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Review Article

CRISPR Technology in Sickle Cell Anemia

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ABSTRACT

CRISPR-Cas technology has emerged as a transformative tool in genome editing, offering an unprecedented level of precision, efficiency, and adaptability. This review highlights the application of CRISPR—particularly the CRISPR-Cas9 system—in the treatment of sickle cell anemia (SCA), a monogenic disorder caused by a point mutation in the HBB gene. CRISPR enables two major therapeutic approaches: direct correction of the sickle mutation through homology-directed repair (HDR) and the reactivation of fetal hemoglobin (HbF) by targeting regulatory elements such as BCL11A. These strategies have led to the development of advanced gene-edited cell therapies, including the first FDA-approved CRISPR-based treatment, Casgevy, marking a major breakthrough in clinical gene therapy. Additionally, the review discusses the molecular mechanisms of Cas9 and Cas12a, their comparative advantages, off-target considerations, and delivery challenges. Broader applications of CRISPR in medicine, agriculture, and biotechnology are explored, emphasizing its growing impact across scientific disciplines. Despite significant progress, challenges remain regarding off-target activity, safe in vivo delivery, and ethical implications, particularly in the context of germline editing. Continued innovation, ethical oversight, and interdisciplinary integration are essential to ensure the safe and responsible advancement of CRISPR technologies. Overall, CRISPR- Cas systems hold enormous potential to revolutionize the treatment of genetic diseases, with sickle cell anemia serving as a landmark example of their clinical success.

INTRODUCTION

Definition

The CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) – Cas (CRISPR-associated protein) systems are part of the adaptive

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immune system used by archaea and bacteria against foreign genetic elements (e.g., viruses or plasmids). The foreign genetic material is stored in the CRISPR sequence of the host's genome and is referred to as a "Spacer." Different spacers are separated by short palindromic repeats. Spacers can be transcribed to form crRNA (CRISPR-RNA). crRNA is then attached to the Cas protein by tracrRNA (trans-activating CRISPR RNA), this complex is used to guide the Cas proteins to the foreign genetic material, which the nucleases (Cas) will then cleave [1].

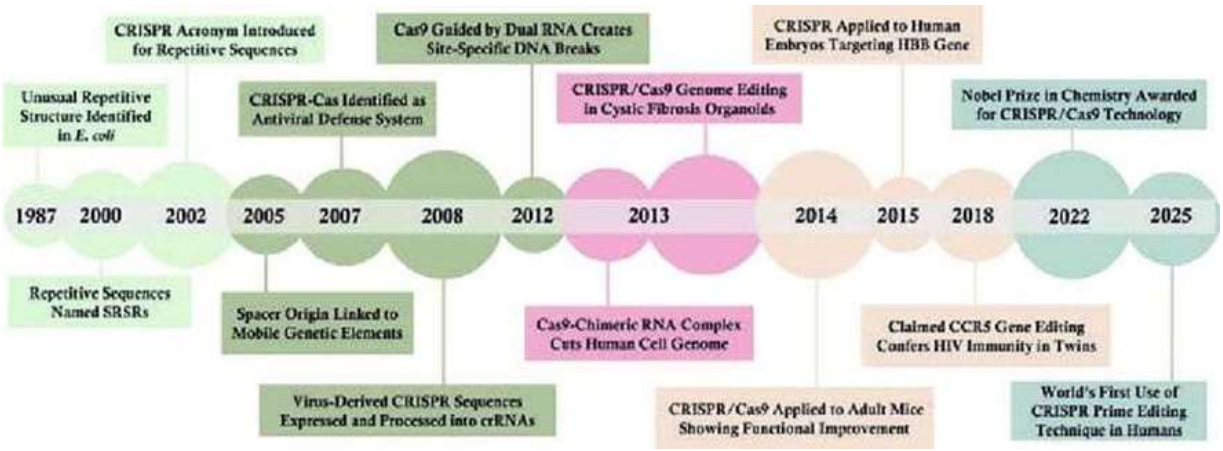

Figure:1 Timeline of CRISPR/Cas9 Discovery and Development [2]

CAS-9 Enzyme

Cas9 (CRISPR-associated protein 9) is a specialized RNA-guided DNA endonuclease enzyme that plays a central role in the CRISPR-Cas9 genome editing system. Cas9 can be programmed with a sequence of guide RNA to recognize and bind to specific locations on a DNA molecule, where it then makes precise double-strand cuts, allowing for targeted genetic modifications or gene editing. Originally, Cas9 evolved in bacteria as part of their adaptive immune response to combat viruses and plasmids but is now widely employed in genetic engineering and biomedical research to edit genes with high specificity. The simplicity, efficiency, and versatility of the CRISPR-Cas9 system have revolutionized molecular biology, making it a powerful tool for applications ranging from correcting genetic disorders to developing disease-resistant crops and advancing cancer research [3].

Key Features of CAS9

- Cas9 acts as molecular scissors, enabling researchers to cut, remove, or add segments to a genome at a chosen site.
- It requires a guide RNA (gRNA) that directs Cas9 to the exact DNA sequence to be modified.
- Its activity depends on the presence of specific short DNA sequences in the target called "Protospacer Adjacent Motifs" (PAM).

Importance in Research

Cas9, a key component of the CRISPR-Cas9 system, has dramatically advanced the field of genetic engineering due to its ability to make precise, targeted cuts in DNA. This RNA- guided endonuclease can be programmed to recognize specific genetic sequences, allowing scientists to edit genes with remarkable accuracy and efficiency. One of the most transformative applications of this technology is in the treatment of sickle cell anemia a genetic disorder caused by a single-point mutation in the HBB gene. By using

CRISPR-Cas9, researchers can target and correct this mutation at the DNA level, enabling the production of normal hemoglobin and alleviating the disease's debilitating symptoms. This approach has already led to the development of groundbreaking therapies like Casgevy, which became the first FDA-approved CRISPR-based treatment for sickle cell disease. The success of such therapies not only offers hope for a permanent cure but also highlights the immense potential of CRISPR-Cas9 in treating a wide range of genetic disorders.

Sickle Cell Anemia

Sickle cell disease is caused by a single base substitution at the 6th codon of the HBB gene. A thymine is switched to an adenine, resulting in a glutamate (hydrophilic) being replaced with a valine (hydrophobic). This mutation, under hypoxic (low oxygen) conditions, causes the red blood cells to have their characteristic rigid sickle shape, allowing them to stick to vessel walls and clump together (sickling). This sickling can lead to vaso-occlusive crises, also known as pain crises. SCD refers to the full range of potential genotypes involving the mutated HBB gene, not to be confused with sickle cell anemia which refers specifically to being homozygous for this mutated HBB gene. Symptoms of sickle cell anemia usually show up at a young age. They may appear in babies as early as 4 months old, but generally occur around the 6-month mark. While there are multiple types of SCD, they all have similar symptoms, which vary in severity [4].

Management Treatments

- It including pain management, blood transfusions, antibiotics for infections, and hydroxyurea to increase Fetal hemoglobin (HbF), which alleviates symptoms.
- Curative options such as bone marrow transplant are pursued in select cases.

Tests and Diagnostic Approaches

- New born screening
- Complete blood count (CBC)
- Peripheral blood smear
- Hemoglobin electrophoresis
- Hemoglobin solubility testing
- Genetic testing

These Include:

- Excessive fatigue or irritability during anemia.
- Fussiness in babies.
- Bedwetting from associated kidney problems.
- Jaundice which is yellowing of the eyes and skin.
- Swelling and pain in hands and feet.
- Frequent infections pain in the chest, back, arms, or legs.

What Are the Types of Sickle Cell Disease?

Hemoglobin is the protein in red blood cells that carries oxygen. It normally has two alpha chains and two beta chains. The four main types of sickle cell anemia are caused by different mutations in these genes.

Hemoglobin SS Disease

Hemoglobin SS disease is the most common type of sickle cell disease. It occurs when you inherit copies of the hemoglobin S gene from both parents. As the most severe form of SCD, individuals with this form also experience the worst symptoms at a higher rate.

Hemoglobin SC Disease

Hemoglobin SC disease is the second most common type of sickle cell disease. It occurs when

you inherit the Hb C gene from one parent and the Hb S gene from the other. Individuals with Hb SC have similar symptoms to individuals with Hb SS. However, the anemia is less severe.

Hemoglobin SB+ (BETA) Thalassemia

Hemoglobin SB+ (beta) thalassemia affects beta globin gene production. The size of the red blood cell is reduced because less beta protein is made. If inherited with the Hb S gene, you will have hemoglobin S beta thalassemia. Symptoms are not as severe.

Hemoglobin SB 0 (BETA-ZERO) Thalassemia

Sickle beta-zero thalassemia is the fourth type of sickle cell disease. It also involves the beta globin gene. However, sometimes the symptoms of beta zero thalassemia are more severe. It is associated with a poorer prognosis. Hemoglobin SD, hemoglobin SE, and hemoglobin SO These types of sickle cell diseases are rare and usually don't have severe symptoms.

Rare Types in Sickle Cell Disease

There are also several rare forms of SCD that occur when the Sickle gene is inherited with other unusual abnormal hemoglobin genes, such as:

- Hemoglobin SD Disease
- Hemoglobin SE Disease
- Hemoglobin SO Disease

MECHANISM

During gene editing, the CRISPR-Cas9 system follows the recognition, cleavage, and repair process. The CRISPR locus is initially transcribed into a long RNA molecule known as pre-crRNA. Next, the tracrRNA hybridises with the repeat part of the pre-crRNA, resulting in the cleavage within the repeat by RNase III polymerase. As a result, mature crRNAs are formed. Cas9 then cleaves and forms Double-Strand Breaks (DSBs) three base pairs upstream of the Protospacer Adjacent Motif (PAM) sequence by using His-Asn-His (HNH) and Crossover Junction Endo Deoxyribonuclease (RuvC) domains for cleaving the complimentary and non-complimentary strands, respectively. The resultant DSBs are repaired by two mechanisms, namely Non-Homologous End Joining (NHEJ) and HDR pathways. In the NHEJ pathway, which is the dominant repair mechanism, short insertions or deletions (indels) are incorporated, resulting in frameshift or exon-skipping mutations and causing the target sequence to be disrupted. The Cas12a (also known as Cpf1) protein, a part of the Type V-A CRISPR-Cas9 systems, offers unique features distinct from the more commonly known Cas9. Unlike Cas9, Cas12a can process its own crRNA due to its RNase site, eliminating the need for tracrRNA. This makes Cas12a a dual-function protein with both endoribonuclease and endonuclease activities. A significant aspect of Cas12a is its recognition of specific PAM sequences (TTTV) and its ability to create staggered DNA breaks downstream of these sites. These breaks result in overhanging ends, which are advantageous for precise gene insertion. Cas12a is noted for its enhanced activity, greater specificity, and reduced off-target effects compared to Cas9. More recently a new Cas12m base editor has been developed GoCas12, that lacks any DNA cleavage activity, minimizing the possibility of DSBs and large genomic rearrangements However, the choice between Cas12a and Cas9 should be based on the specific requirements of the gene editing task, as each system has its own strengths and applications. As sickle cell disease is caused by a genetic mutation, it is a perfect candidate for CRISPR- mediated gene therapy. Treating sickle cell anemia with CRISPR involves an ex vivo procedure known as gene-edited cell therapy, where hematopoietic stem cells are extracted from

the patient, corrected, and then replaced. In this section, we'll discuss the main approaches scientists are using to create CRISPR sickle cell gene-edited cell therapies [5].

Type 1: Mechanism Involved in Beta Globin Gene Editing to Produce Adult Hemoglobin

The mechanism involved in beta globin gene editing to produce adult hemoglobin using CRISPR involves the following key steps:

Targeting: The CRISPR-Cas9 system, guided by a CRISPR RNA (crRNA), binds specifically to exon 2 of the Beta-Hemoglobin (HBB) gene where the sickle cell mutation occurs. Cas9 recognizes a Protospacer Adjacent Motif (PAM) sequence and induces a precise Double-Strand Break (DSB) in the DNA near the mutation site.

DNA Repair Activation: The DSB activates the cell's natural DNA repair mechanisms, predominantly Homology-Directed Repair (HDR) when a correct DNA template is present. This repair pathway uses the homologous donor DNA template supplied exogenously, which contains the normal sequence of the beta-globin gene.

Gene Correction: During HDR, the cell copies the corrected sequence from the donor template into the genomic DNA at the site of the break, thereby repairing the mutation. This precise editing restores the gene's normal sequence.

Expression Of Adult Hemoglobin: The corrected beta-globin gene produces normal beta-globin mRNA and protein, which pairs with alpha-globin chains to form functional Adult Hemoglobin (HbA). This results in restored red blood cell function.

Outcome: Edited erythroid precursor cells show increased Beta-Hemoglobin protein production with a significant reduction of the excess free alpha-globin chains, which are otherwise pathological in sickle cell disease. This correction can reach levels near those seen in healthy individuals [7].

Type 2: Using Crispr To Promote Fetal Hemoglobin Production

The production of Fetal Hemoglobin (HbF) involves several key steps that are crucial in researching therapies for sickle cell anemia. These steps include genetic regulation, erythropoiesis, and targeted gene editing to induce or increase HbF levels in adult humans.

Key Steps in Fetal Hemoglobin Production

1. Genetic Regulation of Globin Switching

Fetal Hemoglobin (HbF) is predominantly produced during fetal development, starting around the 6th week of gestation and becoming the main hemoglobin in fetal red blood cells by approximately 3 months of gestation. The switch from fetal to Adult Hemoglobin (HbA) occurs postnatally, with HbF levels naturally declining to less than 1% by around one year of age.

2. Inhibition of Hbf Suppression Via Bcl11a

In sickle cell disease therapies, a major strategy involves silencing or knocking out the BCL11A gene, which is a transcriptional repressor of HbF production. This silencing reactivates HbF synthesis in adult erythroid cells, effectively reversing the fetal-to-adult hemoglobin switch. CRISPR and other gene-editing technologies are being employed to inactivate BCL11A, leading to significant increases in HbF levels.

3. Gene Editing Approaches

Gene editing techniques, such as CRISPR-Cas9, are used to modify hematopoietic stem cells

outside the body (ex vivo). These cells undergo targeted modifications to disable BCL11A or other regulatory genes, then are reintroduced into the patient, where they produce HbF-rich red blood cells that resist sickling [7].

4. Erythropoiesis and HBF Expression

During erythropoiesis, erythroid precursor cells in the bone marrow undergo differentiation and globin gene expression shifts. Under stress erythropoiesis or genetic manipulation, the expression of Gamma-Hemoglobin Genes (HBG) is increased, leading to higher HbF levels that suppress sickling [8].

5. Therapeutic Induction of HBF Production

Pharmacological agents or gene therapies are designed to induce HbF expression. These approaches aim to either reactivate the natural HbF production pathway or mimic the genetic modifications that sustain high HbF levels, providing a therapeutic benefit in sickle cell patients by reducing hemoglobin polymerization and cell sickling.

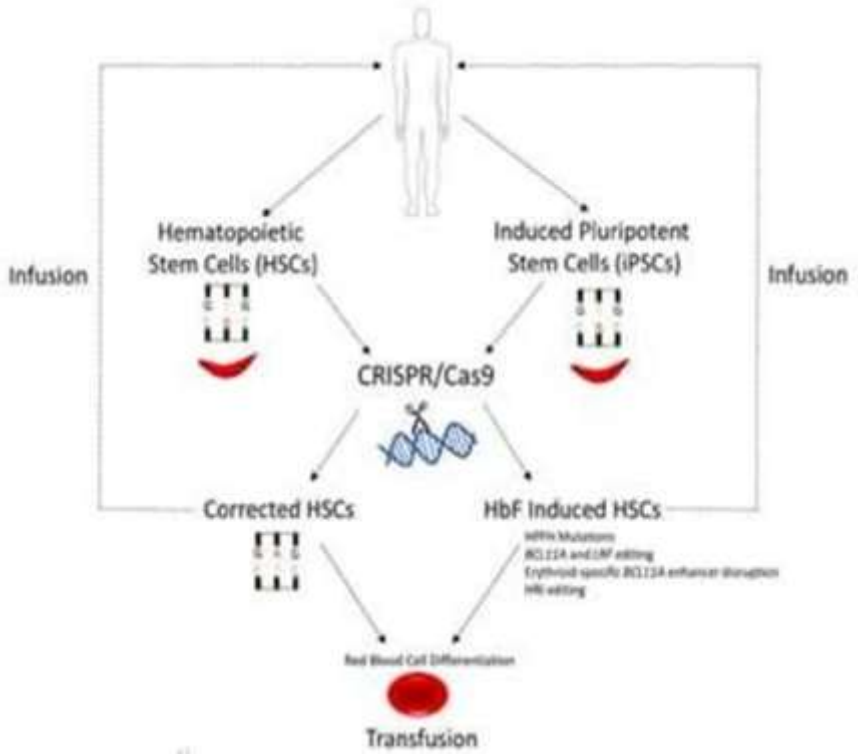

Figure:2 Mechanism of CRISPR [9]

Applications

- Agriculture
- Medicinal
- Industrial
- Bio Medical
- Bioinformatic

The specificity of CRISPR-Cas9 technology has been a topic of great interest and improvement. Studies have explored strategies to reduce off-target effects and enhance precision. Fu etal developed a modified Cas9 variant, termed SpCas9-HF1, which exhibited reduced off-target activity while maintaining high on-target efficiency. This study demonstrated the feasibility of engineering the Cas9 nuclease to improve its

specificity [10]. The versatility of CRISPR-Cas9 extends beyond gene knockout. Researchers have harnessed the technology for a range of applications, including gene activation and repression. In a study by Gilbert etal, the Cas9 protein (a catalytically inactive form of Cas9) was fused to transcriptional activators or repressors, enabling precise control of gene expression. This approach has facilitated the investigation of gene function and regulatory networks [11]. CRISPR-Cas9 technology has transformed the field of genome editing. Key studies by Doudna and Charpentier have paved the way for its widespread adoption and optimization. Advances in specificity, versatility, and applications continue to propel the field forward, opening up new possibilities for scientific research, therapeutic interventions, and agricultural advancements [12].

CRISPR-CAS9 Technology Application In Agriculture

CRISPR-Cas9 technology holds significant promise for revolutionizing agriculture by enabling precise and efficient genetic modifications in crops and livestock. This sub-section highlights the applications and advancements of CRISPR-Cas9 in agricultural settings. Crop improvement is a major focus of CRISPR-Cas9 technology in agriculture. The ability to precisely edit plant genomes allows for the enhancement of desirable traits, such as disease resistance, yield, nutritional content, and stress tolerance. For example, in a study by Shan etal, CRISPR-Cas9 was used to successfully target the susceptibility gene in rice), leading to enhanced resistance against bacterial blight [13]. CRISPR-Cas9 has also shown promise in accelerating the breeding process. Traditional breeding methods often require multiple generations to achieve desired traits. With CRISPR- Cas9, targeted genetic modifications can be introduced directly into the germline of plants, significantly reducing the breeding timeline. In a study by Li et al., CRISPR-Cas9 was utilized to enhance rice grain yield by precisely editing genes associated with grain size regulation [14]. Furthermore, CRISPR-Cas9 technology has the potential to address global food security challenges. By modifying crop genomes, it is possible to develop crops that are more resistant to pests, diseases, and environmental stresses, thereby increasing productivity and reducing yield losses. This technology also offers opportunities for the production of nutritionally fortified crops, which can help combat malnutrition and improve human health [15]. The use of CRISPR-Cas9 in livestock breeding is another emerging area in agricultural applications. Researchers are exploring the potential to introduce specific genetic modifications in livestock to improve traits such as meat quality, disease resistance, and animal welfare. While progress in this field is still in its early stages, CRISPR-Cas9 holds promise for revolutionizing livestock production and addressing challenges faced by the livestock industry [16]. Although the application of CRISPR-Cas9 in agriculture offers immense potential, it is not without challenges. Regulatory frameworks and public acceptance remain important considerations, particularly regarding Genetically Modified Organisms (GMOs). Efforts are underway to address these concerns and establish guidelines for the responsible use of CRISPR-Cas9 in agriculture [17].

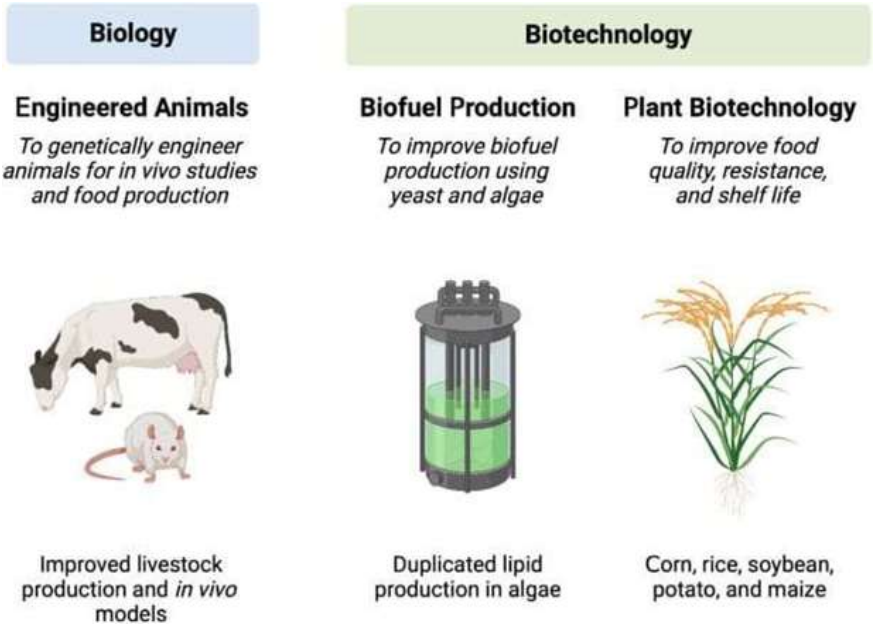

Figure:3 Application in Agriculture [18]

Furthermore, CRISPR-Cas9 technology presents significant opportunities for agricultural advancements. Its precise genome editing capabilities enable targeted modifications in crops and livestock, leading to improved traits, increased productivity, and enhanced food security. As research progresses and regulatory frameworks develop, CRISPR-Cas9 holds the potential to revolutionize agricultural practices and contribute to a sustainable and resilient global food system [19].

CRISPR-CAS9 Technology in Medicine

CRISPR-Cas9 technology has emerged as a powerful tool with transformative implications in the field of medicine. This sub-section provides an overview of the applications and advancements of CRISPR-Cas9 in medical research and therapy [20]. One of the key applications of CRISPR-Cas9 in medicine is in the study of gene function and disease mechanisms. By selectively modifying genes in human cells or model organisms, researchers can gain insights into the underlying causes of genetic diseases. For example, in a study by Shalem et al., CRISPR-Cas9 was employed to systematically knock out each gene in the human genome, leading to the identification of genes essential for cancer cell survival [21]. CRISPR-Cas9 also holds promise for the development of gene therapies. It offers the potential to correct disease-causing mutations directly in the genomes of patient cells. In addition, CRISPR-Cas9 technology is possible to correct a mutation associated with a genetic blood disorder, β-thalassemia, in patient-derived stem cells. Furthermore, CRISPR-Cas9 technology has opened up new avenues for cancer research and therapy. It allows for the targeted disruption of genes involved in cancer progression or the introduction of specific modifications to sensitize cancer cells to existing treatments. In a study, CRISPR-Cas9 was used to knock out a gene involved in chemotherapy resistance, enhancing the effectiveness of the treatment in cancer cells [22]. The potential of CRISPR-Cas9 extends beyond gene editing. It has been harnessed for diagnostic purposes, such as the detection of specific DNA sequences associated with diseases. CRISPR-based diagnostic tools, such as Specific High-Sensitivity Enzymatic Reporter Unlocking

(SHERLOCK) and DNA Endonuclease-Targeted CRISPR Trans Reporter (DETECTR) have been developed to enable rapid and accurate detection of viral infections and genetic mutations [23]. Moreover, the combination use of CRISPR-innovative technology and stem cell research could enable the creation of disease models for the investigation of therapy. The gene editing by using CRISPR-Cas9 was applied to clarify the mechanism Autosomal Dominant Polycystic Kidney Disease (ADPKD) by deleting the PKD2 gene [24]. Furthermore, CRISPR also helped to explain the hypoglycemia by insertion of MEN1 gene with artificial specific point mutation into iPSCs. The CRISPR-Cas9 is also subjected for drug screening by inducing Deoxyguanosine Kinase (DGUOK) knockout to the stem cells which developed as hepatocytes. It generated the mitochondrial dysfunction in hepatocytes as a board for compound screening [25]. While the potential of CRISPR-Cas9 in medicine is immense, there are challenges that need to be addressed. These include off-target effects, delivery methods, and ethical considerations, particularly when it comes to germline editing. Ongoing research aims to improve the specificity and safety of CRISPR-Cas9, and discussions around ethical and regulatory frameworks continue to evolve [26]. However, CRISPR-Cas9 technology holds significant promise in medicine. Its applications range from understanding gene function and disease mechanisms to developing precise gene therapies and diagnostic tools. As research progresses and challenges are overcome, CRISPR- Cas9 is poised to revolutionize the field of medicine, offering new possibilities for treating genetic diseases and advancing personalized medicine [27].

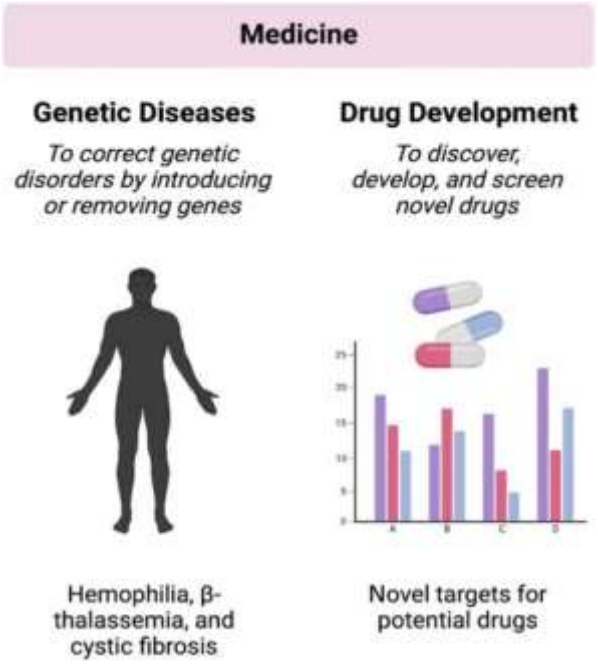

Figure:4 Technology in Medicine [28]

Advantages

Fast, flexible design – Essentially, CRISPR-Cas9 gene editing requires 2 components: the Cas nuclease and a guide RNA. In some cases, researchers opt to use the 2-part crRNA- tracrRNA system while in others they use an sgRNA. Once the guide RNA and endonuclease bind to each other they form a Ribonucleoprotein (RNP) complex. It's within this complex that the guide

RNA can bind to the target sequence and editing can occur.

Multiplexed gene editing – CRISPR-Cas9 can edit multiple genes simultaneously, when the implemented sgRNAs are designed to target different genetic loci. This makes CRISPR-Cas9 gene editing an attractive and efficient approach for manipulating multiple locations in the genome [29].

Cost-effective – Because of the reasons listed above, CRISPR-Cas9 can be extremely cost-effective relative to other gene editing approaches such as Zinc Finger Nucleases (ZFNs) and Transcription Activator-like Effector Nucleases (TALENs), which require protein engineering. The simplicity of CRISPR-Cas9 – relying largely on 2 molecules to induce edits – as well as the flexibility available in designing sgRNAs to bind to any/numerous target(s) means that researchers can start editing quickly and make adjustments easily.

- **Treating Genetic Diseases:**

CRISPR is being used in clinical trials and has received regulatory approval to treat single- gene disorders such as sickle cell disease and cystic fibrosis.

- **Developing Cancer Therapies:**

The technology can engineer a patient's own T-cells to better recognize and attack cancer cells.

- **Fighting Infectious Diseases:**

CRISPR is being explored for diagnosing and potentially treating infectious diseases like HIV by targeting viral RNA.

- **Cardiovascular Diseases:**

Researchers are using CRISPR to target genes related to high LDL cholesterol in an effort to treat cardiovascular disease.

- **Creating Disease Models:**

CRISPR is crucial for quickly creating genetically modified cell and animal models to study diseases and develop new drugs.

In agriculture

- **Improving Crops:**

CRISPR can modify crops to be more tolerant to drought, pests, or other environmental stresses.

- **Livestock Breeding:**

The technology can be used to expedite breeding for desirable traits in livestock. In research.

- **Understanding Gene Function:**

Scientists use CRISPR to perform genetic screens, modify specific genes, and understand their roles in different cells and organisms.

- **Developing Diagnostics:**

CRISPR technology is being developed to create rapid and accurate diagnostic tests for various diseases.

- **Environmental Applications:**

The technology can be used to control disease-carrying insects through gene drives

- **Antimicrobials:**

CRISPR can be engineered to create new antimicrobials to fight bacterial infections.

Disadvantages

Delivery limitations – CRISPR-Cas9 has to be successfully delivered into cells in order to induce the desired edits. Like many other gene editing applications, delivery of the Cas9/gRNA complex into a sufficient number of cells can be challenging because all of the components must be delivered into cells at the right concentrations and at the correct point in a cell's cycle. CRISPR reagent delivery methods include electroporation, lipofection, microinjection, nanoparticles, and viral plasmids.

Efficiency limitations – Another important limitation to consider when using CRISPR-Cas9 gene editing is that when the Cas9/gRNA complex is taken up by cells, the gene editing activity itself may not occur. This is especially true when the goal is to knock-in or insert material into a gene, a process which relies on homology directed repair (HDR). However, researchers have made significant progress in improving HDR efficiency rates with CRISPR. To read more about improving CRISPR-Cas9 gene editing efficiency, check out the DECODED article, Improving efficiency of Homology-Directed Repair (HDR) [30].

Off-target effects – Off-target effects occur when the Cas9 nuclease edits an untargeted section of the genome, resulting in unwanted alterations. Off-target effects are a major concern for CRISPR-Cas9 experiments, and they can be challenging to predict. Efforts have been made to improve in silico off-target prediction tools as well as to reduce off-target effects by improving Cas9 nucleases [31].

Therapeutic Role Of CRISPR/CAS-9

The first CRISPR-based therapy in the human trial was conducted to treat patients with refractory lung cancer. Researchers first extract T-cells from three patient's blood and they engineered them in the lab through CRISPR/Cas-9 to delete genes (TRAC, TRBC, and PD-1) that would interfere to fight cancer cells. Then, they infused the modified T-cells back into the patients. The modified T-cells can target specific antigens and kill cancer cells. Finally, no side effects were observed and engineered T-cells can be detected up to 9 months of post-infusion. CRISPR/Cas-9 gene-editing technology could also be used to treat infectious diseases caused by microorganisms. One focus area for the researchers is treating HIV, the virus that leads to AIDS. In May 2017, a team of researchers from Temple University demonstrated that HIV-1 replication can be completely shut down and the virus eliminated from infected cells through excision of HIV-1 genome using CRISPR/Cas-9 in animal models. In addition to the approach of targeting the HIV-genome, CRISPR/Cas-9 technology can also be used to block HIV entry into host cells by editing Chemokine Co-Receptor Type-5 (CCR5) genes in the host cells. For instance, an in vitro trial conducted in China reported that genome editing of CCR5 by CRISPR/Cas-9 showed no evidence of toxicity (infection) on cells and they concluded that edited cells could effectively be protected from HIV infection than unmodified cells [32].

FUTURE PROSPECTS

Although CRISPR-Cas technology shows great potential in the field of gene editing, it still faces numerous challenges. One of the main limitations is the off-target effects associated with CRISPRCas9 and its related systems, which may lead to unpredictable unintended genetic modifications [33]. Off-target activity might trigger insertions, deletions, or chromosomal rearrangements at unit end endogenomic sites, posing significant risks in clinical applications that require high precision. Although strategies such as developing high-fidelity Cas9 variants, modifying sgRNA design, and pairing with nucleases have

been developed to reduce off-target effects, these risks have not yet been completely eliminated. Another major challenge is delivering CRISPR components to target cells, particularly in therapeutic applications. Although viral vectors such as adeno-associated virus (AAV) are widely used due to their high transduction efficiency and relatively low pathogenicity, inherent risks include immunogenicity, limited packaging capacity (~4.7 kb), and the potential risk of random genomic integration, which may lead to insertion mutations or tumorigenicity. Non- viral delivery methods, such as liposomal nanoparticles and electroporation, offer alternative approaches but often face challenges related to delivery efficiency, tissue specificity, and cytotoxicity [34]. Therefore, developing safe, efficient, and multifunctional delivery platforms remains a priority for the clinical translation of CRISPR technology. These unresolved challenges also limit the development of CRISPR-based diagnostic technologies. Many CRISPR diagnostic platforms, such as systems utilizing Cas12 or Cas13, rely on nucleic acid amplification (e.g., PCR, RPA) to enhance sensitivity. However, amplification steps introduce sample contamination and false- positive results, hindering the application of CRISPR diagnostics in point-of-care testing or resource-limited settings. Currently, amplification-free detection strategies are being developed by leveraging the bypass cleavage activity of Cas proteins and optimizing signal amplification methods; however, sensitivity and specificity still need to be improved. Future research should focus on discovering more novel, customizable Cas proteins while gaining a deeper understanding of the evolutionary drivers behind the diversity of the CRISPR system. Through targeted design and modification, we can also make these Cas proteins safer and more efficient. Furthermore, integrating CRISPR technology with other frontier fields such as synthetic biology, nanotechnology, and computational biology will facilitate the development of next generation gene editing platforms. This integrated system will enable more precise and regulable gene-editing technologies, driving the widespread application of CRISPR in medicine, agriculture, and environmental science [35].

Ethical Challenges

In addition to technical challenges, the application of CRISPR technology has raised complex ethical and regulatory issues. The ability to edit human embryos, germ cells, or even entire species has raised serious ethical questions about unintended ecological consequences, genetic fairness, and the long-term impact on human evolution. A few striking events, such as the controversial case of gene-edited babies, have led to intense global discussions about the boundaries of acceptable genetic intervention and the necessity of collective regulation. To address these concerns, many scientific institutions and governments have called for the establishment of rigorous ethical frameworks. International efforts, such as initiatives led by the World Health Organization (WHO) and the International Committee on Human Germline Genome Editing, emphasize the necessity of achieving global consensus and ensuring transparency when defining the boundaries of CRISPR applications. This means we need to establish stricter regulatory frameworks, introduce long-term follow- up monitoring in clinical trials, and clearly distinguish between somatic cell editing, which only affects individuals, and germline editing, which may affect future generations. Additionally, ethical guidelines must be developed, and public education initiatives launched to ensure the safe and responsible use of CRISPR technology in medicine, agriculture, and the environment [36].

CONCLUSION

CRISPR-Cas technology has revolutionized gene editing, offering precise, efficient, and versatile tools for correcting genetic mutations. Its greatest success so far is in treating sickle cell anemia, where CRISPR enables both direct HBB gene correction and reactivation of fetal hemoglobin, leading to the first FDA-approved CRISPR therapy. While the technology holds tremendous promise in medicine, agriculture, and biotechnology, challenges such as off-target effects, delivery limitations, and ethical concerns must still be addressed. With continued advancements and responsible use, CRISPR is poised to transform the future of healthcare and genetic research.

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