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CRISPR/Cas9 in the treatment of β-thalassemia

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Abstract. β-thalassemia is a congenital hemolytic anemia, a class of hemoglobinopathies caused by a common single-gene recessive condition in which the synthesis of the chain is either totally or partially blocked. About 50% of patients have symptoms within the first three months of life. At birth, the disease is often asymptomatic, and it mostly gets worse during infancy. If left untreated, the disease usually claims its victims by the time they are five years old since its severity rises with age. Currently, there is no cure for this disease, and the main treatment is blood transfusion. With the continuous progress of medical level, gene editing technology is developing rapidly, among which CRISPR/Cas system as a gene editing technology has been applied in the research of single gene genetic disease treatment, and CRISPR/Cas9 has been widely used in the research of treating geopathic anemia because of its high efficiency, low cost, and high specificity, for example, repairing pluripotent stem cells' HBB genes, inducing fetal hemoglobin expression and inhibition of HBA gene expression. This review methodically explains the development of CRISPR/Cas9 through its creation and mode of operation, various research hypotheses tested in animal studies and clinical trials, as well as analyses of safety concerns.

Keywords: CRISPR/Cas9, β-thalassemia, Gene Editing.

1. Introduction

The world has always been plagued by a major problem of single-gene genetic disorders. β-thalassemia, as a single-gene genetic disease, also causes great harm worldwide. The HBB quality, which is located on chromosome 11, is the factor that causes β-thalassemia, and more than 200 different pathogenic alterations have been confirmed to exist. These alterations might be categorized as mild, moderate, or severe [1]. Approximately 300,000 children worldwide are born each year with thalassemia or sickle cell anemia, and 80 to 90 million people are known to carry the β-thalassemia gene, according to measurements. The majority of cases of β-thalassemia are found in China's south of the Yangtze River, with Guangdong, Guangxi, and Hainan having the highest rates. Blood transfusions and constant medication are the main alopecia treatments; nonetheless, no complete cure has been found [1]. Long-term blood transfusions can also result in a significant quantity of iron buildup in the body, necessitating the use of iron removal medicine. However, excessive or prolonged usage of this type of medication can be detrimental to other elements, including eyesight. With the consistent improvement of hereditary designing and the improvement of quality altering innovation, the expansion of qualities, yet additionally the exact remedy and substitution of qualities, it has turned into a possible means to apply this innovation to the treatment of β-thalassemia [2], and CRISPR/Cas9 quality altering innovation has

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turned into a significant arising remedial innovation due to its more exact, more proficient and more secure highlights. This review examines the use of CRISPR/Cas9 innovation in the treatment of β-alopecia, breaks down and discusses the connected security issues, and anticipates the improvement prospect.

2. The history of gene editing technology and the mechanism of action of CRISPR/Cas9

2.1. History of CRISPR/Cas9

Gene editing technology has evolved over the course of history, and so far there are three main generations of technology, zinc finger nuclease (ZFN), transcription activator-like effector nuclease (TALENs), and the CRISPR/Cas system, and there is even a 3.5 generation of the emerging single base gene editing technology [3]. To achieve its purpose, CRISPR/Cas technology only requires the design of a segment of sgRNA complementary to the target sequence, unlike the previous two generations of technology. After the experimental success of CRISPR/Cas technology in 2013 in the precise cutting of endogenous genomic loci in mouse mammalian eukaryotic cells, it officially started the gene editing boom [2], and the Nobel Prize in Chemistry in 2020 is for gene editing-related research. It is evident that gene editing technology has been rapidly developing and has made significant progress in editing efficiency, safety, and other aspects.

2.2. Mechanism of action of CRISPR/Cas9

CRISPR, also known as clustered regularly interspaced short palindromic repeats, was first identified in bacteria and is an acquired immune defense system that has evolved during the growth of archaea. It has the ability to transform specific DNA sites into editable targets for gene editing and is used to protect against plasmids, viruses, and other invasive organisms [4]. According to the different types of Cas, CRISPR/Cas system can be mainly divided into three types, of which Cas9 is a type II system, and the action process can be mainly divided into three stages.

The first stage is the recognition and capture of exogenous DNA by Cas protein, the exogenous DNA is cleaved by Cas into a proto-spacer sequence and then integrated into the host gene after the lead sequence of CRISPR, there is a neighboring sequence PAM at the 3' end of the proto-spacer sequence, which is the recognition site of Cas to distinguish whether it is an exogenous sequence or not. The second stage is the transcription of the CRISPR gene to synthesize crRNA, which is initially transcribed as pre-crRNA, followed by tracrRNA alone and complementary to the former to form a heterodimer, and finally cleaved by RNase III to form a mature crRNA. The third stage is targeted interference, after the above two stages, if the exogenous DNA re-enters the bacteria, tracrRNA and crRNA and Cas9 bind to form a nucleic acid protein complex (RNP) and Cas9 recognizes the PAM sequence and performs double-stranded cleavage. The two main repair pathways for double-stranded broken DNA are NHEJ and HDR [2, 4].

3. CRISPR/Cas9 remedial strategies for thalassemia with a few unique technique for treatment

CRISPR/Cas9 innovation has been used in a large number of fields, particularly in the treatment of monogenic hereditary illnesses, like Huntington's chorea, where extraordinary headway has been made. Therapies based on CRISPR/Cas9 system has been a choice for β-thalassemia with the ceaseless advancement of science and innovation of clinical exploration.

3.1. Induction of fetal hemoglobin expression mediates HBG gene and thus γ-bead protein expression

Reactivation of the expression of fetal hemoglobin is mainly through the formation of γ-globin, which can bind to free α-globin in the body to reduce adverse pathological reactions. Xiangya Clinic of Focal South College has conducted this study, and the primary thought was to relocate HSPCs with CRISPR-Cas9 altered BCL11A enhancers into two youngsters with β-thalassemia (Patient 1: β^0/β^0; Patient 2: β^+/β^+), which came about in persevering HbF enlistment [5]. In this study, two children with TDT were

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screened by certain screening means, and CRISPR-Cas9 and modified synthetic sgRNA electroporation was utilized to prepare CRISPR-Cas9-edited CD34+ HSPCs from these isolated CD34+ cells, and CRISPR-Cas9 gene-edited autologous CD34+ cells were infused into these two patients. By using line plots of relevant pre- and post-transplantation blood content data for each component, therapeutic efficacy was demonstrated, and off-target risk was assessed using deep sequencing technology. According to the data, autologous HSPCs with BCL11A enhancer editing can help restore blood profiles after infusion without any adverse consequences.

3.2. In situ repair of the HBB gene by correcting the expression of the bead protein gene

There are additionally numerous methods of in situ fix, and the locales that can be centered around are exons, introns, and so forth, yet the present status of exploration isn't great or has not been concentrated on top to bottom [6]. There are essentially no clinical trials complete with assessments of the expression levels of β-bead proteins after gene editing and whether they can be transcribed and translated in the long term [2]. However, some animal experiments realized a side-by-side comparison of the potential for HBB gene repair by several different gene editing techniques, and found that CRISPR/Cas9 showed higher DSB efficiency, and its editing efficiency was higher than that of TALENs and ZFNs, among others.

In the study by Peng Xu et al., experiments were conducted using mice to demonstrate the feasibility of direct modification of HBB for therapeutic purposes by directly targeting the HBB IVS2-654 (C>T) mutation in β-thalassemia iPSCs using both TALENs and CRISPR/Cas9, and the two modifications were compared in terms of safety and efficiency [7]. The T7 nucleic acid endonuclease I assay showed that TALEN-1 had higher DSB efficiency than TALEN-2, while sgRNA-2 had higher efficiency than sgRNA-1. Sanger sequencing confirmed that TALENs mediate a higher degree of homologous recombination than CRISPR/Cas9, as confirmed by the selection of four positive TALENs and positive CRISPR/Cas9. Regarding off-targeting, 10 potential off-target sites were amplified by PCR in CRISPR / Cas293-transfected cells, whereas only 2 were present in TALENs, so the off-targeting effect of the TALENs technology was found to be lower after comparison. This experiment demonstrated the potential of the two techniques described above for β-thalassemia gene correction, but more studies are needed to verify whether β-thalassemia iPSCs can be corrected to produce functional and mature hematopoietic progenitor cells.

Lingli Li et al.'s used CRISPR-Cas9 technology to genetically correct pluripotent stem cells derived from α- and β-thalassemia patients [8]. CRISPR-Cas9 technology has successfully repaired the CD41/42 (-CTTT) mutation in the HBB gene. Patient-specific hiPSC has successfully corrected the HBA2 gene with a Hb CS mutation. In this study, it was reported that a patient has a HBB pure deletion mutation (CD41-42) and an HBA heterozygous subpoint mutation (Hb-WS). The objective of the experiments was to identify gRNAs that have long sequences of the 41-42 mutant sequence on the HBB gene and the Hb-WS mutation on the HBA2 gene. corrected clones were selected by antibiotic selection after transfection of the long linearized donor plasmid and Cas9 gRNA into hiPSCs.

3.3. Inhibition of α-bead protein gene expression and correction of α/β-bead protein imbalance

Artificially creating a state of α-thalassemia in the organism is also a therapeutic idea, i.e., inhibiting the expression of the HBA gene. This approach also has a substantial theoretical and experimental basis, among which in vivo mouse experiments by Giulia et al. demonstrated the use of the CRISPR/cas9 system to delete the HBA2 gene to mimic α-thalassemia as well as the targeted integration and expression of an HBB transgene under the control of endogenous HBA to promote it [9]. Using RNP transfection of HSPC and transduction using AVV, the expression of bead proteins was examined during red lineage differentiation and the imbalance was found to be improved. Globin mRNA analysis of individual BFU-E colonies from β^0- and β^+-edited HSPCs showed that the α/β imbalance was ameliorated under all conditions, based on the reduction in the number of HBA2 genes, and the concomitant stronger effect produced by α down-regulation and β^{AS3} expression. Therefore, editing patients' HSPC can improve β^+ and β^0-thalassemia phenotypes. Furthermore, since the edited HSPC can

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be implanted in vivo with the potential for multispectral differentiation, this therapeutic regimen is highly feasible for future clinical trials.

There are even a few novel trial results, as Cosenza et al. effectively revised the HBB quality in red genealogy forerunner cells from patients with the β^{039} transmission box transformation by utilizing CRISPR/Cas9 innovation, and found a lot of HbA protein articulation and a critical decrease in free α-globule protein [2]. Despite this, there are still a few risks associated with the technique.

4. Conclusion

Regardless of which of the above remedial means or exploration strategies, they will ultimately confront the off-target issue, which is additionally a significant thought for whether they can be advanced as the principal single-quality sickness helpful techniques eventually. Despite the use of sgRNA succession sequencing such as CIRCLE-seq and GUIDE-seq, CRISPR off-target risk appraisal techniques have not yet become more mature. There is additionally the issue of DNA harm that ought not be overlooked. In CRISPR/Cas9 innovation, quality altering should be finished under the state of twofold strand breaks, yet twofold strand breaks will set off the apoptosis program, and that implies that the actuation of apoptotic qualities, for example, P53 will be set off. There is an answer for stay away from twofold abandoned DNA breaks - single-base quality altering (BE). Chinese researchers have discovered the TaC9-ABE framework, which is the main recognition of Story restricting to adenine deaminase using BE innovation [10]. Acknowledging A to G transformation can be achieved by employing Cas9 variations that restrict target DNA under gRNA direction and adenosine deaminase that restricts a similar objective under Story direction. The upside of this arising framework is the detachment of quality altering and acknowledgment structures, which are commonly obliged so altering can be started just when the two players have perceived the right objective site, disguisedly keeping away from the event of off-target peculiarities and DNA harm in the outcomes.

This review recognizes the development of gene editing technology, the therapeutic idea of CRISPR/Cas9 technology in β-thalassemia, and the analysis of safety issues, and describes the possibility of β-thalassemia treatment. Although the application of this technology in the treatment of β-thalassemia is still very immature and there are still a lot of problems to be solved, such as the introduction of stem cells, whether it affects other genes, etc., and the most important and difficult problem is the detection and solution of off-targeting, the new findings from the experimental studies confirm the feasibility of these methods for the treatment of this disease, and lay the groundwork for the possibility of the complete cure of the single-gene disease in the future [3, 11, 12]. Currently, HSCT and viral vector-based gene therapy are the only therapeutic options that offer a potentially permanent cure for TDT, and other possible responses include active premarital testing, genetic counseling, and so on, to prevent the birth of affected children and to reduce the pressure on families and health care. With the continuous development of science and technology, gene editing technology will continue to progress, optimize and improve, and in the future, we will find effective and safe therapeutic methods for treating β-thalassemia as well as other genetic diseases, and carry out the treatment of diseases from the genetic level.

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