Proceedings of the 3rd International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/23/20231026

CRISPR-based genomic modification method in the treatment of hematological diseases

Yi Cheng Tao

College of Life Sciences, Shanghai Normal University, Shanghai, 200333, China

1000500751@smail.shnu.edu.cn

Abstract. Mutations in hematopoietic stem cells are the main cause of most blood diseases. Hematopoietic stem cell transplantation, which is the main treatment for hematopoietic stem cell disorders ,brings many negative effects according to the clinical cases, such as high toxic side effects, limited donor source, high recurrence rate, and high treatment cost .CRISPR/Cas acts as an immune system in prokaryote that grants them an ability to resist invasion by exogenous mobile genetic elements (MGE), which is a new generation of gene editing technology after the appearance of TALEN and ZFN. The immune system functions in three steps: adaptation, expression, and interference. It enables genomic modification by unwinding the double strands of DNA and inducing DNA repair processes such as homologydirected repair (HDR) and/or non-homologous end joining (NHEJ). So, this paper discusses the utilization of Crispr system in the treatment of β-hemoglobinopathy, Chronic myeloid leukemia (CML) , Hemophilia and summarizes the current limitations of this technology, such as the editing efficiency, potential immunogenicity of editing tools and off-target effects , while it still remains promising as to its future prospects for research. This technology is very likely to replace traditional therapies and achieve a complete cure for the blood disease.

1. Introduction

Blood and hematopoietic organs constitute the blood system. As a class of pluripotent stem cells, hematopoietic stem cells (HSC) can subsequently differentiate into various blood cells and immune cells, and are highly self-renewing and can differentiate into all types of blood cells. As adult stem cells after birth, HSCs are mainly found in bone marrow, which is therefore the main hematopoietic organ, while almost no HSCs are found in peripheral blood. Hematopoietic stem cell transplantation (HSCT) can alleviate the symptom of blood diseases originated from the mutations of hematopoietic stem cell by removing abnormal bone marrow hematopoietic tissues and transplanting in hematopoietic stem cells with normal functions, supplemented with chemotherapy and radiotherapy, it is a comprehensive treatment program that may cure hematogenetic diseases and hematological malignancies However, the negative effects cannot be ignored. Radiotherapy and chemotherapy bring harm to normal cells and affect organ functions. The mutagenic effect of drugs and ionizing radiation makes long-term use risky, rejection reaction after hematopoietic stem cell transplantation, low immune function, etc.

The CRISPR technology is a new generation of genetic modification technology, which is an immune system in prokaryote that grants them an ability to fight against exogenous genes and viruses [1]. The CRISPR system integrates fragments of invading exogenous DNA into CRISPR Array and the homologous sequences are degraded by CRISPR RNAs (crRNAs) to achieve immune function.

DOI: 10.54254/2753-8818/23/20231026

The CRISPR system incorporates single-guide RNA and Cas9 proteins. Similar to ZFN and TALEN, by unwinding the double strands of DNA and inducing DNA repair processes such as homologydirected repair (HDR) and/or non-homologous end joining (NHEJ), CRISPR/Cas technology also makes it possible to modify genes. Among them, homologous recombination repair necessitates the existence of late S and G2 sister chromatids, and DNA are then repaired precisely according to the template. By comparison, Non-homologous end joining does not need a homologous template and is not able to accurately repair DNA double-strand breaks, which can result in mutant sites being deleted, key DNA sequences being altered, or nucleotide insertions and deletions(indel) at double-strand breaks that can happen at any point in the cell cycle. The selection between the two pathways is largely determined by the homologous template and the cell cycle, and is difficult to regulate artificially [2]. This technology differs from previous means of gene editing: a Cas9 nuclease reaches the target site with the help of RNAs with specificity, cuts the specific site and achieves targeted mutation of genes, which is fast, efficient and economical, while gene editing of hematopoietic stem cells can cure many genetic and acquired diseases.

The CRISPR system serves as the host's immune system, guarding it against any Mobile Genetic Element (MGE)[3]. Due to this universality, it has been discovered that the majority of bacteria and archaea include this defence system, which determines the diversity of the CRISPR/Cas system. The Crispr-based system currently has 2 major classes, 6 types and 33 isoforms. Two main classes are included in Crispr according to types of the Cas proteins: Class 1 systems are effector complexes made up of several effector proteins, including type I, III, IV; Class 2 CRISPR/Cas systems are single Cas protein complexes, including type II, V, VI. Compared with type 1, type 2 systems have greater potential for genome editing and gene screening applications, and therefore have gained the attention of many researchers and been fully developed and applied, such as type II Cas9 (Csn1), type V Cas12a (Cpf1), type VI Cas13a (C2c2) and Cas13b (C2c6) systems. Mechanism of action of each type depends on the respective different Cas proteins and gRNAs, which provides a variety of options for genetic modification. Among them, the classical type II CRISPR/Cas9 system, which has been continuously developed and improved, is by far the most widely used genomic modification method. Also, the type V Cas12a system is considered as one of the genome editing technologies with good application prospects and research value.

Recent methodological advances in hematological disease research have been revolutionized with the advent of the CRISPR-Cas9 precision genetic modification method, which has been shown to delete and rectify genes or mutations and to introduce specific genes which is for therapy in human cells. Thus, this system-mediated gene therapy seems to be a new method for the treatment of inherited hematological diseases and is expected to alleviate the related symptoms by correcting the mutated genes in the near future. So, the Crispr-based system is a flexible genomic modification technology that may be used to fix, disrupt, or introduce genes in HSPCs and iPSCs from patients. Below is a summary of recent preclinical studies on the targeting of HSPCs and iPSCs in vivo by CRISPR/Cas9.

2. β-hemoglobinopathy

Sickle cell disease and β-thalassemia are both subordinate to β-hemoglobinopathies, which are brought on by the abnormalities of the HBB gene in hematopoietic stem cells. Daniel P. Dever ’s team deliver a homologous donor to perform NHEJ of the HBB gene using the Crispr-based technology. The study showed that the mutation of Glu6Val, which causes sickle cell disease can be effectively corrected by using the patient's own stem and progenitor cells for editing, and that these stem and progenitor cells expressed adult β-bead protein (HbA) messenger RNA after differentiation into erythroid cells. Their findings confirmed that the edited HBB allele could successfully achieve complete transcriptional regulation [4].

DOI: 10.54254/2753-8818/23/20231026

3. Chronic myeloid leukemia (CML)

CML is another blood disease originated from hematopoietic stem cell. It is caused by a translocation of the chromosome: When the ABL proto-oncogene on chromosome 9's long arm moves to chromosome 22's long arm's breakpoint cluster region (BCR), a fusion gene is then produced. The expression of this fusion protein leads to the oncogenesis of downstream signaling proteins in hematopoietic stem cells [5]. Currently, small molecule inhibitors of the BCR-ABL protein are employed in clinical settings to exert anti-leukemic effects and prolong patient life, such as the first-generation drug imatinib and the second-generation drug dasatinib, but this approach can gradually develop resistance. Garcia-Tunon et al. designed three sgRNAs targeting the BCR- ABL fusion sequence, tyrosine kinase sequence-1 and tyrosine kinase sequence-2, and combined them with Cas9 and lentivirus to form a lentiviral vector transfected with Boff-p210 cell line. Subsequently they cultured CRISPR/Cas9-edited cells for 3 weeks in mice in a tumor model, and no tumor growth was detected. Therefore, their study suggests that the knockdown of this mutated gene by CRISPR-based methods may be able to replace conventional drug therapy in the future and achieve a complete cure for CML.

4. Hemophilia

Hemophilia has an approximately 1 frequency in 10,000 people [6]. Hemophilia A(HA) or hemophilia B (HB) is caused by the VIII (FVIII) or IX (FIX) genes'mutations. Due to the gene fragment with shorter length (1.5 kb), HB is more suitable than HA for the gene therapy that is virus-based, and has a higher incidence rate of 85%. Previous research have used human FVIII or FIX-expressing AAV vectors extensively for gene therapy and the results are ideal, including the safey of it [7].

Pavani's team recently integrated therapeutic genes into human HSPCs using CRISPR/Cas9[8]. Treatment options for hemophilia and other hereditary illnesses are made possible after normal protein has been successfully expressed by editing the HSPCs. Furthermore, genome editing in iPSCs to correct the mutating site is also feasible and another choice. As previously established, recessive congenital hemorrhagic disorders like HA are brought on by mutations in FVIII gene. B domain of FVIII has 475 kinds of mutations that cause HA, according to the Human Gene Mutation Database. In order to cure the error of B domain in HA-iPSCs, Hu's team reframe the 4-bp frameshift loss with targeted deletion of 50 bp utilizing CRISPR/Cas9. [9]. The endothelial progenitor cells, which is reframed and derived from iPSC showed FVIII expression and activity in vivo test. Additionally, the 1st and the 22nd introns in FVIII gene are respectively involved in 140-kbp or 600-kbp of two extensive inversions of chromosomes that account for around half of all severe HA cases [10]. Park's team used the CRISPR system to correct two inversions of chromosome in IPSCs derived from the hemophilia patients and the consequences were demonstrated in mice that were HA-letha l[10]. Another kind of serious HA is caused by insertions, deletions, and point mutations [11]. By specifically inserting the correct gene fragment which is fuctional into the human H11 site or FVIII locus using the Crispr-based technology, some researchers revealed a flexible method to fix these various mutations in iPSCs derived from HA patients [12, 13]. The gene-corrected iPSCs produced a functionally active FVIII protein in vivo as a result of the addition of the FVIII gene. However, scientists were unable to verify the functional outcomes of the corrected iPSCs after being implanted into the body. But there have been no inversion reports in HB. In the FIX gene, missense or frameshift mutations account for about 80% of severe HB cases [14]. As was already established, HB's monogenicity and suitable viral payload make it an excellent option for gene therapy. In order to fix these mutant types utilizing CRISPR-centered methods to modify the gene. According to the reports, a FIX cDNA was inserted into the AAVS1 region or the 1st exon of native FIX gene in iPSCs derived from the HB patients [15, 16]. In a short-term experiment, the altered iPSCs were differentiated into hepatocytes that could release FIX in a stable manner, and these liver cells were tested to be active when being transplanted into the body of the animal model with HB [15]. However, these universal gene-correction techniques should be given further thought as a secure therapy that based on cell. Mizuno's team has proved a concept of the therapy for the HB gene through the CRISPR-based

DOI: 10.54254/2753-8818/23/20231026

technology [17]. Their research proved that the CRISPR-based genomic modification technology can successfully differentiate iPSCs into the liver and correct the mutation of the FIX genes.

5. Challenges

Despite the great prospect presenting by the CRISPR-based genomic editing methods according to a large number of related researches, many issues concerning it still need improvement.

First, for example, the efficiency of shearing and editing is an issue. For editing at a particular gene locus, there are not many options for gRNA, so the main hinder for using various kinds of cleavage of the CRISPR system is the original spacer adjacent motif (PAM) sequence [18]. Nineteen isoforms of the CRISPR system with Cas proteins that recognize different PAM sites have been introduced to recognize diverse PAM sequences, expanding the genomic loci that can be targeted, but not all are forms are guaranteed when it comes to their effectiveness and security, so the researchers tend to utilize the most common Cas types in their studies (eg. Cas9 -SpCas9 or Cpf1-Cas12a), which also directly contributes to the low editing efficiency.

Second, editing tools may be potentially immunogenic. In 2019, preliminary clinical trial data published in the U.S suggest that the application of CRISPR/Cas9 in curing SCD and thalassemia may be detrimental, mainly because the guide RNA and Cas9 protein will have an effect on the immune system and can be cytotoxic. According to the tests on the normal people in 2018, Charlesworth's team tested the presence of antibodies to Cas9 from Staphylococcus aureus (79%) or Cas9 from Streptococcus pyogenes (65%). The same year, Simhadri et al. measured antibodies against SaCas9 and SpCas9 with prevalence rates of 10% and 2.5%, respectively. Though it was not out of expect, the immune system’s being activated by the CRISPR system is potentially detrimental to humans, and therefore the levels of expression, methods of delivery, vector types required for transduction, and target cell populations of Cas9 need to be optimized to reduce immune responses as much as possible [19].

Finally, off-target effects are currently the biggest problem of this genomic modification method, mainly due to a fact that a Cas9 ribonucleoproteasome’s recognition can allow a 5-base mismatch. So it may result in unexpected genomic abnormalities, and the resulting set of safety issues cannot be ignored[20]. There are two main approaches to increase the specificity of this system, one of which is to target the design of guide RNA (gRNA) by chemically modifying it during its synthesis or by shearing Spacer RNA, which can effectively increase the specificity of Cas9 nucleic acid endonuclease. The other approach uses a Cas9’s variant that can only cut one DNA strand in a single pass, the incision enzyme (Cas9n) to enhance the recognition specificity of Cas9. Even so, clinical trials have revealed a number of problems: chromosomal deletions, insertions and rearrangements, the mechanisms of which are still unclear, and therefore a large number of clinical studies still need to be implemented to elucidate this series of safety issues [21].

6. Conclusion

CRISPR technology has revolutionized genome editing. Currently, the Cas protein of type II is more welcomed by the researchers. The type V CRISPR/Cas12a, though discovered a little later, has its unique advantages, such as the ability to target multiple genetic loci for editing, recognition of T-rich PAM sites, and nucleic acids. The ability to target multiple loci for editing, recognize T-rich PAM sites, process crRNA on its own by nucleases and generate sticky end gaps for repair, etc., completes the versatility of the CRISPR/Cas system together with Cas9. Data also indicate that Cas12a appears to be safer and more reliable than Cas9. Although Cas12a has achieved promising results in human cells and disease treatment, etc., the potential off-target rate still cannot be ignored, and genome editing in humans involves safety and ethical issues and cannot be applied in the clinic at this stage. as mentioned above. However, as more types of CRISPR/Cas are mined and genome editing tools continue to mature, the technology could one day be safely and effectively applied in more fields.

This paper presents cases of β-hemoglobinopathies, chronic myeloid leukemia and hemophilia treated with the Crispr/Cas system. The treatment of these diseases is achieved through targeted

DOI: 10.54254/2753-8818/23/20231026

editing of pathogenic gene loci using Crispr/Cas system, including HBB gene, BCR- ABL fusion sequence and FVIII or IX (FIX) genes. In terms of the limitations, the efficiency issues of shearing and editing, potential immunogenicity, and off-target effects have led to the immaturity of this Crispr technology for the treatment of hematologic diseases in medicine. However, the utilization of the Crispr-based method to treat hematologic disorders is still extremely promising, with the possibility of replacing traditional hematologic therapies and achieving a complete cure for hematologic diseases in the coming few decades.

References

[1] Fu, B., Liao, J., Chen, S. et al. "CRISPR–Cas9-mediated gene editing of the BCL11A enhancer for pediatric β0/β0 transfusion-dependent β-thalassemia." Nat Med 28, 1573–1580 (2022).
[2] Bao LW, Zhou YY. et al. "Advances in CRISPR/Cas9-based gene therapy for β-thalassemia and hemophilia. "Heredity 10, 949-964(2020).
[3] Bao XR, Chen MS, Zhong J. et al. "CRISPR/Cas12a genome editing technology and applications". China Journal of Biological Engineering
[4] Dever, D., Bak, R., Reinisch, A. et al. "CRISPR/Cas9 β-globin gene targeting in human haematopoietic stem cells." Nature 539, 384–389 (2016).
[5] i WY, Gao QP, Liu H. "CRISPR/Cas9 system and its research progress in gene therapy for hematological diseases". Chinese Journal of Experimental Hematology 06, 1863-1867(2018)
[6] Srivastava A, Brewer AK, Mauser-Bunschoten EP, et al. "Guidelines for the management of hemophilia." Haemophilia.19: e1–47 (2013).
[7] Nathwani AC, Reiss UM, Tuddenham EG, et al. "Long-term safety and efficacy of factor IX gene therapy in hemophilia B." N Engl J Med. 371:1994–2004 (2014).
[8] Pavani G, Laurent M, Fabiano A, et al. "Ex vivo editing of human hematopoietic stem cells for erythroid expression of therapeutic proteins." Nat Commun. 11:3778 (2020).
[9] Hu Z, Zhou M, Wu Y, et al. "ssODN-mediated in-frame deletion with CRISPR/Cas9 Restores FVIII function in hemophilia a-patient-derived iPSCs and ECs." Mol Ther Nucleic Acids. 17:198–209 (2019).
[10] Park CY, Kim DH, Son JS, et al. "Functional correction of large factor VIII gene chromosomal inversions in hemophilia a patient-derived iPSCs using CRISPR-Cas9." Cell Stem Cell. 17:213–20 (2015).
[11] Payne AB, Miller CH, Kelly FM, et al. "The CDC hemophilia A autation project (CHAMP) mutation list: a new online resource." Hum Mutat. 34: E2382–91 (2013).
[12] Sung JJ, Park CY, Leem JW, et al. "Restoration of FVIII expression by targeted gene insertion in the FVIII locus in hemophilia A patient-derived iPSCs." Exp Mol Med. 51:1–9 (2019).
[13] Park CY, Sung JJ, Cho SR, et al. "Universal correction of blood coagulation factor VIII in patient-derived induced pluripotent stem cells using CRISPR/Cas9." Stem Cell Rep. 12:1242–9 (2019).
[14] Li T, Miller CH, Payne AB, et al. "The CDC hemophilia B mutation project mutation list: a new online resource." Mol Genet Genomic Med. 1:238–45 (2013).
[15] Lyu C, Shen J, Wang R, et al. "Targeted genome engineering in human induced pluripotent stem cells from patients with hemophilia B using the CRISPR-Cas9 system." Stem Cell Res Therapy.9:92 (2018).
[16] Ramaswamy S, Tonnu N, Menon T, et al. "Autologous and heterologous cell therapy for hemophilia B toward functional restoration of factor IX." Cell Rep. 23:1565–80 (2018).
[17] S, Mizuno S, Ozawa H, et al. "CRISPR/Cas9-mediated gene correction in hemophilia B patient-derived iPSCs." Int J Hematol. 111:225–33 (2020).
[18] Wagenblast, E., Azkanaz, M., Smith, S.A. et al. "Functional profiling of single CRISPR/Cas9-edited human long-term hematopoietic stem cells." Nat Commun 10, 4730 (2019).
[19] Demirci S, Leonard A, Haro-Mora JJ, et al. "CRISPR/Cas9 for Sickle Cell Disease: Applications, Future Possibilities, and Challenges." Adv Exp Med Biol. 1144:37-52 (2019).

DOI: 10.54254/2753-8818/23/20231026

[20] Tran NT, Danner E, Li X, et al. “Precise CRISPR-Cas-mediated gene repair with minimal off-target and unintended on-target mutations in human hematopoietic stem cells.” Sci Adv. 2022 Jun 3;8(22): eabm9106.

[21] Daniel-Moreno, A., Lamsfus-Calle, A., Raju, J. et al. “CRISPR/Cas9-modified hematopoietic stem cells—present and future perspectives for stem cell transplantation.” Bone Marrow Transplant 54, 1940–1950 (2019).