Dean&Francis

ISSN 2959-409X

Application and Research Advances of CRISPR/Cas9 in the Treatment of Sickle Cell Anaemia

Nan zhang[1,*]

[1]Department of Biotechnology, Jilin Normal University, Siping, China
*Corresponding author: zhang.nan2027@gmail.com

Abstract:

The conventional treatment of sickle cell anaemia is based on the traditional methods including blood transfusions, the use of hydroxyurea and supportive measures. These various strategies improve symptoms, but neither is a cure, and all are associated with various limitations such as transfusion-related infections and drug toxicities. In gene therapy, the strategy used currently is based on the addition of genes into cells by means of lentiviral vector, with the more promising approach being that of gene editing which has been pioneered by the CRISPR/Cas9 technology. By means of this technology, the editors can be targeted towards the hematopoietic stem cells of the patients either by the actual repairs of the causal (sickle mutation) or the aerobic change which involves a reactivation of the fetal Hb production so as to restore natural function. With a very high targeting ability as well as prospects for long lasting effects from a single procedure, the CRISPR/Cas9 technology opens up a possible way of correcting the genetic defect of hereditary disease. The core challenges are primarily fourfold: low editing efficiency, off-target effects, the difficulty of expanding hematopoietic stem cells in vitro, and high treatment costs. This might be a possible solution to a form of treatment for sickle cell anaemia, and mainly lays down the theory of how to treat various forms of monogenic disorders.

Keywords: CRISPR/Cas9; Sickle cell anaemia; Gene therapy.

1. Introduction

Sickle cell disease (SCD) is an autosomal recessive single-gene disorder caused by specific point mutations in the β-globin gene (HBB) on chromosome 11. These mutations lead to the production of structurally abnormal hemoglobin (HbS). HbS polymerizes easily under hypoxemic conditions resulting in the production of sickle-shaped red blood cells. Such morphological changes lead to vaso-occlusive crises,

chronic hemolysis, and gradual organ-system damage which represent considerable morbidity and mortality risks of patients with SCD [1]. Current standard of care therapeutic interventions including routine transfusion regimens, restate and hydroxyurea therapy do not address the underlying genetic cause of the disease but serve to comply symptomatic relief. Hydroxyurea can increase the content of HbF within red blood cells by activating HbF expression, thereby reducing the aggregation of HbS and the sickling of red blood cells. This therapy alleviates vascular obstruction and hemolysis at their root cause[2]. Conventional therapy cannot correct mutations in the HBB and require lifelong medication. Prolonged use of conventional treatment regimens carries the risk of complications such as infections and toxicity related to medication, thus the need for more definitive forms of treatment is apparent. The further advance of gene-editing platforms has opened up exciting avenues towards a potential cure for sickle cell disease. The method specifically behind the CRISPR/Cas9 system has become a major focus of research and is touted for its relative simplicity of operations, high efficacy and precision of targeting. A custom designed guide RNA (gRNA) directs the Cas9 nuclease to specific areas of the genome, eg. either to the mutated transcript of the HBB gene or regulatory sites such as the BCL11A inhibitor that activate the fetal glow (HbF) production [3]. This process can either rectify mutation of gene, activate silenced transcription or knock out inhibitory genes, thus facilitates the restoration of function of glow or enhances the levels of HbF and attenuates the sickling tendency of red blood cells [4]. This study investigates the underlying theory and therapeutic possibilities of CRISPR/Cas gene therapy for sickle cell anemia. The fundamental rationale for this technology is examined in detail in this work from every angle, following the whole process, from the design of the sequence specific gRNAs that guide the enzyme Cas9 to its target genomic sites to the subsequent post–DNA repair processes. In this study, some significant advances in biological research are discussed: preclinical investigations substantiate the efficacy of this technology in terms of its ability to correct HBB gene defects efficiently in hematopoietic stem cells (HSCs), while clinical studies have shown that it is able to produce persistent levels of normal hemoglobin (HbA) and to lessen complications; at the same time, editing processes are now being improved which can be of assistance in enhancing genomic editing fidelity. By optimizing gRNA design to reduce off target effects, using Cas9nickese to reduce the risk of double strand breaks, and developing base editing techniques (such as BE4) to achieve precise single base repair [5]. This study attempts to demonstrate the revolutionary importance of CRISPR/Cas technology as a means of controllably effecting a cure for genetic disease-something in which there is a great difference from the conventional methods. It is because of its precision, for example, that this technique is able to effect alteration of the essential genes which produce disease through action which is entirely specific on its own part, while the destruction of surrounding genomic areas is lessened as a consequence. It is directed at the foundation of the trouble, too, in the correction of abnormal sequences or substitution for them so as to effect cures rather than symptomatic cure. Finally, one of the most important aspects of it is that by the editing of the self-renewing hematopoietic stem cells, it is possible to provide a constant source of genetically renovated blood cells-bounty of genetically renovated blood cells, thus affording the great chance of a genuine and possibly life-long healing of the ultimate condition.

There are still the ever-constant problems which surround so many aspects of biological therapy which at present inhibit a more general use of CRISPR/Cas type therapy for sickle cell disease and certain other genetic defects. The difficulties which still remain to be solved are as follows: the need for more efficiency in editing phenomena, particularly in the case of the heterogeneous nature of the primary patient cells where the cells have but a limited power of growth expansion leading to correction rates which if remedied would be subtherapeutic; the necessity for even more improvement of the off-target activity of the systems-an item as to which the entire series of improved enzyme variants are that they are capable of ever becoming more efficient and this at a time when deleterious off-targeting in oncogenic or areas of functional importance remain clearly retaining oncogenic powers and requiring modified pre-safety engineering and also requiring very delicate and meticulous screening studies. The phenomenal expenses attached to the present ex vivo editing methods render them entirely out of the reach of the majority. It is both necessary and desirable, therefore, that this type of complex, technical and burdensome procedure should be simplified and rendered more economically possible if the equitable use of a class of therapy for simple technical disease is to continue. The conclusion to be drawn from this analysis is that clinically operative curative forms of therapy for sickle cell anemia are in sight. With trial and error and research into the optimization of gRNA libraries, the conditions to be imposed on culture systems for hematopoietic stem cells so as to best allow implantation, and the development of adjunctive therapies impreventing the post-transplant complications attack, all tend to go to the greater fulfillment of this ambitious end. The scientific value of the economic significance of the whole mature problem also is beyond this class of dis-

ISSN 2959-409X

ease and can also present quite novel therapeutic methods of approaches for the construction of blood laboratory therapy in disease of a monogenic nature-rate distortion from, e.g., thalassemia or cystic fibrosis. A general analytical frame has been laid by ATT which throws light not only on the question of curative therapy or not, but also the efficacy and safety which is implied in the whole CRISPR/Cas approach to sickle cell disease for curative therapeutic effects. By this study has been made, what is in its way, a preparable model which can lend itself for change via various genetic defects, this machinery for audit in itself holds out the possibility of an enormous quickening both in research and in practical amelioration of the mileage within the field of gene medicine between all parts. This will be the first basis for the initiation of a new therapeutic basis for the antagonism of more general morbidity. Gene production will have become an accepted standard procedure and within reach of not only those afflicted with every degree of genetic defect, but also for those multi-morbid-type patients suffering probably from dozens of affections due to acquired genetic aberrations. These all present the greatest of chances of remedy being given to them by a standardized briefing level of thorough remediability concerned by the positive ‘cure’ ancestry retrospects or future policies thus indirectly to provide a healing drug for enthusiasm and generation of millions of patients throughout the world.

2. CRISPR/Cas9 Technology

2.1 The developmental history of the CRISPR-Cas9 system

The story of CRISPR-Cas9 unfolds as a progressive journey of discovery, beginning with the identification of specialized repetitive DNA sequences in bacterial genomes in 1987-later named CRISPR-and extending to the domain of archaea in 1995. Early research identified several associated proteins, including the initially designated Cas5 (now known as Cas9). Guided by a single-stranded guide RNA (sgRNA) and mediated by the R-loop structure, the HNH domain and RuvC domain of the Cas9 protein precisely localize to the complementary and non-complementary strands of target DNA, jointly inducing DNA double-strand breaks. A critical breakthrough occurred with the revelation that conserved nucleotide motifs adjacent to some of the CRISPR sequences were discovered and which were named the protospacer adjacent motif (PAM). The PAM is the receptor of the molecule that allows the system to differentiate the incoming DNA from that of the host and induce the immune defense. The RNA guided studies continued and the RNA guided technology of the system was brought to light. It was shown that the CRISPR associated RNAs (crRNAs) directed the Cas proteins to complementary foreign gene targets, and that the precursor crRNAs (pre-crRNAs) are transcribed and processed to guide molecules and defective transformation. After these studies it was confirmed that a complementary crRNA site of only about 20 nucleotides is needed to guide the Cas9 complex to perform a selective cleavage of the specific or target DNA. The processing of the RNA mediated technologies needed a trans transactivating crRNA (tracrRNA) that is necessary and important in processing the crRNA and for induction of subsequent DNA cleavaging. A critical advance followed: researchers managed to merge crRNA and tracrRNA into sgRNA. This streamlined design simplified efficient in vitro assembly of the CRISPR-Cas9 system and paved the way for its broad adoption [6].

This series of discoveries from the initial identification of loci and proteins, through the functional characterisation of the two RNA molecules, to their ultimate fusion-collectively constituted a fully functional adaptive immune system. It laid the foundation for the Nobel Prize and the subsequent revolutionary applications of this technology.

2.2 CRISPR/Cas9 Technical Principles

CRISPR/Cas9 is a precision genome editing technology derived from bacterial adaptive immune systems, comprising the core components of the Cas9 nuclease and gRNA. The gRNA localises to target sequences within the genome via complementary base pairing. Following recognition of the PAM sequence (pro-spacer adjacent motif, typically NGG) adjacent to the target sequence, Cas9 cleaves the DNA at the target site, creating a double-strand break (DSB).

Cells repair DSBs via two pathways: Non-Homologous End Joining (NHEJ), which readily introduces insertions-deletions (INDELs) to achieve gene knockout; and Homologous Directed Repair (HDR), requiring a donor DNA template to precisely introduce or replace sequences for gene knock-in or gene correction [7]. The host cell repair process is outlined in Figure 1. This technology has been widely utilised in different ways - from functional genomics and generation of disease models to the design of genetic treatments for diseases such as sickle cell anaemia - due to its ease of engineering, high efficiency and extraordinary precision of targeting.

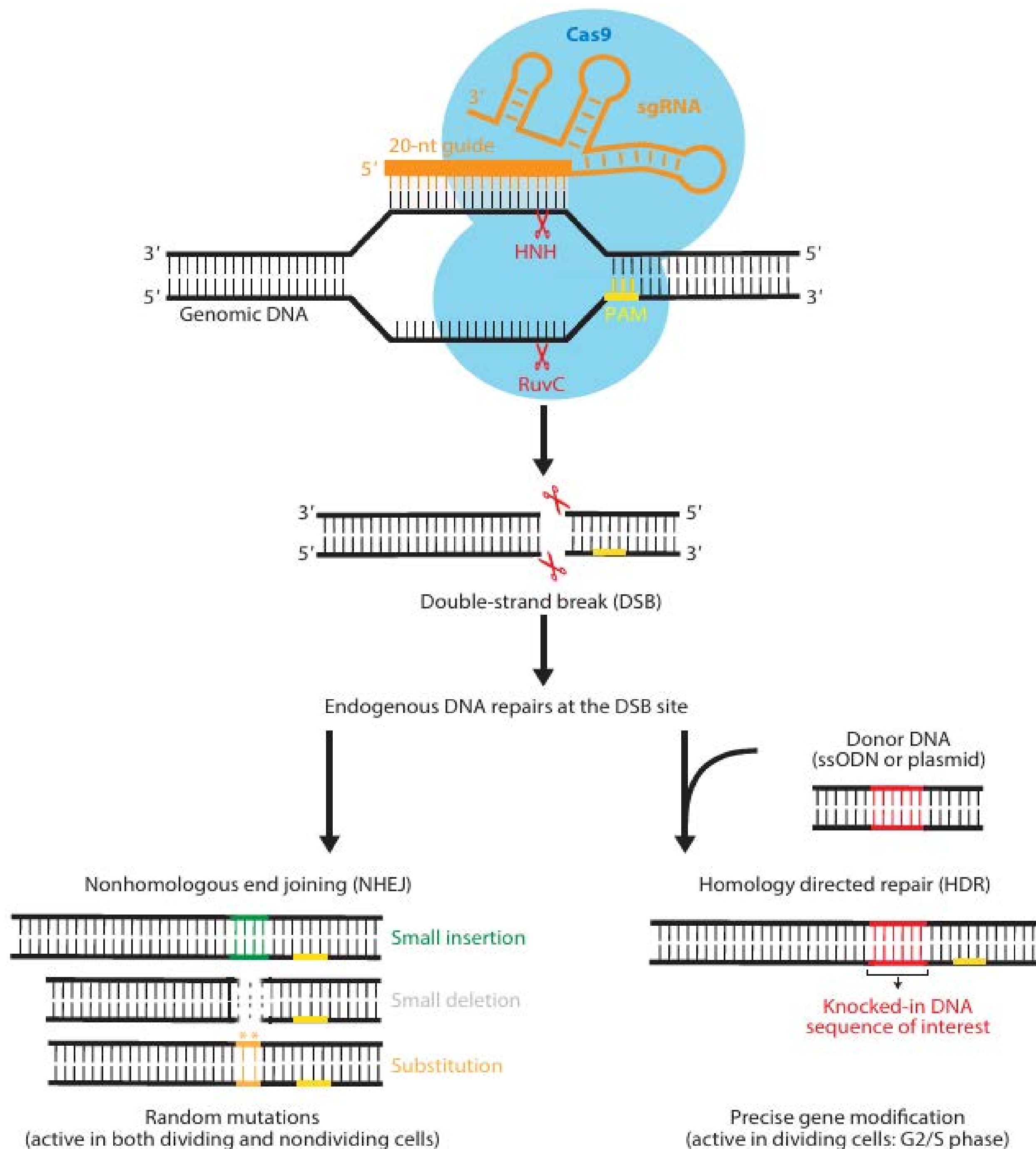

Fig. 1 CRISPR-Cas9 Mechanism of Action and DNA Repair Pathways [8]

2.3 Reasons for and Advantages of Application in Gene Editing

CRISPR/Cas9 has emerged as a revolutionary gene editing platform that is characterized by its programmable accuracy and operational versatility. One major strength is its easy accessibility of design: targeting new loci in the genome requires only the synthesis of a complementary gRNA and is therefore an accessible and cost-effective technology. In addition, it is able to induce highly effective and accurate alterations in the complex cellular environment. Many types of genetic manipulations are enabled by its ability to utilise endogenous DNA repair pathways induced by double strand breaks, from mutation of genes (knockout) and site specific insertions, to precise alter-

ISSN 2959-409X

ations in sequence repair. Further, the simultaneous application of a number of gRNAs allows multiplexed editing to be performed, thereby enabling the researcher to alter a number of genetic targets at one time and thus speeding up massively experimental and therapeutic workflows.

3. Sickle cell anaemia

3.1 Pathogenesis

Sickle cell disease is an inherited haemoglobinopathy caused by a specific A→T transversion in the HBB gene. The mutation produces a single amino acid substitution from valine to glutamic acid at the sixth position of the β-globin chain, resulting in pathological HbS. A characteristic property of HbS is its tendency to polymerise under low-o2 conditions, which drives the characteristic sickling of the red blood cells [9]. When the polymerization of HbS reaches a critical value in sickle red blood cells, destruction of the cells occurs. The massive destruction of these damaged red blood cells leads to the disease phenotype expressing marked haemolytic anaemia and vascular occlusion [1]. The cumulative effect of repeated vaso-occlusion includes progressive impairment of the spleen, kidneys, and liver, alongside an elevated risk of neurological, cardiopulmonary, and systemic organ failure. Notably, splenic dysfunction emerges early, significantly increasing susceptibility to life-threatening infections. Thus, what begins as a haemoglobin disorder evolves into a systemic illness necessitating lifelong care, with substantial impacts on both life expectancy and quality of life.

3.2 Sickle cell anaemia therapeutic approach

The current treatment of sickle cell anaemia revolves around the use of differing therapeutic modalities with supportive standard methods of thyroid supportive therapy, haematopoietic stem cell destruction and newly evolving gene therapies all giving expression to differing phases of therapeutic enhancement. Hydroxyurea is the mainstay of the pharmacotherapeutic conventional therapy, whose action rests mainly on its ability to enhance HbF formation with reduction in the frequency of vaso-occlusive crises. [10]. Transfusion support may help in reducing the burden of anaemia and organ damage. Here again, there are problems in that these support therapies require continued therapy, are not curative and engender risks in themselves, ex iron loading. Of the new drugs, a new class of oral drugs, consisting of pyruvate kinase activators, SNH-119014, have just come into therapeutic evaluation clinically. These drugs act to stimulate energy metabolism, reduce haemolysis and occlusion of the vessels in red cell type cells, and may reflect a new wave of therapy where repeated transfusion will not be the order of the day. At this time, the only established curative regime remains haematopoietic stem cell transformation. The area of its beneficial employment is still restricted to limited application because of the impossibility of getting donors, the number eligible being very limited. Newer insights into genetic transformation are seen as very exciting horizons. Gene splicing methods, CRISPR methods exa-cel and lovo-cel, are now OK'd for use in the U.S., while the new gene editing product CS-101 injection of Zhengxu Bio. proposes yet again another area of innovative therapy. These ideas remain differing in methods of working, exa-cel working by inactivation of the BCL11A to elicit production of HbF, lovo cel imparting a functional β-globin gene through lentivirus stimulus type, while CS -101 working to effect repairs at the HBG1/2 promoters to restore fetal Hb activity [11]. By modifying patients' hematopoietic stem cells, these therapies hold promise for achieving long-term remission of the disease. Clinical outcomes demonstrate significant increases in HbF levels and decreases in HbS levels in patients' blood, with some individuals resuming normal daily activities [12]. Despite these advances, several challenges persist. The required bone marrow ablation therapy may induce side effects including infertility and heightened infection risk. Furthermore, ongoing monitoring remains critical to fully establish the long-term safety profile of these interventions.

4. Conclusion

With its targeted, flexible, and cure potential, CRISPR/Cas9 is an important technological breakthrough in the treatment direction of sickle cell anaemia. This technology renders the disease-causing HBB gene in patients' haematopoietic stem cells precisely correctable, or enables the precise reactivation of normal haemoglobin production by regulating key genes such as BCL11A and HBG1/2, through the mechanisms of NHEJ or HDR, in order to promote effective production of HbF. This technological breakthrough not only opens up a new way to treat sickle cell anaemia, but also lays a solid technological foundation for the treatment of other single-gene hereditary diseases such as thalassaemia. At present, there are many bottlenecks in the clinical development of this technology: inadequate editing efficiency leads directly to therapeutic instability; potential off-target effects bring risks to safety; the in vitro culture system of HSCs is not yet perfect; coupled with the enormous cost of treatment, these constraints restrict its wide application in areas where the disease is prevalent. For instance, high costs primarily restrict its application in low-income areas, while insuf-

ficient editing efficiency limits treatment success rates. To solve these problems requires further continuous and comprehensive research: improvement of the design strategy of guide RNA, improvement of the delivery system of gene editing tools, establishment of more efficient stem cell expansion platform, optimisation of treatment methods to control cost. Through these systematic innovations, the early maturity of CRISPR/Cas9 should be accelerated in clinical application, so that sickle cell anaemia patients can receive safe, effective and inexpensive curative treatment all over the world.

References

[1] Steinberg H M. Sickle Cell Anemia, the First Molecular Disease: Overview of Molecular Etiology, Pathophysiology, and Therapeutic Approaches. The Scientific World Journal, 2008, 8: 1295-1324.

[2] T P M,E R W. Hydroxyurea therapy for sickle cell anemia. Expert opinion on drug safety, 2015, 14 (11): 1749-58.

[3] Fontana L,Martinucci P,Amistadi S, et al.Multiplex base editing of BCL11A regulatory elements to treat sickle cell disease.Cell reports. Medicine,2025,6(10):102376.

[4] Oscar B Q,Sara B F,Mercedes R D, et al. Gene Editing for Inherited Red Blood Cell Diseases. Frontiers in Physiology, 2022, 13: 848261-848261.

[5] Congting G ,Xiaoteng M ,Fei G , et al. Off-target effects in CRISPR/Cas9 gene editing. Frontiers in Bioengineering and Biotechnology, 2023, 11 1143157-1143157.

[6] Irina G. CRISPR–Cas9: A History of Its Discovery and Ethical Considerations of Its Use in Genome Editing. Biochemistry (Moscow), 2022, 87 (8): 777-788.

[7] Magdalena H,Daniel L,Joanna Z, et al. CRISPR/Cas9 Immune System as a Tool for Genome Engineering. Archivum immunologiae et therapiae experimentalis, 2017, 65 (3): 233-240.

[8] Jiang F, Doudna JA. CRISPR-Cas9 Structures and Mechanisms. Annu Rev Biophys, 2017;46:505-529.

[9] Rees C D,Williams N T ,Gladwin T M . Sickle-cell disease. The Lancet, 2010, 376 (9757): 2018-2031.

[10] Steinberg H M,Barton F,Castro O, et al. Effect of Hydroxyurea on Mortality and Morbidity in Adult Sickle Cell Anemia: Risks and Benefits Up to 9 Years of Treatment. JAMA: The Journal of the American Medical Association, 2003, 289 (13): 1645-1651.

[11] B E,E L,Alessandra B, et al. Post-Transcriptional Genetic Silencing of BCL11A to Treat Sickle Cell Disease. The New England journal of medicine, 2020, 384 (3).

[12] Javed D,Rai K A,Th A, et al. Efficacy of traditional medicine in the management of sickle cell disease: a scoping review of current evidence. Journal of complementary & integrative medicine, 2025.