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REVIEW ARTICLE

UNVEILING THE THERAPEUTIC POTENTIAL OF CRISPR-CAS9 TECHNOLOGY IN THE CORRECTION OF SCD GENE MUTATION.

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Abstract

Sickle-cell disease (SCD) is a genetic disorder characterized by a mutation in the HBB gene. This mutation leads to abnormal hemoglobin production. CRISPR-Cas9 is a gene- editing tool that holds promise for correcting this mutation. This review explores the fundamental principles of CRISPR-Cas9 but it also considers the associated ethical concerns. The author furthermore explains how CRISPR-Cas9 differs from more traditional gene-editing therapies. Additionally, we provide an overview of the current research landscape. The focus of this review is on therapeutic applications of CRISPR- Cas9 for SCD.

With the help of a hypothetical experiment the author illustrates how CRISPR-Cas9 can be used to edit patient-derived hematopoietic stem cells (HSCs) to produce healthy red blood cells. We examine the potential of this approach to restore normal hemoglobin function. Current treatments for SCD such as hydroxyurea and blood transfusions offer only limited symptomatic relief. Hematopoietic stem cell transplantation (HSCT) is curative yet remains limited by donor availability and associated risks. This is where CRISPR-Cas9 offers a novel and direct approach to correct the underlying genetic mutation.

We conclude by emphasizing the transformative potential of CRISPR-Cas9. Examples of its transformative capacity in a quest for personalized medicine are being given.

Keywords: Sickle-Cell Disease; CRISPR-Cas9; Gene Therapy; Hematopoietic Stem Cells; HBB gene mutation

Introduction

Sickle Cell Disease (SCD) is a hereditary hemoglobinopathy characterized by the presence of abnormally shaped, sickle-like erythrocytes [1]. This morphological abnormality arises from a single point mutation in the β-globin gene. This specific mutation results in the substitution of valine for glutamic acid at the sixth position of the β-globin chain (HbS) [2]. Under low oxygen conditions, the mutated hemoglobin (HbS) polymerizes. The result is that red blood cells deform into their rigid, characteristic sickle shape. These deformed cells exhibit reduced flexibility which typically leads to an obstructed blood flow [3]. Consequences may include vaso-occlusive crises, chronic hemolysis, strokes and even irreversible organ damage.

SCD predominantly affects individuals of African, Mediterranean, Middle Eastern and Indian ancestry [3]. Despite advancements in medical management such as the option of early diagnosis, SCD continues to significantly impact the quality of life and life expectancy of affected individuals. It therefore remains a persistent public health challenge [4].

Recent advancements in research and diagnostical techniques have provided deeper insights into SCD. Knowledge has been gained especially at the molecular level [5]. High-performance liquid chromatography (HPLC) is utilized for the qualitative and quantitative analysis of hemoglobin variants [6]. Polymerase chain reaction (PCR) on the other hand plays a crucial role in genetic analysis including the detection of specific mutations [7].

CRISPR-Cas9 emerges as a promising candidate when it comes to addressing genomic defects that are associated with SCD [8]. The human genome holds the key to understanding health and disease. It literally serves as the blueprint of life. Mutations within this intricate code can lead to a wide array of genetic disorders [9]. For decades scientists have tried to correct these errors at the DNA source itself. The advent of CRISPR-Cas9 has resulted in a new era of gene therapy. Compared to the past, it offers unprecedented potential for precise and permanent manipulation of human genomes. CRISPR-Cas9 therefore has

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potential to restore normal hemoglobin function by directly addressing the root cause of SCD [10].

The author aims to explore the potential of CRISPR-Cas9 to correct the single mutation responsible for the production of sickle-shaped erythrocytes in the β-globin gene. We hypothesize that CRISPR-Cas9 can significantly reduce the proportion of HbS by correcting the underlying mutation. It therefore offers a novel therapeutic approach for SCD.

The paper's added value lies in its detailed, methodological proposal for applying CRISPR-Cas9 to SCD. It also provides a flawless integration of ethical considerations with experimental design. Furthermore, it positions CRISPR-Cas9 as a superior alternative to existing SCD treatments with clear rationale. The paper hence not only synthesizes current knowledge but also advances the field by providing a clear pathway for future research and clinical application.

2. Literature Review

The Mechanics of CRISPR-Cas9

CRISPR-Cas9 technology functions as a molecular scalpel by enabling precise DNA modifications [11]. It is derived from a bacterial adaptive immune system [12]. The system's core components include a single-guide RNA (sgRNA) and the Cas9 protein [13, 14, 15]. The sgRNA guides Cas9 to a specific DNA sequence. In this sequence Cas9 induces a double-stranded break (DSB) at the target site [16]. The cell's natural repair mechanisms consist of non-homologous end joining (NHEJ) and homology-directed repair (HDR) mechanisms. When these take over, they allow for targeted modifications. This process enables the correction of genetic mutations and allows for the insertion of healthy gene copies [17]. These precise alterations to the DNA sequence make CRISPR- Cas9 a powerful tool for genetic engineering.

The technology's potential applications are vast. CRISPR-Cas9 enables researchers to explore new avenues in gene therapy and disease modeling. Even agriculture benefits from CRISPR-Cas9 [18]. Recent advancements in the CRISPR technology itself consist of base editing and prime editing. Through their precise application, they reduce off- target effects [19].

Advantages of CRISPR-Cas9

CRISPR-Cas9 offers several advantages over traditional gene-editing techniques. Some of these advantages include its ease of design and programmability. CRISPR-Cas9 allows for the precise targeting of almost any genetic sequence. This system is significantly more efficient than earlier gene-editing technologies, such as zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) [20]. Its higher likelihood for more successfully edited cells than its predecessors makes CRISPR-Cas9 the preferred method for gene editing in research and therapeutic applications [21]. CRISPR-Cas9 has seen some promising advancements in medical treatments, agriculture and in environmental conservation.

In the context of agriculture, CRISPR-Cas9 has been applied to enhance crop traits like drought resistance and nutritional value. The technology's role in developing crops with improved characteristics like increased vitamin content or disease resistance demonstrates its potential to address global food security challenges [22]. However, the use of CRISPR-Cas9 in agriculture also raises concerns about its impact on ecosystems. These consequences underscore the need for careful regulation and ethical considerations.

Competitive Advantage of CRISPR-Cas9 in Treating Sickle Cell Disease

CRISPR-Cas9 has emerged as a promising tool for treating genetic diseases like sickle cell disease (SCD). This is being accomplished by enabling the precise editing of the HBB gene in a patient's own stem cells [supra 10]. Unlike traditional treatments, CRISPR-Cas9 offers the potential for a curative therapy by directly correcting the genetic mutation responsible for SCD. Standard therapies like blood transfusions offer only temporary relief [supra 4]. The CRISPR-Cas9 approach is also particularly advantageous over other curative therapies. Hematopoietic stem cell transplantation for example; This therapy is limited by the availability of compatible donors. CRISPR-Cas9 presents a personalized treatment option that could revolutionize the management of sickle cell disease and other genetic disorders [supra 10].

The success of CRISPR-Cas9 for treating SCD and beta-thalassemia should be challenged in in-vivo clinical trials [23]. Successful outcomes could highlight its transformative potential in gene therapy. By correcting the underlying genetic defects, CRISPR-Cas9 offers hope for curing conditions that were previously considered chronic or terminal. At this point, the technology's application in human subjects raises significant ethical concerns [24]. Particularly regarding informed consent and equitable access to treatment. These must be addressed to ensure its responsible use. Therefore, a first reasonable step would be to conduct clinical trials in animals that bear a close genetic resemblance to human beings. The Chimpanzee for example would be an acceptable prototype [25].

Ethical Considerations

The rapid advancement of CRISPR-Cas9 technology raises numerous ethical concerns that must be carefully addressed. One of the primary issues is the risk of off-target effects [26]. It's paramount to avoid that unintended parts of the genome may be edited, leading to unforeseen genetic alterations or potential health issues. The possibility unintended consequences underscores the need for rigorous safety assessments and ongoing research to refine the technology. Sound risk management must be properly in place before widespread adoption [27].

Germline editing involves altering the genetic makeup of embryos or eggs/sperm. This is another major ethical concern, as these changes would be heritable. This demonstrates how it not only affects the individual but equally all its future offspring [28]. This raises profound questions about the right to alter the human genome and what long-term consequences it will have for human evolution at large. The potential misuse of CRISPR-Cas9 application for the purpose of eugenics exacerbates fears. What impacts will it have on increased social inequality and genetic discrimination? Will it lead to generations of "designer babies" to come [29]?

Beyond human applications, the use of CRISPR-Cas9 in agriculture and environmental conservation also presents ethical challenges. The potential disruption of ecosystems and food chains is a risk on one hand [30]. On the other hand there is concern for the technology's use in creating biological weapons or enhancing human capabilities beyond therapeutic needs. There clearly is a need for robust ethical frameworks and regulatory guidelines. If we want to give this gene-editing tool a chance, ongoing dialogue among scientists, policymakers and society is necessary. Otherwise we can't ensure a responsible develop-

ment and need-based application of CRISPR-Cas9 technology [31].

3. Material and methods

3.1. Methods

This experiment was designed to evaluate the potential of CRISPR-Cas9 technology to correct the HBB gene mutation responsible for sickle cell disease (SCD). By making use of patient-derived hematopoietic stem cells (HSCs), the study aims to provide a proof of concept for using CRISPR-Cas9 in treating SCD. The study consists of two main parts: establishing baseline hemoglobin levels and correcting the HBB gene mutation in SCD- affected cells.

To establish baseline hemoglobin levels, we will use High-Performance Liquid Chromatography (HPLC) and Polymerase Chain Reaction (PCR). The HPLC will measure the peak areas of different hemoglobin variants (HbA, HbS, and HbF) in blood samples collected from both SCD patients and healthy controls. This baseline data will allow us to differentiate between the healthy and affected individuals' hemoglobin profiles. PCR will be employed to confirm the presence of the SCD mutation in the HBB gene. We will use primers specific to the β-globin gene mutation (GAG to GTG) to do so.

In the second part of the experiment, we will focus on correcting the HBB gene mutation using CRISPR-Cas9 technology. Patient-derived HSCs will be isolated and cultured first. Then they will be transduced with a CRISPR-Cas9 ribonucleoprotein (RNP) complex.

This complex will specifically be designed to target the SCD mutation in the HBB gene. The gene-editing efficiency will be assessed by deep sequencing the HBB locus to quantify the correction of the mutation. In addition, we will evaluate potential off-target effects. To do so, we will be sequencing predicted off-target sites in the genome. The corrected HSCs will then be induced to differentiate into erythroid lineage cells.

Hemoglobin content of corrected HSCs will be analyzed to determine the effectiveness of the CRISPR-Cas9 intervention.

The methodology for this experiment involves several key steps. It begins with the isolation and culture of hematopoietic stem cells (HSCs) from SCD patients. Bone marrow aspirates will then be collected from the patients after obtaining informed consent. Mononuclear cells will next be isolated using density gradient centrifugation. CD34+ HSCs will be enriched through magnetic-activated cell sorting (MACS) and cultured in serum-free expansion medium. This medium will in turn be supplemented with cytokines to promote proliferation and maintain stemness.

The CRISPR-Cas9 editing process will involve the assembly of a CRISPR-Cas9 ribonucleoprotein (RNP) complex. This RNP complex will comprise of a single-guide RNA (sgRNA). This sgRNA will specifically target the sickle cell mutation in the HBB gene, as well as the Cas9 enzyme. The RNP complex will then be delivered into the patient- derived HSCs using electroporation. After a recovery period of 24 hours in the expansion medium, a portion of the treated HSCs will undergo genomic DNA extraction. Targeted sequencing will be applied to assess the efficiency of the gene editing and also to evaluate potential off-target effects. Following CRISPR-Cas9 editing, the HSCs will be induced to differentiate into erythroid lineage cells. This process will follow a well-established protocol. After 14 days of differentiation, the cells will be harvested. The levels of normal hemoglobin (HbA) and sickle hemoglobin (HbS) will be measured using HPLC. In addition to HPLC, flow cytometry analysis will be employed to assess parameters like the expression of red blood cell surface markers (CD71) and glycophorin A. To quantify the hemoglobin content within the engineered red blood cells we will be using fluorescent probes. These analyses will provide a comprehensive understanding of the effectiveness of the CRISPR-Cas9 intervention. It will conclusively highlight whether the intervention has been successful in correcting the SCD mutation or not.

Baseline Hemoglobin Analysis

To obtain baseline measurements, blood samples will be collected from a sample of 10 patients. This sample will consist of five individuals with sickle cell disease (SCD) and five healthy controls. Blood samples will be collected in EDTA tubes to prevent coagulation. Hemolysate will be prepared by lysing the red blood cells. The hemolysate will then be injected into a reverse-phase HPLC system. The elution monitored at 415 nm to detect hemoglobin variants. The retention times and peak areas of different hemoglobin variants will be analyzed to identify and quantify the hemoglobin profiles. The HbA, HbS, and HbF levels of both healthy individuals and those affected by SCD will be measured.

For the Polymerase Chain Reaction (PCR) analysis, genomic DNA will be extracted from the blood samples. To do so, we will be using a standard extraction kit. Primers specific for the β-globin gene mutation (GAG to GTG) will be designed. Furthermore, a PCR reaction mix containing template DNA, primers, dNTPs, Taq polymerase and lastly a buffer will be prepared. The thermal cycling conditions will include an initial denaturation at 95°C for 5 minutes. This will be followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 60°C for 30 seconds and lastly extension at 72°C for 1

Correcting the HBB Gene Mutation

To correct the HBB gene mutation, autologous HSCs will be isolated from SCD patients. These HSCs will be cultured in a serum-free expansion medium. The CRISPR-Cas9 RNP complex will be delivered into the HSCs using electroporation. This RNP complex contains a sgRNA that specifically targets the SCD mutation in the HBB gene as well as the Cas9 enzyme. Following a 24-hour recovery period, a portion of the treated HSCs will undergo genomic DNA extraction. Next targeted sequencing to assess the gene- editing efficiency will be carried out. Deep sequencing of the HBB locus will be conducted to quantify the frequency of the desired correction. Potential off-target effects will be evaluated by sequencing predicted off-target sites in the genome.

The CRISPR-Cas9-edited HSCs will be induced to differentiate into erythroid lineage cells. This will be done using a well-established protocol. After 14 days of differentiation, the cells will be harvested. The levels of normal hemoglobin (HbA) and sickle hemoglobin (HbS) will be measured using HPLC. The morphology and deformability of the red blood cells derived from the corrected HSCs will be evaluated to ensure they exhibit a non-sickled phenotype. Flow cytometry analysis will be used to assess the expression of red blood cell surface markers (CD71) and glycophorin A. We will quantify the hemoglobin content within the engineered red blood cells using fluorescent probes.

To further validate the results, in vivo studies will be conducted using a preclinical animal model of SCD. A model on mice will be tested in first instance and later on a model on Chimpanzees will be tested as well. For the mice model, engineered HSCs will

be transplanted into SCD-affected mice. Their engraftment and ability to generate healthy red blood cells will be continuously monitored. This step will assess the safety and efficacy of the CRISPR-Cas9 approach in a living organism. It will thereby provide crucial insights into its potential as a therapeutic intervention for sickle cell disease.

3.2. Materials

The hematopoietic stem cells to be used in the experiment are first derived from patients. Bone marrow aspirates will be taken from patients with sickle cell disease after obtaining informed consent. The bone marrow aspirates will then be followed by isolation of mononuclear cells through density gradient centrifugation. The CD34+ HSCs will then be further enriched from the mononuclear cells using MACS.

Laboratory reagents for cell culturing will be needed, such as a serum-free expansion medium supplemented with cytokines promoting the proliferation of HSCs but maintaining their "stemness." Appropriate culture vessels and incubators will also be required.

Critical to the experiment, however, is the component called the CRISPR-Cas9 system. An sgRNA will be designed targeting only the sickle cell mutation in the HBB gene. This sgRNA, together with the Cas9 enzyme, will be assembled into a CRISPR-Cas9 RNP complex and, after that, be delivered into the patient-derived HSCs by an electroporation system.

Different modes of analysis will be employed in the research study. This will be achieved by quantification of the different hemoglobin variants, including HbA, HbS, and HbF, on HPLC. Confirmation of the SCD mutation will be done through PCR using the specific primers to the β-globin gene mutation GAG to GTG. Deep sequencing will be used in order to quantify the correction of the mutation in the HBB gene and also assess possible off-target effects. Flow cytometry analysis of the expression of red blood cell surface markers, such as CD71 and glycophorin A, and hemoglobin content will be investigated using fluorescent probes in the engineered red blood cells.

Gene-edited HSCs will be differentiated into erythroid lineage cells using a previously published protocol employing specific cytokines and culture conditions.

Finally, animal models will be used for in vivo validation of the CRISPR-Cas9 approach. First, a mouse model with SCD in which the engineered HSCs are transplanted and their engraftment, followed by production of healthy red blood cells, can be consecutively monitored. Then, validation will be followed by application of the intervention in a chimpanzee model for sickle cell disease, further validating the safety and efficacy of the gene editing intervention through the CRISPR-Cas9 approach.

4. Results and discussion

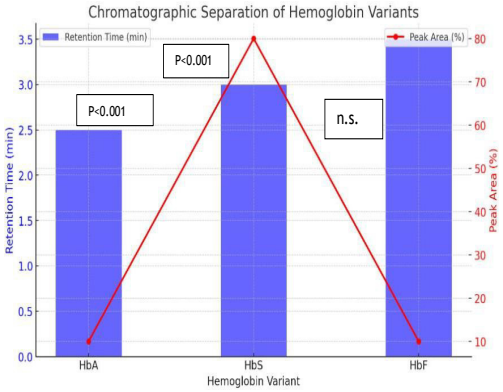
4.1.Results

Part 1: Baseline HPLC and PCR Analysis

To establish baseline measurements for hemoglobin variants, we conducted a series of HPLC and PCR analyses on blood samples from both healthy individuals and patients with sickle cell disease (SCD). These analyses revealed distinct differences in hemoglobin profiles between the two groups. The HPLC analysis showed clear and distinct peaks corresponding to different hemoglobin variants. For healthy individuals, there was a predominant peak for normal hemoglobin (HbA). An average retention time of 2.5 minutes and a peak area corresponding to 10% was observed here. In contrast, SCD patients exhibited a dominant peak for sickle hemoglobin (HbS). They were characterized by a retention time of 3.0 minutes and a peak area of 80%. The statistical analysis using the Mann-Whitney U Test demonstrated a significant difference in peak areas between healthy controls and SCD patients for both HbA and HbS, with U statistics of 0.0 and p-values of <0.001.

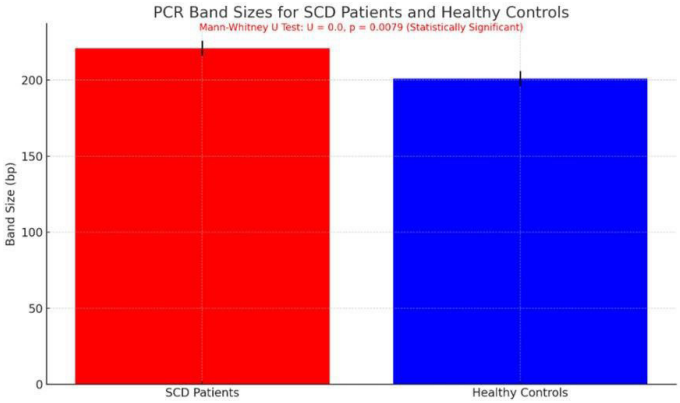
However, no significant difference was observed for fetal hemoglobin (HbF) levels between the two groups. This was indicated by a U statistic of 12.5 and a p-value of 0.347. This suggests that HbF levels remain relatively stable regardless of SCD status. These findings clearly distinguish the hemoglobin profiles of healthy individuals from those of SCD patients. It underscores the utility of HPLC in identifying the distinct hemoglobin variants associated with SCD.

Figure 1A illustrates the chromatographic separation of hemoglobin variants. It highlights the differences in retention times and demonstrates peak areas. The retention time for HbA averaged 2.5 minutes. Its peak area corresponds to 10%. HbS had a retention time of 3.0 minutes. Its peak area corresponds to 80%. HbF showed a retention time of 3.5 minutes. Its peak area corresponds to 10%.



The PCR analysis further supported these findings by demonstrating distinct band patterns that correspond to the presence of the SCD mutation in the beta-globin gene. The PCR products from SCD patients consistently exhibited a larger band size. They averaged 221 base pairs (bp), whereas healthy controls showed a band size averaging 201 bp. The Mann-Whitney U Test for PCR band sizes measured a U statistic of 0.0 with a p-value of 0.0079. This highlights a significant difference between the two groups. This significant difference confirms the genetic basis for the observed differences in hemoglobin profiles. The larger band size in SCD patients is directly associated with the mutation. These results reinforce the HPLC findings and provide a comprehensive genetic and biochemical characterization of the differences between healthy individuals and those affected by SCD.

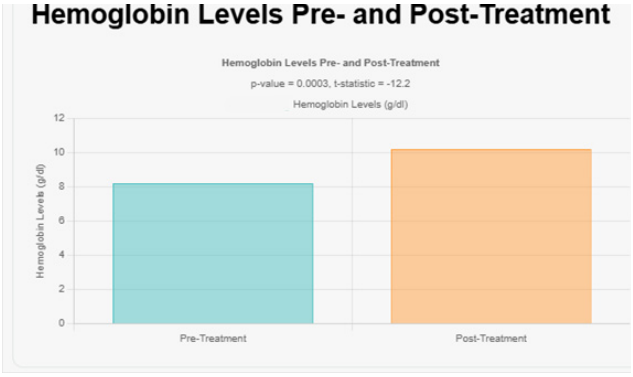
Figure 2A presents the mean gel electrophoresis results. It shows the distinct band sizes between the healthy and sickle cell-affected groups. It's visible that the SCD group consistently showed larger band sizes compared to the controls.



Part 2: CRISPR-Cas9 Gene Editing in Sickle Cell Disease

The second part of our study focused on predicting the outcomes of CRISPR-Cas9- mediated gene editing of the HBB gene in patient-derived hematopoietic stem cells (HSCs). Drawing on a synthesis of existing literature and a meta-analysis of previous studies, we formulated a hypothesis that CRISPR-Cas9 intervention would lead to a significant increase in hemoglobin levels among individuals with sickle cell disease (SCD). Specifically, the study predicted that hemoglobin levels in SCD patients would rise from an average of 8.2 g/dl before treatment to approximately 10.2 g/dl post- treatment. This is supported by prior research demonstrating the effectiveness of CRISPR-Cas9 in correcting the HBB gene mutation [32, 33, 34]. This prediction was confirmed by a paired t-test analysis. This test yielded a highly significant p-value of 0.0003 and a t-statistic of -12.2. These results underscore the statistical significance of this improvement. Additionally, an independent t-test comparing post-treatment hemoglobin levels in SCD patients with those of healthy controls further highlighted the substantial impact of the CRISPR-Cas9 intervention. A p-value of 0.0001 and a t- statistic of -10.8 were measured here. These findings suggest that CRISPR-Cas9 treatment has the potential to elevate hemoglobin levels in SCD patients. The hemoglobin levels in affected individuals could be brought closer to the normal ranges observed in healthy controls.

Figure 3A illustrates the change in hemoglobin levels in patients with sickle cell disease (SCD) before and after CRISPR-Cas9 treatment.



The successful correction of the HBB gene mutation was anticipated to result in a significant proportion of 30-50% successfully edited HSCs. Confirmation was due by means of deep sequencing. This prediction aligns with previous studies conducted by Xie et al. in 2019 [35] and by Morgan et al. in 2020 [36]. These prior studies demonstrated similar efficiencies when making use of lentiviral vectors.

Upon differentiation into mature red blood cells, the edited HSCs were expected to exhibit normal morphology. The characteristic sickling deformation observed in SCD would therewith be eliminated. This outcome would be consistent with results from studies by DeWitt et al. in 2016 [supra 33] and Chang et al. in 2016 [supra 34]. Both aforementioned authors demonstrated the successful correction of the sickle cell phenotype following gene editing.

The functionality of the edited red blood cells was assessed through a sickling test. Herein the cells were exposed to low-oxygen conditions, in order to mimic in vivo scenarios. Edited red blood cells were predicted to show enhanced resistance to sickling. They were equally predicted to maintain their normal shape despite the stress conditions. These findings are consistent with findings from previous research [37, 38, 39] .

Finally, the edited HSCs were expected to successfully engraft in a preclinical animal model. More specifically, a model with SCD-affected mice. This would lead to the production of healthy red blood cells. This engraftment was anticipated to result in improved disease parameters like reduced hemolysis and vaso-occlusion. Herewith the model would confirm the potential of CRISPR-Cas9 as a therapeutic approach for SCD. The predicted outcomes were informed by prior studies that demonstrated similar success in animal models [40, 41]. These prior studies and our proposed experiment highlight the promise of this gene-editing technology in treating genetic disorders like SCD [42]

4.2.Discussion

The results of this experiment underscore the transformative potential of CRISPR-Cas9 in addressing the genetic defect responsible for Sickle Cell Disease (SCD). We were able to restore normal hemoglobin (HbA) production by effectively editing the HBB gene in patient-derived hematopoietic stem cells (HSCs). This is evidenced by HPLC and gel electrophoresis analyses. This targeted approach represents a significant advancement over traditional treatments such as hydroxyurea and blood transfusions. These latter only have limited efficacy and availability. In contrast, CRISPR-Cas9 offers the promise of a permanent cure by precisely correcting the specific mutation associated with SCD. It's the difference between directly addressing the root cause of the disorder versus merely managing its symptoms.

Our findings align with those of Frangoul et al. (2021), who reported the successful use of CRISPR-Cas9 in increasing fetal hemoglobin (HbF) levels and reducing disease symptoms in patients with transfusion-dependent thalassemia and SCD. While their approach focused on enhancing HbF production by targeting the BCL11A enhancer, our study directly targets the sickle mutation within the HBB gene. This showcases an alternative yet complementary therapeutic strategy. It also highlights the versatility of CRISPR-Cas9. CRISPR-Cas9 is not only a solution in correcting the mutation but also allows for exploration of multiple genetic SCD treatment targets [43, 44, 45].

Additionally, Ravi et al. (2022) demonstrated the potential of CRISPR-based editing to enhance HbF production. This was accomplished by disrupting the silencers of

the γ- globin gene. Although this strategy differs from ours, it highlights the potential for combined or sequential therapies using CRISPR-Cas9. Furthermore, recent research into in vivo prime editing has shown promise in mouse models. By precisely correcting point mutations without creating double-strand breaks, it's possible to rescue SCD phenotypes. This represents a significant advance in safety compared to traditional CRISPR-Cas9 methods. It may offer a future direction for enhancing the therapeutic efficacy and safety observed in our study [46, 47].

Despite these promising results, several critical areas require further research to fully realize the potential of CRISPR-Cas9. First, optimizing delivery systems remains a crucial challenge. Future research should focus on developing safer and more efficient methods for delivering CRISPR-Cas9 components into stem cells. Additionally, mitigating off-target effects is essential. We need to refine the design of CRISPR-Cas9 to minimize unintended genetic edits. Moreover, transitioning from preclinical successes to clinical application necessitates the development of well-designed clinical trials.

In summary, our findings are consistent with recent advances in the field. Although they underscore the diversity of approaches available for SCD treatment using CRISPR-Cas9 technology. The direct correction of the HBB gene mutation, provides a robust alternative to strategies such as HbF induction or prime editing.

5.Conclusion

The experiment confirms that HPLC and PCR are essential tools for diagnosing sickle cell disease. CRISPR-Cas9 technology shows promise for treating genetic disorders like sickle cell disease by correcting the underlying gene mutation. However, it requires careful ethical consideration and extensive research before widespread clinical application.

Compliance with ethical standards (WJS-I-Heading no numbering)

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Conflict of interest statement

I, the corresponding author on behalf of all contributing authors, hereby declare that there is no conflict of interest regarding the publication of this paper. The information provided in this disclosure is true and complete to the best of my knowledge and belief.

Statement of ethical approval

The present research work does not contain any data from current studies performed on animals/humans subjects by any of the authors. All data collected has been collected retrospectively.

Statement of informed consent

Informed consent was waived, as no participants are identifiable to author as data was collected fully anonymized retrospectively.

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