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Review Article

The Fabulous Impact of CRISPR Method in Sickle Cell Disease Treatment

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HIGHLIGHTS

- Sickle cell disease (SCD) is a type of monogenic blood disorders.
- The CRISPR/Cas9 technology can be used to treat SCD.
- RBC sickling is reversed by mutation correction or fetal hemoglobin induction.

ABSTRACT

Sickle cell diseases are the most prevalent monogenic blood diseases with complications such as severe end-organ harm, pain, and reduced life expectancy. Dealing options for sickle cell diseases are inadequate, as there are just two FDA-approved drugs to decrease acute manifestation. The only sickle cell diseases curative procedure is bone marrow transplantation, frequently from a harmonized, related donor. *Ex vivo* manipulation of autologous hematopoietic stem and progenitor cells and subsequent transplantation of genetically altered cells theoretically offer an everlasting therapy appropriate to all sickle cell anemia patients, regardless of the accessibility of fit donors and graft-versus-host disease. In this review, we emphasize applying CRISPR gene editing strategies for sickle cell anemia treatment, containing the genetic modification and rectification of sickle cell disease mutation in β-globin and the stimulation of fetal hemoglobin to protect cells against sickling. We summarize the importance of stem cell manipulation to cure sickle cell anemia having likely lifetime efficacy for cell and gene therapies.

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Introduction

Sickle cell disease (SCD) is a homozygous state-inherited disease controlled by a single pair of genes and is caused via a single replacement of glutamic acid (Glu) by valine (Val) on chromosome 11 in the sixth site of the β-globin gene. The SCD includes a set of conditions with various clinical manifestations, but sharing the same pathophysiologic result originated from a sole pair of genes alteration. The altered β-chain hemoglobin gene is translated to an irregular hemoglobin S (HbS) that is fast polymerized in the anaerobic condition and can change the red blood cell (RBC) lifetime. This sole replacement results in several downstream effects and distressing clinical difficulties, including chronic anemia,

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acute and long-lasting pain, chronic inflammation, stroke, organ dysfunction, recurrent vaso-occlusion, and sooner death (Paulukonis et al., 2016).

Gene therapy employing hematopoietic stem cell transplantation (HSCT) appears to be a technique to lessen disease load, recover consequences and life quality for SCD cases, and theoretically decrease medical costs in prolonged period (Ballas, 2009; Bhatia et al., 2015; Saenz and Tisdale, 2015; Arnold et al., 2017). The gene therapy strategy either in the platform of DNA editing or gene insertion into hematopoietic stem and progenitor cells (HSPCs), obtained from the same patient's body, increases the potential of a harmless treatment for SCD that is accessible to all patients. After years of technical development, gene therapy for SCD treatment is now tested through several clinical trials with positive early outcomes (Demirci et al., 2019).

Prokaryotes utilize numerous defense machineries, both innate and adaptive, to protect themselves against the attacker genetic elements such as bacteriophages and parasitic plasmids (van Houte et al., 2016; Hampton et al., 2020). Among these, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPRs) are brilliant as they are able to uninterruptedly update and adapt immune responses to fit the invaders' specific nucleic acid sequences (Hashemi, 2018, Nussenzweig and Marraffini, 2020). CRISPR, plus the CRISPR-associated proteins (Cas endonucleases), guard their hosts against external attacker nucleic acids by an adaptive immune system (Hashemi, 2020). Defense initiates with an acquisition phase in which, afterward infection, small fragments of the invader's genome, entitled spacers, are collected and joined to the CRISPR locus, embedded in the bacterial genome. Next, spacers are transcribed into CRISPR RNAs (crRNAs) to recognize and disable the cognate targets throughout the targeting stage, leading Cas endonucleases to cut the DNA or RNA of the attacker (Ebrahimi and Hashemi, 2020). The Cas9 endonuclease, as the best-characterized effector unit, comprises two dissimilar nuclease domains, RuvC and HNH, which is in charge of target DNA strands cut. A trans-acting CRISPR RNA (tracrRNA), needed for pre-crRNA processing as well as target recognition, is as well encoded from the type II loci (Mohanraju et al., 2016). CRISPR methodology has been utilized to create effective genome editing in animals, plants, and microorganisms. First, a 2- to 4-bp fragment called the protospacer-adjacent motif (PAM) flanking a target DNA position is identified by the Cas endonuclease under the RNA supervision. Then, the Cas9 searches the flanking DNA for base-pairing complementarity with a single guide RNA (sgRNA) after the PAM attachment, leading to DNA cleavage (Barrangou and Doudna, 2016).

In this review, the tremendous influence of CRISPR on translational genome editing is going to be introduced. The importance of stem cell exploitation having likely lifetime durability for cell and gene therapies is stressed.

History of medicine: Innovative medicines result in new cures

The Hippocratic medical practice weighed on modern medicine's growth and revived illness management approaches for more than 2000 years (Chast, 2008). Advances in health and medical procedures encompass steps via which patients worldwide with various diseases are treated (Waddington, 2015). The history of medicine starts from sanitation and clean water to the current advances involving living drugs. Clean water and sanitation have undoubtedly saved millions - maybe billions - of lives, since they were widely employed in the 19th and 20th centuries (Khalifa and Bidaisee, 2018). Clean water and public hygiene programs have significantly decreased the occurrence of lethal waterborne pathogens, such as cholera, and enhanced sanitation and have significantly reduced the health consequences of parasitic infections and other environmental health conditions (Ramírez-Castillo et al., 2015). Surgery used to be, without a doubt, a far lower position than it is now. One of the critical explanations for this is that anesthetics was not an option until the middle of the 19th century. Although anesthetics was a tremendous development, it became much more advantageous with another advance that happened around the same time, antisepsis, or the formation of a sterile surgical setting (Nakayama, 2018). Throughout history, contagious diseases have had a great impact on human health. The development of vaccination is one of the most effective ways to defend against widespread infections (Madhav et al., 2020). The pharmaceutical market, long containing small-molecule medicines, was revolutionized by the introduction of biologics. Biomedicine is at the forefront of a recent advent today, through the use of microbial and human cells as useful therapeutic agents.

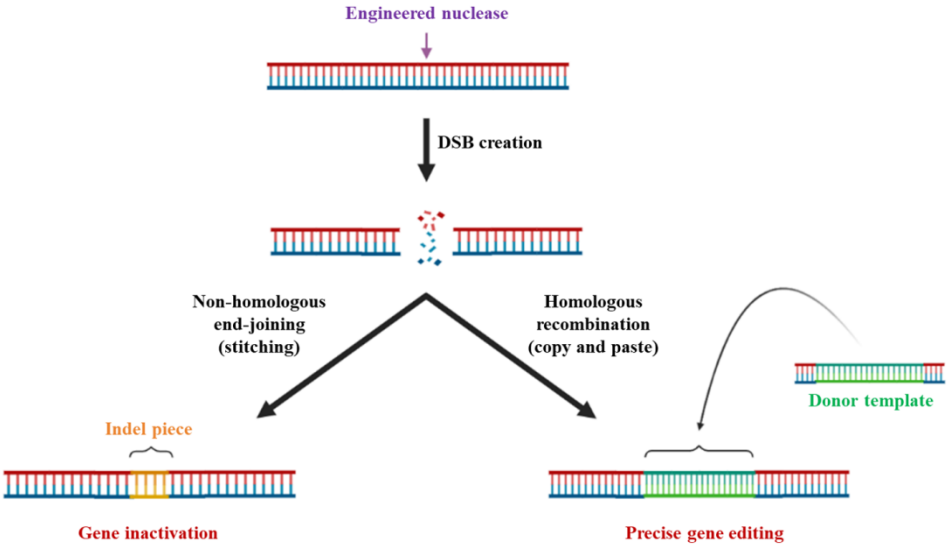

Figure 1. DSB repair systems. The double-stranded break (DSB) is formed by an engineered nuclease. 1) The non-homologous end joining (NHEJ) method which is an error-prone system of DSB repair do not require a donor template. 2) The homology-directed repair (HDR) method necessitates an exogenous DNA template, comprising the sequence of interest compartment and homology arms with DSB-adjacent sequences, to accurately repair the DNA.

Altogether, sanitation, antisepsis, vaccines, small molecules, and biomedicines helped lots of patients all over the world; but they've certain limitations, and there are still many diseases in many people for whom these therapies are not adequate (Fischbach et al., 2013). The two new types of living drugs, cell or gene medicines and manipulated microbiome, are new era in medicines. Living therapies consist of completely functional cells chosen and regularly modified for the treatment of particular disorders, such as cancer (Kitada et al., 2018). These drugs are able to migrate, divide and respond to their environment. Moreover, if the genetically modified stem cells are used as therapeutic agents, they are potential to give life-time durability making it distinguishable from small molecules and biologics such as enzymes or antibodies (Fischbach et al., 2013).

Precise genome editing via partaking cellular repair systems

The way preferred to genetically engineer stem cells is genome editing (Mali and Cheng, 2012). The fundamental process for genome editing is to use an engineered nuclease (Porteus, 2016). The CRISPR/Cas9 has democratized an accelerated system to use genome editing because it's effortless to design highly active and specific nuclease making a precise break at a particular site in the genome (Paix et al., 2017; Malech, 2021). Once the gap is made, the cell then repairs the break in two fundamental ways. There're some subvariants for each of these ways (Iliakis et al., 2004). The first method is non-homologous end joining (NHEJ), which cases small indel mutations could be made at the site of the break allowing the specifical inactivation of genes or genetic elements. This method is being applied in the translational setting or as a powerful research tool (Román-Rodríguez et al., 2019). The second pathway, being preferred for precise editing, is the homologous recombination method in which the break is healed by recombination machinery using a provided donor template. The recombination machinery uses this template as a substrate to make a copy and thereby paste the sequence of the donor into the sides of the break, and this way makes it possible to change even a single nucleotide, as well as, a cassette of genes (Zhang et al., 2017) (Fig. 1).

The process of recombinational genome editing can be used in a number of different ways. This method could be used for single nucleotide changes, such as a treatment for SCD (Tasan et al., 2016). The second application of recombinational-based genome editing is the correction of functional genes, in which the gene is driven by its own promoter. For instance, Pavel-Dinu et al. performed a knock-in of cDNA in long-term hematopoietic stem cells (LT-HSCs) to treat X-linked severe combined immunodeficiency (SCIDX1) in

human (Pavel-Dinu et al., 2019). The third usage of precise genome editing is safe-harbor gene addition, in which the gene is driven by an exogenous promotor. For example, Gomez-Ospina et al. utilized this method for knocking-in the promoter-gene expression cassette into a single safe location through CD34+ hematopoietic stem and progenitor cells' genome, called safe harbor, to treat Mucopolysaccharidosis type I (Gomez-Ospina et al., 2019). The other application of this method is for targeted transgene addition, in which the gene is driven by the endogenous promoter. As an example, Liu et al. inserted a gene encoding CD19-targeting chimeric antigen receptor (CAR) into T-cell receptor α subunit constant (*TRAC*) to generate CAR T-cells, which are given to treat chemo-refractory or relapsed B-cell cancers (Liu et al., 2017).

Drug development is a highly risky and expensive process as reportedly only 10% of products can pass the steps through the clinic. The reason of this problem partly is that target identification and measuring the effectiveness of the drug hitting the target are quite difficult. The drug development based on genome editing is quite easier and faces fewer failures than in conventional formulations; Since target identification, specifically for genetic diseases, is done and validated and it's quite easy now to measure the effectiveness and specificity of the genome editing process at drug target which is, of course, the genetic sequence that causes the disease (King et al., 2019). Genome editing that is adapted for translational purposes and researches, is actually one of nature's major ways of creating genetic diversity. The CRISPR system is responsible for this diversity in bacterial populations (Sniegowski and Raynes, 2013; Karthika et al., 2020).

Sickle cell disease (SCD), a common monogenic complication

Sickle cell anemia is globally of the most prevalent types of inherited monogenic disorders. In 1949, Pauling et al. introduced sickle-cell anemia as the first molecular disorder based on the discovery of a state caused by a defect in a protein responsible for oxygen-carrying, hemoglobin (Pauling et al., 1949). SCD is considered as a condition in which inflexible, "sickled" red blood cells (RBCs) appear that have trouble moving through the blood circulation and do not efficiently transfer oxygen (Ji, 2020). The annual number of patients diagnosed with SCD reaches approximately 300,000 cases (Piel et al., 2017). Around 90,000 people in the US suffer from the disease (Brousseau et al., 2010). This complication is genetically caused by a single nucleotide mutation through the hemoglobin β subunit gene (HBB) on chromosome 11. This point mutation leads to the replacement of glutamic acid with valine in the sixth codon of the beta globin gene increasing hemoglobin molecule hydrophobicity (Orkin and Bauer, 2019). The SCD clinical manifestation is caused by polymerized deoxygenated sickle hemoglobin. This disorder is indicated by hemolysis, erythrocyte deformation, anemia, permanent end-organ damage, painful vaso-occlusive episodes, and a reduced life expectancy (Pasricha and Drakesmith, 2018).

Treatment choices mostly consist of transfusion, pain management, and hydroxyurea in SCD patients (Platt et al., 1984). Only two medications are approved by FDA to lower SCD severity, including hydroxyurea and L-glutamine (Demirci et al., 2019). Newly-agreed therapies such as crizanlizumab, significantly reduce the prevalence of vaso-occlusive episodes, since neither treatment stops the underlying reason of the disease nor completely ameliorates SCD manifestations (Ataga et al., 2016).

Gene editing strategy for SCD treatment

Allogeneic bone marrow transplantation is a cure strategy for SCD, but fewer than 20% of receptor individuals access a doner with matched status of human leukocyte antigen (HLA) (Baronciani et al., 2016; Gluckman et al., 2017; Eapen et al., 2019). This strategy may cause severe complications such as graft-vs-host disease (GVHD) and graft rejection. These unintended effects are eliminated when HSPCs, originated from patient's own body, are genetically edited and then transplanted. Such procedure eradicates two main hurdles in the treatment of SCD: absence of appropriate donors, and GVHD-associated death. There are two main strategies of gene editing for SCD cure: (i) Fetal hemoglobin (HbF) induction, and (ii) SCD mutation correction (Demirci et al., 2019, Frangoul et al., 2021) (Fig. 2).

HbF induction

Fatal hemoglobin is baby's principal globin category after the first trimester of pregnancy and is exchanged via HbA until six months postpartum. Both fatal and adult hemoglobin genes are kept on chromosome 11, having regulated switch from HbF to HbA primarily by an upstream enhancer locus, named locus control region (LCR), which activates the expression of each gene promoter by looping (Li et al., 2002).

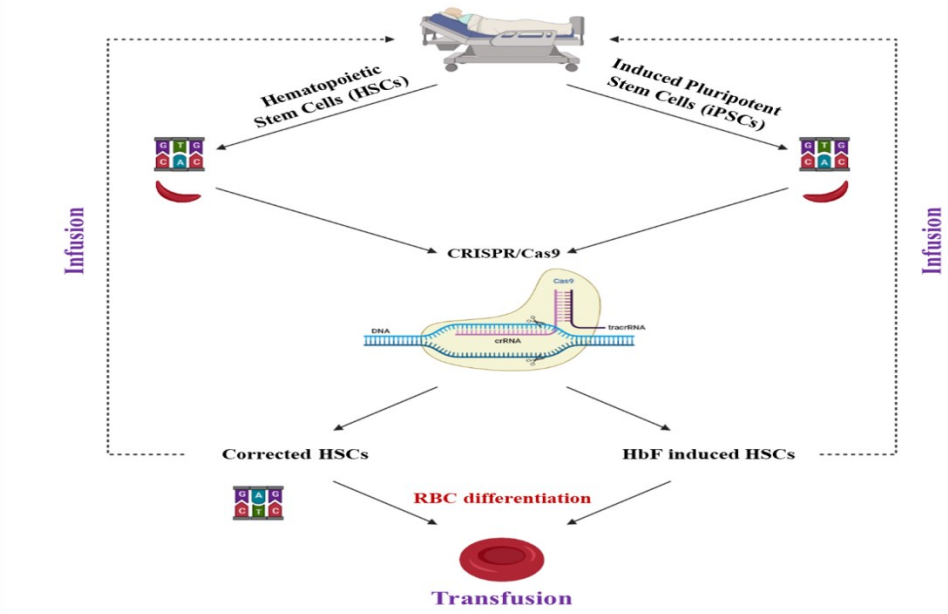

Figure 2. Potential therapeutic applications of CRISPR/Cas9 for sickle cell disease (SCD). This application can be performed by two main mechanisms in SCD patients-derived HSCs and iPSCs containing the SCD-related mutation correction and fetal hemoglobin (HbF) induction. The resulted normal RBCs is utilized for transfusion purposes.

Subsequent to the shift to HbA, HbF is not completely repressed, however, not regularly dispersed among RBCs. When no genotypic reason for HbF persistence in every RBCs exists, HbF could be produced minimally in limited cells or focused in special cells known as F-cells (Demirci et al., 2018). The protection against SCD made by the elevated HbF was first known by Janet Watson et al. in 1948 when they observed a lag-time for initiation of SCD complications in newborn babies (Watson, 1948). This resistance against SCD was further observed in researches displaying asymptomatic cases with SCD together with higher HbF expression as a consequence of co-inheriting genetic persistence of HbF mutations (Stamatoyannopoulos et al., 1975; Forget, 1998). Suchlike an elevation can occur as a result of large deletions in the HBB gene, or smaller deletions in quantitative trait loci (QTL), responsible for HbF regulation, or γ-globin promoter (Paikari and Sheehan, 2018). The hereditary persistence of fetal globin (HPFH) can be mimicked by deletion/inversion mutations in HSPCs, obtained from SCD patients, leading to an increased level of HbF expression and ameliorated sickling phenotype *ex vivo* (Antoniani et al., 2018). Transcriptional regulators such as *GATA1*, *KLF1*, and *SCA/TAL1* can be considered as targets of manipulation as an alternative method to enhance infrequent naturally happening HPFH mutations to regulate HbF expression (Sankaran and Orkin, 2013). In another approach, point mutations in the γ-globin promoter (-115 and -200 positions) using CRISPR/Cas9 tool makes this promoter resistant to HbF repressors, such as BCL11A and LRF rescuing the HbF expression from silencers effect (Liu et al., 2018; Martyn et al., 2018; Wang and Thein, 2018). Both of these HbF silencers can be targeted for manipulation directly (Shim et al., 2017; Humbert et al., 2018).

SCD mutation correction using DNA donor template

As mentioned before, the underlying genetic cause of SCD is completely identified. So, it seems that the most feasible way of SCD treatment is mutation correction. This editing is possible with CRISPR methodology, in which the Cas endonuclease is responsible for double-stranded break (DSB) creation and this break can be repaired by a homology-directed repair (HDR) system. A donor template having homology with DSB-adjacent sequences is required for break repairing. This template provides the right sequence of β-globin and edits the genome without needing exogenous transgene activation (Demirci et al., 2019). The correction of SCD-related mutations using different gene-editing systems is one of the scientists' top search interests these days (Huang et

al., 2015; Dever et al., 2016; Hoban et al., 2016). Most of these studies focus on CRISPR/Cas9 methodology since, it has performed effectively in precise correction and appears to have lower off-target effects than other gene-editing strategies like TAL-effector nucleases (TALENs) (Hoban et al., 2016; Bak et al., 2018). The efficient editing ability of SCD- related mutation by CRISPR/Cas9 tool in comparison with TALENs and zinc finger nucleases is clearly observed in a study in 2015. In this study, human induced pluripotent stem cells (iPSCs), obtained from SCD patients, were manipulated using specific sgRNA and Cas9 nuclease for DSB generation and wild type *HBB* DNA sequence as a donor template for HDR way of DNA repair (Huang et al., 2015). In another study, bone marrow hematopoietic stem and progenitor $CD34^+$ SCD-mutated cells were targeted for *HBB* gene editing by TALENs and CRISPR/Cas9 tools, and associated on-target and off-target effects in both methods were assessed using several TALENs pairs and various sgRNAs. The higher performance of the CRISPR technique was obvious (Hoban et al., 2016). Animal studies are an important research field before any clinical trial. The SCD treatment using CRISPR gene editing is also explored in animal models especially by mouse transplantation experiments. For example, in two separate studies, the engraftment of enriched $CD34^+$ modified cells were explored via flow cytometry and RNA-seq methods, respectively in bone marrow. The enrichment of $CD34^+$ cells by GFP-expressing donor was in order to differentiate between gene-edited HSPCs and only GFP-expressing donor receivers (Dever et al., 2016; Magis et al., 2018)

Conclusions

Recently, engineered nucleases, especially CRISPR/Cas9, have been the focus of devotion among the research community owing to their several applications in multipurpose model systems. Addition of any virtually-designed favorite artificial genetic sequence into the genome is one such usage that is related to genetic conditions therapy. Being a monogenic disorder, SCD has a high possibility of being treated by programmable nucleases.

Ethical Statement

This article does not contain human subjects and/or animals study.

Competing Interests

The authors have declared no conflict of interest.

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Authors' Contribution

Both authors involved in the conceptualization, validation of resources, and data extraction, V. Ebraimi prepared the draft of the manuscript and both authors reviewed and edited the manuscript and approved the final manuscript for submission.

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