Research Progress and Future Directions of CRISPR Gene Editing Technology in β-thalassemia

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***Abstract*—β-thalassemia is a hereditary hemolytic illness where the synthesis of hemoglobin gets reduced. It mainly comes about because of mutations in the β-globin gene. In the recent few years, CRISPR gene editing technology has been brought in and used to correct the mutations related to β-thalassemia. This paper gives a full look at how CRISPR gene editing technology has been applied in treating β-thalassemia over the past five years. The mechanisms and the latest clinical progress of CRISPR gene editing technology when it comes to treating this disease are explored in a systematic manner through three ways: mending the HBB mutation sites, aiming at the BCL11A enhancer to get fetal hemoglobin activated, and holding back the expression of α-globulin. On November 16, 2023, the UK Medicines and Healthcare Products Regulatory Agency gave the green light to the therapeutic drug Casgevy, which makes use of CRISPR-Cas9 gene editing technology, for treating transfusion-dependent β-thalassemia patients. This marks the start of a new era in gene therapy. But CRISPR gene editing technology still has risks like off-target effects. The appearance of new technologies such as base editors brings a new angle for treatment.**

Keywords—β-thalassemia, CRISPR gene editing technology, HBB, BCL11A

I. Introduction

Beta thalassemia is an inherited hemoglobin disorder caused by mutations in the beta globin gene. These mutations cause an excessive build-up of alpha-globin within red blood cells, resulting in ineffective erythropoiesis and hemolysis. There are over 300 mutations that can bring about beta thalassemia. Each year, more than 60,000 patients are diagnosed with this disease across the world [1, 2]. In clinical settings, hemoglobin is seen as playing a crucial role in enhancing the phenotype of beta-thalassemia. Current mainstream treatments include blood transfusion, iron removal therapy, and bone marrow transplantation [3]. However, the first two methods only manage symptoms and cannot cure the disease. While bone marrow transplantation offers a potential cure, the rate of donor matching is less than 20%.

Given that β-thalassemia is caused by mutations in the β-globulin gene, gene editing technology has emerged as the most promising and effective approach. CRISPR-Cas technology, being one of the most widely studied gene editing methods, has made significant breakthroughs from the laboratory to clinical applications in the past five years. On November 16, 2023, the Medicines and Healthcare Products Regulatory Authority (MHRA) in the UK approved the first CRISPR-Cas9 gene editing therapy, Casgevy, for treating transfusion-dependent beta thalassemia patients [4].

This article presents beta-thalassemia and explains the mechanism of CRISPR-Cas9 technology through three treatment ways: correcting mutations in the HBB gene, aiming at the BCL11A enhancer to activate fetal Hemoglobin (HbF), and suppressing alpha globulin expression [5–7]. It also delves into the future development direction and the current challenges that CRISPR-Cas9 technology is facing.

II. Beta-Thalassemia

β-thalassemia is an inherited blood disorder that arises due to one or more mutations in the β-globin gene, making it an autosomal recessive genetic disease. The immediate cause for thalassemia is an imbalance between the number of α-chains and β-chains [3]. In patients with β-thalassemia, the synthesis of β-chains is reduced or even non-existent resulting in a relative overabundance of α-chains. The term “thalassemia” originates from its frequent appearance in the Mediterranean region [8].

The pathogenesis of β-thalassemia is multifactorial and complex. Genetic factors play a central role, as they disrupt the normal production of hemoglobin in the body. Additionally, other modifying elements may interact with these genetic ones, causing all sorts of changes that ultimately lead to the development of β-thalassemia.

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Adult hemoglobin consists of two pairs of globin subunits. When the synthesis of globin chains goes wrong, it leads to abnormal hemoglobin synthesis [2]. The way β-thalassemia comes about has to do with mutations in the β-globin gene. Because of these mutations, the synthesis of β-globin chains either drops or stops altogether. Then, there will be too much free α-globin, which will form cytotoxic precipitates. These precipitates will set off ineffective erythropoiesis and also make mature red blood cells die [8]. The pathophysiological mechanisms behind the clinical manifestations of β-thalassemia are shown in Fig. 1 [8].

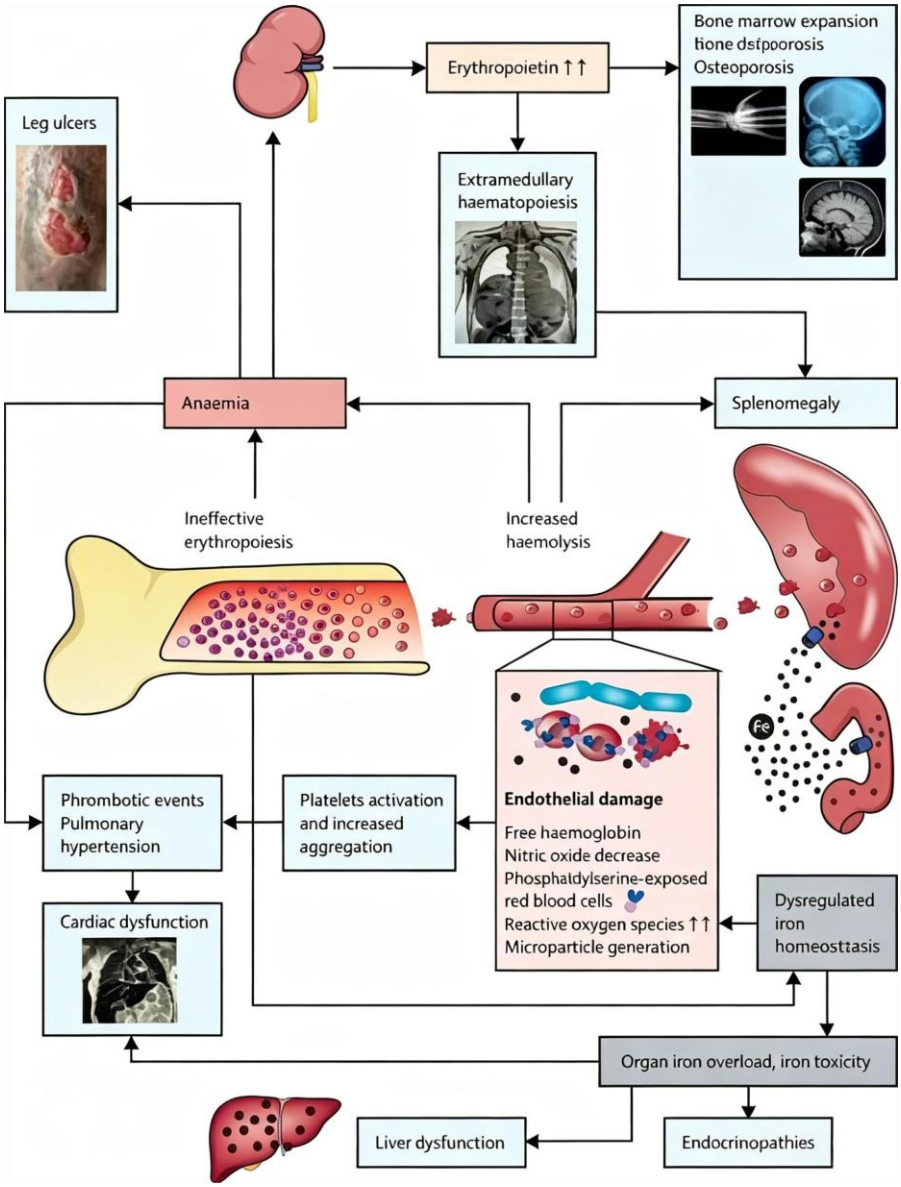

Fig. 1. Pathophysiological mechanisms underlying the clinical manifestations of β-thalassemia [8].

III. The CRISPR Gene Editing Technology

CRISPR gene editing technology has been widely studied and applied in various fields. This technology plays a crucial role in genetic research and related applications. Scientists are constantly exploring its potential and making efforts to further develop and optimize it to achieve better results in different research and practical scenarios. It has brought about many changes and opportunities in the realm of genetics, influencing the way people understand and manipulate genetic information.

Gene editing is a technology that can precisely modify the genome sequence, making insertions, deletions, or substitutions occur within the genome. Among current gene editing tools, CRISPR technology is an efficient editing tool. The CRISPR/Cas9 system originally evolved in bacteria and archaea to defend against phage infections and plasmid

transfers. When foreign phages or plasmids first get into bacteria or archaea, these organisms will get a part of the invading DNA sequence and put it into the CRISPR spacer. If the same homologous DNA shows up again, the bacteria will start the transcription of the CRISPR region. After going through a series of processing and maturation steps to produce a single guide RNA (sgRNA), the sgRNA then guides Cas9 to recognize and cleave the matching DNA sequence. By changing the nucleotide sequence of a small part of sgRNA, CRISPR can achieve precise cutting at almost any site, which makes it possible to correct mutant genes [9].

The target sites of DNA include the 5' end of sgRNA, which matches up with a 20-nucleotide (nt) sequence in the pre-spacer region, and the Protospacer Adjacent Motif (PAM) that's right next to this region and interacts with the endonuclease Cas9 [1]. The way the CRISPR-Cas system works is depicted in Fig. 2 [1].

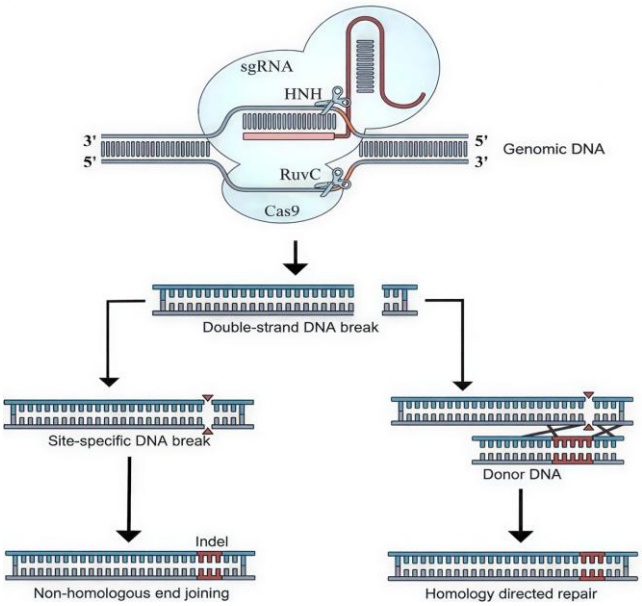

Fig. 2. Mechanism of action of the CRISPR-Cas system [1].

A. The Targeted Activation of the BCL11A Enhancer Can Boost the Production of Fetal Hemoglobin

BCL11A is a transcription factor that has zinc fingers, and it can stop the expression of γ-globin and fetal hemoglobin in red blood cells. Once a baby is born, fetal hemoglobin gets silenced because the transcription factor BCL11A suppresses the γ-globin gene (HBG). If CRISPR gene editing technology is used to target and disrupt the BCL11A erythroid-specific enhancer, make the expression of BCL11A go down, get the γ-globin gene to express again, bring back γ-globin synthesis, and in this way, restart the production of fetal hemoglobin [6, 10]. In the experiment that Frangoul and others reported, the levels of fetal hemoglobin in the donors that were edited were clearly higher than those in the controls that weren't edited. In an experiment conducted by Fu et al., autologous hematopoietic stem cells and progenitor cells with the BCL11A enhancers that were edited were transplanted into two patients. After more than 18 months, both patients didn't need transfusions anymore. Their Hb levels went up from 8.2 and 10.8 g/dL to 15.0 and 14.0 g/dL, respectively [7, 10].

B. Fixing the Mutation Sites of the Beta-Globin Gene

For mutation sites on the β-globin gene—such as the point mutation IVS2-654 C > T or the small deletion CD41/42-CTTT—the CRISPR-Cas9 system can be utilized to precisely correct the mutated gene [5]. Two main strategies have been developed for this purpose.

The first approach involves gene correction through induced Pluripotent Stem Cell (iPSC) repair. Initially, the patient's somatic cells are reprogrammed into iPSCs, which undergo differentiation into Hematopoietic Stem Cells (HSCs) after correcting the β-globin gene mutation in vitro. These cells are then reintroduced into the patient's body, facilitating autologous transplantation. In a study conducted by Lingli Li and colleagues, a novel iPSC line was derived from fetal amniotic cells carrying a homozygous β41–42 (TCTT) deletion mutation in the HBB gene and a heterozygous Westmead mutation (C > G) in the HBA2 gene [5]. The CRISPR-Cas system was employed to target and correct the mutations. The repaired iPSCs retained pluripotency and could differentiate into CD34+ hematopoietic stem/progenitor cells, successfully re-establishing the erythroid lineage in a mouse model. However, the differentiation efficiency of iPSCs was insufficient, and the long-term engraftment ability of the differentiated HSCs was inferior to that of natural HSCs.

The second approach involves in situ gene correction, directly repairing mutations in the patient's HSCs. Yang and colleagues designed two pairs of sgRNAs (targeting the 5' and 3' ends of the abnormal exon) for the IVS2-654 splice site mutation [11].

C. Suppression of α-Globin Expression

The main pathophysiological mechanism of beta-thalassemia involves a serious imbalance in the synthesis between α-globin and β-globin chains. In patients with beta-thalassemia, the synthesis of β-globin chains is significantly cut down or is completely gone, while the synthesis of α-globin chains stays relatively normal. As a result, an excess of free α-chains piles up abnormally inside red blood cells and forms insoluble deposits (inclusion bodies). These deposits harm the erythrocyte membrane, which then leads to ineffective hematopoiesis and hemolytic anemia, and these are the direct reasons for the disease to progress [8].

So, fixing this α/β globin chain imbalance has turned into one of the crucial treatment strategies. According to this principle, the CRISPR-Cas gene editing technology offers a strong tool. An innovative way is to target the genomic area that controls α-globin expression, like its enhancer elements, to bring down the expression level of α-globin. The intention

is to ease the relative excess of α-chains and make the imbalance better.

Pavani and colleagues carried out this strategy using the CRISPR-Cas9 system to accurately delete the HBA2 gene, a key gene in the α-globin gene cluster [2]. The experiment was conducted in HUDEP-2 cells (a model of erythroid progenitor cells) that came from patients with β^0/β^0 thalassemia. The experimental results showed that deleting the HBA2 gene worked well to reduce α-globin synthesis. This intervention managed to correct the α/β globin chain imbalance at the cellular level and really cut down on the formation of harmful α-chain deposits. Moreover, when they applied this editing strategy to the patients' own hematopoietic Stem Cells and Progenitor Cells (HSPC), they also saw improvements in the α/β globin imbalance, which paved the way for a possible autologous stem cell transplantation therapy [12].

IV. The Advancement in Clinical Transformation

The treatment landscape for severe genetic blood disorders, such as Transfusion-Dependent Beta-Thalassemia (TDT), is going through a revolutionary change driven by CRISPR-Cas9 gene editing. This breakthrough started getting formal acknowledgment in late 2023 [4]. On November 16th of that year, the UK's MHRA issued the world's first regulatory approval for a CRISPR therapy, Casgevy (exagamglogene autotemcel), specifically for TDT.

The convincing efficacy data that support these approvals come from global clinical trials. In these centers, 91% (32 out of 35) of the 54 TDT patients who could be evaluated reached the crucial therapeutic aim: being independent of transfusions [4, 7]. After treatment, the median level of HbF reached 11.9 grams per deciliter (g/dL), making up a striking 94% of the patients' total hemoglobin. This effectively makes up for the defective adult beta-globin. There's further innovation seen in the methods used. Chinese research using Bangyao Biotech's non-viral vector editing platform (BRL-101) showed promising results, especially in tough cases. Pediatric patients with the severe β^0/β^0 genotype, who don't produce any functional beta-globin, had HbF levels that went over 140 grams per liter (g/L) after treatment. This huge increase has kept these children alive without the need for transfusions for over two years.

Looking ahead, next-generation base editing therapy (like CS-101) seems to offer even more precision and power. By aiming at a specific spot (the −113 position) in the γ-globin gene promoter, CS-101 has shown that it can increase HbF expression about four times more than traditional CRISPR-Cas9 editing in preclinical models. This might give a more effective way to get to therapeutic HbF levels for a broader range of patients. This quick move from the idea to an approved therapy, along with the continuous technological improvements like non-viral delivery and base editing, means a big shift in the way things are done. It gives real hope for a functional cure to patients who are suffering from TDT [10, 11].

V. Challenges and Future Prospects

The CRISPR-Cas9 system has been shown by a whole bunch of studies to be a powerful and quite precise gene-editing tool. Its operation process is straightforward and can be carried out in standard molecular biology laboratories you can think of [9]. But there are still several challenges left. There are problems related to things like how often HDR happens, off-target effects, the ways of delivering it, choosing the target site, and designing sgRNA. If people can deal with these challenges, it will boost the effectiveness and safety of the CRISPR-Cas9 system. Right now, the main problem with the CRISPR-Cas9 system is its delivery mechanism. Coming up with new ideas in delivery systems, like using electroporation along with RNP complexes and Lipid Nanoparticles (LNPs), can make the safety profile of the CRISPR-Cas9 system a lot better [1].

VI. Conclusion

Casgevy (exagamglogene autotemcel), which is based on the revolutionary CRISPR-Cas9 gene editing technology, has reached a milestone in the practical application of gene therapy, especially when it comes to the treatment of β-thalassemia. This serious hereditary blood disorder is caused by mutations in the β-globin gene. It leads to insufficient hemoglobin synthesis and chronic anemia, and for decades, new therapeutic methods have been needed. Casgevy gets around this problem. It precisely edits the patients' own hematopoietic stem cells outside the body to reactivate the expression of fetal hemoglobin. In this way, it can make up for the defective adult hemoglobin and achieve a functional cure. Compared with traditional gene-editing technologies, the CRISPR-Cas9 system has obvious simplicity in design and a big improvement in precision. Its modular feature enables it to target different genomic sites just by changing the guide RNA sequence, making it an adaptable and powerful tool. This advancement does not only bring a transformative treatment for β-thalassemia but also creates a path for new therapies aimed at many diseases that come from specific gene mutations. Even though technology looks quite promising, it's not without its challenges. There are ongoing concerns about off-target mutations, which are those unintended edits that happen on similar DNA sequences. Nevertheless, the clinical success of Casgevy really shows the huge potential of CRISPR-Cas9. Lots of research efforts are busy dealing with these limitations. They are trying hard to come up with more accurate Cas variants and investigate new delivery vectors, including both viral and non-viral ones. Although there are still difficulties, the therapies that rely on CRISPR-Cas9 have remarkable potential. It gives a hint that many genetic diseases that cannot be cured at present might be treatable in the near future. This will completely change the situation of medicine.

Conflict of Interest

The authors declare no conflict of interest.

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