How can CRISPR technologies be used to treat patients with sickle cell disorder?

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ABSTRACT

Sickle cell disease (SCD) is a severe, hereditary blood disorder characterized by abnormally shaped hemoglobin leading to red blood cells that are crescent or sickle shaped. This abnormal shape causes the red blood cells to become rigid, sticky, and obstructs capillary blood flow. SCD causes chronic pain, anemia, pain crises, and serious conditions such as organ damage and higher susceptibility to infections. There are about 8 million SCD patients around the globe, 80% of which are in the Sub-Saharan Africa region. Children ages 5 years or younger diagnosed with SCD have a 50%-80% mortality rate (GBD 2021 Sickle Cell Disease Collaborators, 2023). Although there have been advancements in potential treatments including hydroxyurea, bone marrow transplant, and blood transfusions, a complete or definitive treatment has still not been found. The treatments available today, however, can only alleviate the symptoms of SCD, and not the gene defect which causes the disease. The understanding of the CRISPR-Cas9 system began as an immune defense mechanism of bacteria and archaea against viral and other mobile genetic elements. This powerful technology for gene editing can have a tremendous positive impact when used to correct genetic mutations that are caused by SCD. Early studies and clinical trials have shown that CRISPR based therapies are capable of genetically modifying hematopoietic stem cells to generate healthy red blood cells which brings the possibility of such intervention as a potential cure for SCD. It's anticipated that with further investment in research in this field, the treatment of SCD will almost be eradicated and the mortality rates caused by SCD could be remarkably reduced, potentially saving millions of lives.

INTRODUCTION

Introduction to Sickle Cell Disease

Sickle Cell Disease (SCD), also known as sickle cell anemia is a hereditary disorder of red blood cells caused by a mutation in the hemoglobin which is a protein that transports oxygen in blood (National Heart Lung and Blood Institute, 2024). Under normal conditions, the human body has red blood cells which have a disc shaped and non rigid structure that is designed for free flow within blood vessels to carry oxygen from the lungs to other parts of the body for efficient use. In the instance of SCD, abnormal hemoglobin causes red blood cells to become inflexible, sticky and distorted in shape to that of a sickle or crescent, this would obstruct blood circulation through small capillary blood vessels around the body. This can lead to prolonged episodes of pain, anemia and stroke, episodes of pain known as pain crises and other life threatening conditions that come with organ dysfunction or infections.

Sickle cell disorder has about 8 million occurrences around the globe, however, some racial and ethnic populations are more affected than others. Statistically, sub-Saharan Africa has the highest infection rates and suffers about 80% of the SCD cases, with children under five suffering from the highest mortality rates of 50-80%. In the case of America, it is estimated that the number of people having SCD is approximately 100,000 Americans. The disease is widely

prevalent amongst African Americans, as an African American baby has a probability of 1 in 365 to suffer from SCD at birth. In addition, 1 in 13 African American babies carries SCD trait, which means that the babies might be a carrier of genes that causes the disease (GBD 2021 Sickle Cell Disease Collaborators, 2023).

There are numerous ways in which SCD can be treated. Currently, there are 4 U.S. Food and Drug Administration-approved medicines to treat SCD : (1) Hydroxyurea, (2) Voxelotor, (3) L-glutamine, and (4) Crizanlizumab (National Heart Lung and Blood Institute, 2024). Hydroxyurea and Voxelotor are oral medicines used to prevent the sickling of red blood cells. L-glutamine is also administered orally and reduces oxidative stress to the sickle cells (Niihara et al., 2018). Hydroxyurea and L-glutamine both act to diminish pain crises, and Voxelotor increases patients' hemoglobin and blood flow. Unlike the other medicines, Crizanlizumab is administered intravenously (through an IV) monthly to prevent blood cells from sticking to blood vessel walls and blocking blood flow (U.S. Food and Drug Administration, 2019). It also helps to reduce pain crises in patients. Common side effects of these medicines include low white blood cell counts, headaches, stomach or back pain, diarrhea, nausea, and fever.

While these treatment options can drastically improve the symptoms of SCD, they are not a cure. The only cure for SCD is to undergo stem cell or bone marrow transplantation(National Health Service UK, 2022). A bone marrow or stem cell transplant involves taking healthy cells from a donor and putting them into someone whose bone marrow (the soft tissue inside bones that produces blood cells) is not working properly (Centers for Disease Control and Prevention, 2024). This allows the new bone marrow of the SCD patient to create healthy red blood cells. In order for the transplant to be successful, the donor's bone marrow needs to very closely match the patient's, which makes it difficult finding eligible donors.

In addition, these transplantation methods are more rare because there are significant risks involved. Obtaining stem cells from bone marrow is a complex and painful process, and other complications can also arise. One of the main risks is that the transplanted cells will start attacking other healthy cells within the body, in a condition known as graft-versus-host disease (National Health Service UK, 2022). Alternatively, the patient's immune system might classify the transplanted cells as foreign invaders and attack them. For these reasons, improvements need to be made in the treatment and cure options for SCD.

Introduction to CRISPR

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) is a revolutionary gene-editing tool that has significantly advanced the field of molecular biology. In the late 1980s, scientists discovered unusual repetitive sequences in the genomes of bacteria, which were later termed CRISPR and identified as part of the bacterial adaptive immune system (Ishino et al., 2018). In 2007, it was demonstrated that CRISPR is involved in defending bacteria against viral infection by incorporating viral DNA into the bacterial genome. In this system, when a bacterium is attacked by a virus, the bacterium captures fragments of the viral DNA and integrates them into the CRISPR array within its own genome. This allows the bacterium to have a record of previous infections and "remember" the virus. During subsequent infections, CRISPR-associated proteins (such as Cas9) use these stored sequences to identify and destroy

the viral DNA, preventing further infection. These fragments are used to guide proteins like Cas9, an RNA-guided endonuclease, to recognize and cut viral DNA during subsequent infections, thus providing immunity (Barrangou et al., 2007).

A major breakthrough came in 2012 when Drs. Doudna and Charpentier showed that CRISPR could be adapted for genome editing in eukaryotic cells, earning these two scientists the Nobel Prize in Chemistry. This discovery opened the door for a wide range of applications, including gene therapy, agricultural improvements, and functional genomics. Scientists have adapted this bacterial system for use in mammalian cells by synthesizing specific guide RNAs that direct the Cas9 protein to a target gene within the mammalian genome (Jinek et al., 2012). This enables the precise alteration of DNA sequences in a variety of organisms, ranging from human stem cells to animal models. This ability to accurately target specific DNA sequences has made CRISPR a rising star in modern genetics. Additionally, the versatility of the CRISPR-Cas9 system has led to its application in a wide range of biological research and potential medical therapies.

CRISPR works by utilizing the Cas9 protein, guided by a small RNA molecule known as the guide RNA (gRNA), to identify and cut specific sequences of DNA (Cong et al., 2013). The gRNA contains a sequence complementary to the target DNA, allowing it to bind precisely to the gene of interest. Once bound, Cas9 makes a double-stranded break in the DNA. The cell then attempts to repair the break, either by non-homologous end joining (NHEJ) or homology-directed repair (HDR) (Hsu et al., 2014). These repair mechanisms can introduce mutations that disrupt the gene or allow the insertion of new genetic material, respectively.

Traditional CRISPR-Cas9 technology allows for two key types of genetic modifications: knockout and knock-in. CRISPR knockout involves the use of the error prone NHEJ pathway. When Cas9 introduces a double stranded break in the DNA, NHEJ often creates small insertions or deletions that disrupt the gene's sequence, leading to a loss of function (Cong et al., 2013). This approach is useful for studying gene function and inactivating certain diseases. CRISPR knock-in relies on HDR, a precise mechanism that uses an exogenous DNA template to insert new genetic sequences at the break site. This method allows for the addition of new traits or the correction of genetic mutations, making it valuable for gene therapy.

Advancements to CRISPR technology have led to its increased utility. One advancement involves deactivating the nuclease sites of the Cas9 protein to create dead Cas9 (dCas9), meaning it can no longer create a double stranded break in the DNA (La Russa & Qi, 2015). Instead, another protein, such as an activator or a repressor protein, can be tethered to dCas9. While still using a custom gRNA to direct the dCas9 complex to the specified region in the genome, this allows for targeted overexpression or inactivation of a gene of interest, respectively.
These varying CRISPR techniques are particularly beneficial for advancing treatment options. To do so, CRISPR can be applied in vivo or ex vivo. In vivo CRISPR editing refers to gene modification carried out within a living organism (CRISPR Therapeutics). This approach is being explored for therapeutic purposes, such as correcting genetic mutations in targeted tissues or organs. Ex vivo CRISPR editing involves removing cells from an organism, modifying them in the lab, and then transplanting the edited cells back into the patient. Ex vivo editing offers more

control and less risk of off-target effects compared to in vivo editing, as cells can be screened for successful modifications before being transplanted back. Both of these methods are being explored for sickle cell disease. In this review, I will discuss how CRISPR can be used to advance therapeutic options for sickle cell disease.

MAIN TEXT

The Use of CRISPR in Sickle Cell Disease

Most experiments performed in animal models, known as preclinical studies, utilize ex vivo gene editing. Human hematopoietic stem cells (HSCs), which are cells that develop into various types of blood cells including red blood cells, can be isolated from human tissue such as from the cord blood or bone marrow and edited using CRISPR (Park & Bao, 2021). These edited stem cells can then be transplanted into a mouse model of sickle cell disease to test how well they overtake the sickle cells.

One example of this is a study that utilized CRISPR's ability to precisely target and correct the HBB mutation in patient-derived stem cells. Researchers used CRISPR-Cas9 to correct single point mutations that cause SCD in over 80% of HSCs derived from patients (DeWitt et al., 2021). The researchers noted minimal off-target effects, which is a key consideration for the safety of gene-editing therapies because that means the CRISPR system successfully targets and edits the desired gene with very few or no unintended changes elsewhere in the genome. When these corrected cells were transplanted back into an immunocompromised mouse model, the bone marrow was successfully refilled and normal, and healthy red blood cells were produced.

The ultimate goal is to use CRISPR in SCD patients in a safe and effective manner. Studies have found that reactivation of fetal *HBG1* and *HBG2* (γ-globin) genes can serve as a therapeutic approach for treating SCD. Multiple studies have focused on the disruption of the *BCL11A* or *ZBTB7A* gene using CRISPR-Cas9, in order to block repression (or induce expression) of fetal γ-globin. This can be performed in HSCs (Wu et al., 2019) or directly in red blood cells (also known as erythrocytes) (Martyn et al., 2018). This approach is very promising, as HSCs or erythrocytes can be taken directly from a patient, edited inside a lab, and then transfused back into the patient's body as a method of treatment for SCD.

Another method focuses more on in vitro modeling, which means the experiments are all performed in the lab and cells are grown on a petri dish. Cells can be collected directly from patients with SCD and then programmed backwards into stem cells, known as induced pluripotent stem cells (iPSCs). iPSCs are beneficial because they retain any mutations that the patient had. Specifically, these patient-derived iPSCs have a mutation in the HBB gene, which encodes for adult β-globin proteins. CRISPR-Cas9 can be used to edit and correct this mutation in a more efficient manner compared to other gene editing methods (Huang et al., 2015). The goal of this is to create human-specific model systems for SCD so that gene therapies can be tested in these cells and verify their efficacy and safety before being used in patients.

The most challenging therapeutic method is in vivo CRISPR editing due to the difficulty in delivering the treatment to specific cells. However, this has shown promise in treating sickle cell disease by directly editing the defective hemoglobin gene in the bone marrow stem cells of a mouse model of SCD, aiming to correct the genetic mutation that causes abnormal hemoglobin production (Newby et al., 2021). Nearly 80% of cells containing the SCD mutation were corrected. This could mean that a one-time treatment for SCD is possible for patients, with the possibility of less side effects compared to other treatments.

Clinical Trials on CRISPR Gene Therapy for SCD

The gene therapy has remarkable outcomes in the management of genetic disorders such as SCD through eliminating the mutation in the HBB gene that causes the disease. Among these gene-editing techniques, CRISPR-Cas9 is at the forefront of these gene-editing strategies and is currently undergoing various clinical trials to explore its effectiveness in correcting the SCD mutation. Early clinical and preclinical trials have indicated that red blood cells can be produced in patients after re-introduction of changed HSCs, which were modified using CRISPR (Dever et al., 2016). In this case, the patient derived HSCs before reintroduction into the patient, had their Glu6Val mutation that causes Sickle Cell Disease corrected using CRISPR-Cas9. These erythrocytes were then differentiated from the stem cells and transplanted back into the patient. The study confirms expression of adult β globin, which is consistent with ongoing advances made towards developing better SCD therapies using CRISPR.

Other clinical trials have similarly focused on ex vivo gene therapy, where patients' HSCs are harvested, modified, and reinfused. One documented strategy involves disrupting the BCL11A gene, which normally inhibits the production of fetal hemoglobin (HbF) (Frangoul et al., 2021). By using CRISPR-Cas9 to inhibit BCL11A, researchers were able to reactivate HbF, which is not sickle shaped and can replace the defective adult hemoglobin in SCD patients. This method significantly improved the prognosis of patients, with some clinical trial participants reporting complete elimination of the painful vascular occlusive crisis. A vascular occlusive crisis occurs as a result of sickle shaped cells obstructing small blood vessels resulting in pain and decreased oxygen diffusion to parts of the body. The elimination of this crisis is the most important goal of the study, since it is necessary to manage not only the pathology of the SCD disease, but also to improve patient’s symptoms.

Gene therapy involving the use of CRISPR-based methods can forever change how SCD is treated because it incorporates a one time treatment plan. Clinical trials so far have demonstrated that CRISPR edited stem cells are able to retain the ability of continuously producing healthy red blood cells after being implanted for a long time (University of California, San Francisco, 2022). But off-target effects have been observed including unintended insertions or deletions at non-target regions, potentially leading to the activation of oncogenes, and immune responses to CRISPR proteins like Cas9. As research in this area continues, the primary concern will be on ensuring safety, reducing off target effects, and targeting CRISPR therapy to specific tissues. With future research and efforts, SCD treatment using CRISPR can be more efficient and significantly improve patient’s survival rate and quality of life.

Cost Effectiveness of CRISPR Compared to other Treatments

While CRISPR stands out as a highly effective treatment that could potentially cure SCD, the cost remains high for widespread usage. Blood transfusions, along with hydroxyurea, have been viewed as the most effective primary treatments for SCD but unfortunately require a continuous, lifetime burden of cycles of therapy and costs (Ballas & Darbari, 2020). For instance, while monthly blood transfusions lower stroke risk and other complications, they are regularly administered every month and may lead to iron overload thus requiring further treatment with iron chelators that further contribute to the financial burden over the lifetime.

On the other hand, the cost of CRISPR therapies, however, reflects the complexity of gene editing and the equipment required to deliver the treatment. Current estimates indicate that it may cost one million dollars to treat just one SCD patient using the CRISPR technique for its single treatment. That comes from the cost of collecting the patient's HSCs from the blood followed by ex vivo gene editing, then infusing the edited cells back into the patient's body following a necessary myeloablative therapy (high-dosage chemotherapy that aims to kill cells within the bone marrow to create space for the edited cells to reenter).

Although CRISPR has a high initial cost, its long-term cost- effectiveness comes from its ability to treat the disease with a one time cure, thus avoiding multiple therapies and treatments for the long term. In one study, cost analysis was conducted to estimate and compare lifetime costs of standard SCD management alongside CRISPR based therapy (Demirci et al., 2019). The study indicated that while CRISPR has high initial costs, the therapy could save healthcare systems up to $500,000 per patient over a lifetime. This is because the treatment significantly reduces the number of hospitalizations, the need for emergency interventions, and the need for chronic treatments which include but are not limited to transfusions or iron chelation therapy.

Along with the direct costs of healthcare, CRISPR therapy can also mean high economic returns considering the escalation in the quality of life and higher economic output. SCD patients are frequently hospitalized and severely suffering from pain episodes that prevents them from attending school or engaging in work. A one shot curative treatment like CRISPR would greatly relieve these burdens, allowing patients to live healthier, more productive lives, which contributes to the long term cost savings associated with the treatment.

CONCLUSION AND FUTURE DIRECTIONS

CRISPR can be considered a novel approach in the treatment of SCD as it addresses the problem from the genetic root instead of merely the pathology. For SCD, therapeutic options lie with CRISPR revolutionizing the field of genetic engineering through its capability of correcting the mutated β-globin gene, actively producing healthy red blood cells, and possibly employing a single treatment approach to cure the disorder. The preliminary results of the clinical trials have been favorable with patients demonstrating a considerable decrease in the disease symptoms and better life quality (Frangoul et al., 2021). One of the most notable outcomes was the rise in fetal hemoglobin levels after genes such as BCL11A were edited to stop red blood cells from sickling (Ribeil et al., 2017).

Although promising in its potential, CRISPR is also met with constraints. One of the factors that is raised in this regard is that there might be changes in unpredicted parts of the genome, which can have negative side effects including cancer or other diseases (Guo et al., 2023). In addition, reaching target cells in human patients with CRISPR, especially in hematopoietic stem cells, remains a great challenge. Other limitations include long term safety, immune reactions towards CRISPR associated proteins, and universal access to the technology.

Ethics associated with the application of CRISPR in SCD treatments is also concerning. One major concern is the skepticism whether the CRISPR applications would be accessible to the general public. Considering the high expenses, most of the people would be unable to afford this therapy except the wealthy class. The other question that arises is that will the populations most affected by SCD, in the sub-Saharan region, be able to utilize gene editing when the health care structure available may limit the potential (Memi et al., 2018).

Future Directions

In order for CRISPR to maximize its full potential in the treatment of SCD, additional research should be directed toward enhancing the advancement of the technique and decreasing the risks associated with off-target effects (DeWitt et al., 2017). The emergence of novel CRISPR inventions like Prime editing and Base editing techniques improves the precision of modifications and lowers invasiveness, which decreases the risks associated with current CRISPR technologies (Anzalone et al., 2020). Also, enhancing the use of viral vectors or nanoparticles to the delivery of gene editing in HSCs of patients is also achievable (Yin et al., 2016).

On the ethical side, however, there must be some development of the guidelines and regulations to prevent the misuse of the technology. Societal aspects of gene editing can be addressed through public involvement; for example, the prospects of gene enhancement and disparities in availability of such advanced therapies (Coller, 2019). Solving such problems is vital for the ethical progress and effective integration of CRISPR technology in treatment of SCD and other genetic diseases.

In conclusion, although the road to the implementation of SCD’s treatment with CRISPR is multidimensional, the steps taken up till now are very encouraging. Given this expansion of knowledge, enhancement of techniques, and moral issues, CRISPR has the potential to be a game changer in the field of genetic engineering and provide a definitive solution for people suffering from SCD and possibly revolutionize treatment for other inherited conditions in the future.

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