SCD) stands out as one of the most compelling areas.

2 CRISPR-Cas9 gene editing technology

The development of this gene editing technology was awarded the Nobel Prize in 2020 to two scientists for their hard work and research on CIRSPR-Cas 9, the so-called “genetic scissors”. The abbreviation “CRISPR” stands for Clustered Regularly Interspaced Palindromic Repeats which was first referred to a sequence found in bacterial genomes. These repeats were discovered in the gene sequences of Escherichia coli bacteria; and was then described by Ishino et al in 1987 [1]. This kind of immune system is used for the protection of virus and other extraneous genetic materials. CRISPR locus are composed of multiple short and nearly identical DNA sequences, which are called "repetitive sequences". These sequences act like bookmarks that mark specific locations in the genome. In between these repeated sequences, a lot of different short sequences called "interval" sequences take place. These spacer sequences do not appear randomly--they are fragments of DNA from phages that previously invaded the bacteria. When a bacterium cell encounters a viral invasion (e.g. from a phage), it has the ability to take a small piece of the invader's DNA and insert it into a CRISPR site in its own genes as a new spacer sequence. The bacterium will create a database among the genes of the previous invaders; by doing this, the bacterium would recognize the invaders more efficiently whenever they appear again.

In type II CRISPR systems, the CRISPR region is transcribed into a pre-CRISPR RNA (pre-crRNA). TracrRNA subsequently associates with crRNA to assume the task of drawing the Cas9 enzyme and steering it to pinpoint the specific DNA sequence that aligns with the spacer (the portion of crRNA produced from the target DNA) [2]. The Cas9 enzyme acts as an endonuclease capable of severing two strands of DNA. For cutting to happen, the Cas9 enzyme must recognize a particular DNA sequence, which is 2 to 5 nucleotides in length and changes based on the type of bacteria that generates Cas9. This specific sequence must reside near the 3' end of the guide RNA (gRNA), referred to as the Protospacer Adjacent Motif (PAM). Post-DNA cleavage, repair can happen through two routes: non-homologous end joining, which commonly results in random insertions or deletions in the DNA; or homology-directed repair, where a homologous DNA segment acts as a blueprint for repair.

After that, scientists found out that the CRISPR system was not just a bacterial defense system, it can also be applied in clinical medicine for the goal of cutting a specific gene fragment. In bacterial immune system, crRNA and transcrRNA are two individual molecules. Because of the needs for facilitating the experiment, scientists integrated the functions of the two molecules to one – and named the new molecule as single guide RNA (sgRNA). This RNA also has a region called "spacer" that is complementary to the targeted DNA fragment, which is typically 18-20 nucleotides in length. After a double strand cutting of the DNA, homology-directed repair facilitates accurate genomic modification: by delivering a homologous DNA fragment with the desired sequence changes along with Cas9 nuclease and sgRNA, base-pair precision editing could be achieved theoretically [3].

3 Sickle cell disease

Sickle cell disorder is a overarching category encompassing various conditions (such as sickle cell anemia (SCA), HbSC, and HbSβ -thalassemia) linked to alterations in the genetic code for the hemoglobin subunit β. Hemoglobin (Hb), a quadruple-unit protein formed by various globin subunit assemblies, has each globin subunit linked with the coenzyme heme, facilitating the carriage of an oxygen molecule. Hb A, the most prevalent (>90%) type of adult hemoglobin, includes two α-globin subunits (resulting from the duplicate HBA1 and HBA2 genes) and two β-globin subunits [4]. The β -globin subunit encoded by the HBB gene is an important component of adult hemoglobin (HbA). The primary trigger for SCD stems from a single nucleotide change in the HBB gene on chromosome 11, which causes the creation of irregular hemoglobin named HbS [5].

This specific gene mutation includes a substitution of a single nucleotide, where adenine(A) is replaced by thymine(T). As a result, the encoded amino acid change from glutamic acid to valine at the sixth position of the beta-globin chain. Glutamic acid is a polar amino acid with a negative charge; while valine is a non-polar aliphatic amino acid, so the substitution leads to the surface charge properties of the β-globin chain. Such a change will have a significant impact on the overall structure and function of the hemoglobin. Under conditions of deoxygenation, which is when hemoglobin is not bounded with oxygen molecules, the protein could polymerize and turn into sickle shaped (which is where the disease take it ' s name).

SCD is characterized by a wide range of symptoms that can vary in severity and presentation among individuals. [5] The symptoms include severe pain in various body parts, anemia, infections, stroke, etc. The SCD is a globally spread disease that have significantly impacted the individual and social health all around the world. According to current estimations, in Sub-Saharan alone, there are approximately 300,000 new born infants being affected by SCD; while on the other side of the world, SCD presents in 1 out of 365 African American birth [5]. Due to the widespread nature of SCD, its curation has become one of the most tough problem to solve among the medical field.

4 Application of CRISPR-Cas9 in treating sickle cell disease

The CRISPR-Cas9 system can be utilized to repair mutations in the β -globin gene or enhance fetal hemoglobin expression, thereby reducing the formation of sickle-shaped cells and alleviating disease symptoms.

One approach to treating SCD with CRISPR-Cas9 involves correcting the mutated gene on the HBB. By using AAV (Adeno-Associated Virus) as a carrier, Cas9 protein would be sent to CD34+ hematopoietic stem cells in order to cut the mutating gene fragment as a piece of normal gene fragment without mutation would be sent for replacement. Researches have shown achievement rates ranging from 18% to 94% [6]. This strategy not only addresses the root cause of SCD but also holds the potential for long-term therapeutic benefits by restoring normal hemoglobin function.

Another way is called Casgevy - to use CRISPR-Cas9 to interfere genes that restrain the growth of HBF, such as BCL11A. In the experiment, scientists created a sgRNA that would direct Cas9 protein to the enhancer (which is a piece of DNA that can significantly increase the transcriptional efficiency of the genes) of BCL11A. After a double-strand breaks at a specific genomic location, there would be a decrease in the activity of BCL11A, thus increase the formation of HBF. While HBF could carry oxygen molecules efficiently and won ' t turn into sickle shaped, there would be a big leap in the living quality of patients with sickle cell disease [7]. Casgevy has been developed as a treatment option for individuals aged 12 years and older suffering from sickle cell disease (SCD), particularly those who frequently encounter vaso-occlusive crises. Research findings indicate that in a continuing clinical study with 44 SCD patients, the therapy proved effective, with 93.5% of assessable participants remaining free from severe VOC episodes over a period of at least 12 months. Among the commonly reported adverse effects are reduced platelet and white blood cell counts, oral ulcers, feelings of nausea, musculoskeletal discomfort, abdominal distress, vomiting, fever-related neutropenia, headaches, and skin itching. The research on SCD carried out by the Innovative Genomics Institute highlights encouraging outcomes, such as the restoration of functional hemoglobin levels, alleviation of SCD-related pain, and a reduction in the rate of disease progression [11]. This kind of treatment marks a significant breakthrough as the first gene therapy developed specifically for SCD. One of its key benefits is the potential to eliminate the need for frequent blood transfusions and bone marrow transplants which offer patients a more sustainable and less invasive management option. By doing so, it substantially

improves the overall quality of life for those living with SCD [12].

According to a study in The New England Journal of Medicine by Frangoul et al., two patients --one with Sickle Cell disease and the other with Transfusion-dependent β-thalassemia (TDT)-- received autologous CD34+ cells edited with CRISPR-Cas9 targeting the BCL11A enhancer. Over a year, both patients exhibited high levels of allelic editing in their bone marrow and blood, with substantial increases in fetal hemoglobin distributed across all cells. For the patient with TDT, this resulted in transfusion independence, while the patient with SCD experienced complete elimination of vaso-occlusive episodes [13].

5 Potential challenges of CRISPR-Cas9

Even though the technology of CRISPR-Cas9 has shown significant benefits on the curation of Sickle Cell Disease and also other genetic diseases, multiple challenges still exist.

The first challenge is off-target effect. Off-target effects remain one of the major concerns for CRISPR-Cas9 technology, as they can lead to unintended genetic mutations. This phenomenon arises when the Cas9 protein targets incorrect genomic areas and generates cuts that could result in detrimental effects. The off-aim sites are typically influenced by sgRNA, considering Cas9 can accommodate up to three mismatches between sgRNA and the genomic DNA [8]. These mutations may result in abnormal cellular changes and ultimately cell death [9]. Additionally, off-target mutations pose a risk to environmental stability. Gene transfer between organisms can occur through a continuous mechanism called gene drive. In this process, unintended mutations can also be passed on to other species, hence disrupting the integrity of the environment [9].

The other problem lies in potential influences on human embryos after editing the genes, this use raises significant ethical issues and can lead to irretrievable effects on the future generations. For example, although we have full evidence that an unborn infant would have SCD, we are still unable to use CRISPR-Cas9 on the genes of a human embryo. The controversy of editing genes of an embryo is not about CRISPR-Cas9 itself, but about the unclear status of when the human embryo got a personality. Even though some scientists believe that it's immoral to edit the genes of a human embryo that is older than 14 days, different governments and organizations all around the world did not have a uniform standard toward when a human embryo start to have human rights [10]. Editing the genes of a human embryo using CRISPR-Cas9 and other genetic technologies are still ethically arguable.

6 Summary and outlook

As a revolutionary gene editing tool, CRISPR-Cas9 has demonstrated significant potential in disease treatment, particularly in the area of sickle cell disease. By precisely correcting or modulating disease-causing genes, CRISPR-Cas9 not only addresses genetic defects but also targets unique markers on cancer cells or adjusts risk factors for diseases. Advances in this technology have made the path from basic research to clinical translation more feasible, contributing to the growth of the biopharmaceutical industry, improving public health and disease prevention, promoting global health equity, and generating positive socioeconomic impacts.

References

[1] Gostimskaya I. CRISPR-Cas9: A History of Its Discovery and Ethical Considerations of Its Use in Genome Editing. Biochemistry (Mosc). 2022 Aug; 87(8):777-788.

[2] Ma Y, Zhang L, Huang X. Genome modification by CRISPR/Cas9. FEBS J. 2014 Dec;281(23):5186-93.

[3] Redman M, King A, Watson C, King D. What is CRISPR/Cas9? Arch Dis Child Educ Pract Ed. 2016 Aug;101(4):213-5.

[4] Kato GJ, Piel FB, Reid CD, Gaston MH, Ohene-Frempong K, Krishnamurti L, Smith WR, Panepinto JA, Weatherall DJ, Costa FF, Vichinsky EP. Sickle cell disease. Nat Rev Dis Primers. 2018 Mar 15;4:18010.

[5] Elendu C, Amaechi DC, Alakwe-Ojimba CE, Elendu TC, Elendu RC, Ayabazu CP, Aina TO, Aborisade O, Adenikinju JS. Understanding Sickle cell disease: Causes, symptoms, and treatment options. Medicine (Baltimore). 2023 Sep 22;102(38):e35237.

[6] Ceglie G, Lecis M, Canciani G, Algeri M, Frati G. Genome editing for sickle cell disease: still time to correct? Front Pediatr. 2023 Nov 2; 11: 1249275.

[7] Park SH, Bao G. CRISPR/Cas9 gene editing for curing sickle cell disease. Transfus Apher Sci. 2021 Feb;60(1):103060.

[8] Guo C, Ma X, Gao F, Guo Y. Off-target effects in CRISPR/Cas9 gene editing. Front Bioeng Biotechnol. 2023 Mar 9;11:1143157.

[9] Shinwari ZK, Tanveer F, Khalil AT. Ethical Issues Regarding CRISPR Mediated Genome Editing. Curr Issues Mol Biol. 2018;26:103-110.

[10] Brokowski C, Adli M. CRISPR Ethics: Moral Considerations for Applications of a Powerful Tool. J Mol Biol. 2019 Jan 4;431(1):88-101.

[11] Tariq H, Khurshid F, Khan MH, Dilshad A, Zain A, Rasool W, Jawaid A, Kunwar D, Khanduja S, Akbar A. CRISPR/Cas9 in the treatment of sickle cell disease (SCD) and its comparison with traditional treatment approaches: a review. Ann Med Surg (Lond). 2024 Aug 14;86(10):5938-5946.

[12] Singh A, Irfan H, Fatima E, Nazir Z, Verma A, Akilimali A. Revolutionary breakthrough: FDA approves CASGEVY, the first CRISPR/Cas9 gene therapy for sickle cell disease. Ann Med Surg (Lond). 2024 May 15;86(8):4555-4559.

[13] Frangoul H, Altshuler D, Cappellini MD, Chen YS, Domm J, Eustace BK, et al. CRISPR-Cas9 Gene Editing for Sickle Cell Disease and β -Thalassemia. New England Journal of Medicine [Internet]. 2020 Dec 5;384(3).