Gene Editing: A New Approach to Treat Genetic Disorders

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Abstract:

Gene editing has emerged as a groundbreaking method in tackling genetic disorders/diseases by precisely altering DNA sequences. Genetic disorders like Turner Syndrome and Sickle cell disease occur from mutations or altered genetic material. Gene editing technologies, including TALENs, ZFNs, and CRISPR-Cas9, have introduced the power to fix mutations at precise locations in the genome. The CRISPR-Cas9 system can efficiently modify genes with unprecedented precision. As shown by research conducted on Leber Congenital Amaurosis type 10 and Sickle cell disease, gene editing holds promise for correcting disease-causing mutations. However, ethical concerns and safety considerations, including germline editing and off-target effects, indicate that caution is necessary. As the field moves forward, recent advancements like prime editing and tackling antimicrobial resistance highlight the hidden potential of gene editing. Gene editing will continue to grow and unleash a field of innovative and personalized medicine, revolutionizing healthcare.

Introduction to Genetic Disorders:

Genetic disorders occur when a mutation affects your genes or when there is an incorrect amount of genetic material in a certain gene. Mutations can be harmless, helpful, or harmful. Most mutations will have no effect on the health of the body. The human body can also repair several mutations. Helpful mutations can improve a person's health. For example, a helpful mutation could be one that protects against heart disease. Disease-causing mutations are the harmful changes to a gene. By changing a gene's instructions for making a protein, a protein can be produced incorrectly or not be produced at all ("How can gene variants"). If a protein is produced incorrectly, the shape of the protein will also be incorrect. This can be detrimental because the proper shape of a protein directly correlates to its function in the body. (MedlinePlus Genetics)

Genes are made of DNA or deoxyribonucleic acid, so they contain instructions for cell functioning and all characteristics of a person. A wide array of mutations can cause many different types of genetic disorders. These disorders can be chromosomal, complex, or single-gene in origin. Several examples of chromosomal disorders are Down Syndrome, Trisomy 18, and Turner Syndrome. Cancer, Arthritis, and Diabetes are multifactorial disorders. Cystic fibrosis and Sickle cell disease (SCD) are single-gene disorders ("Genetic Disorders: What Are They").

Turner syndrome is a condition that only affects biological females (assigned female at birth). It occurs when one of the X chromosomes (sex chromosomes) is missing or partially missing. People typically have two sex chromosomes: biological females have two X chromosomes and biological males have one X and one Y chromosome. In normal conditions, a biological female will normally receive one X chromosome from each parent. Turner syndrome

results when one normal X chromosome is present and the other is structurally altered or missing (“Turner Syndrome”).

SCD is a blood disorder that affects red blood cells. In SCD, hemoglobin (a protein in red blood cells) is abnormal. This causes the shape of the red blood cell to change from round and flexible to a rigid crescent shape. SCD is caused by a genetic mutation in the HBB gene. The HBB gene is responsible for manufacturing hemoglobin. People with SCD inherited two mutated HBB genes, one from each parent. SCD is inherited in an autosomal recessive manner, meaning that the parents of the child will carry a copy of the mutated gene (“Sickle Cell Disease (SCD)”).

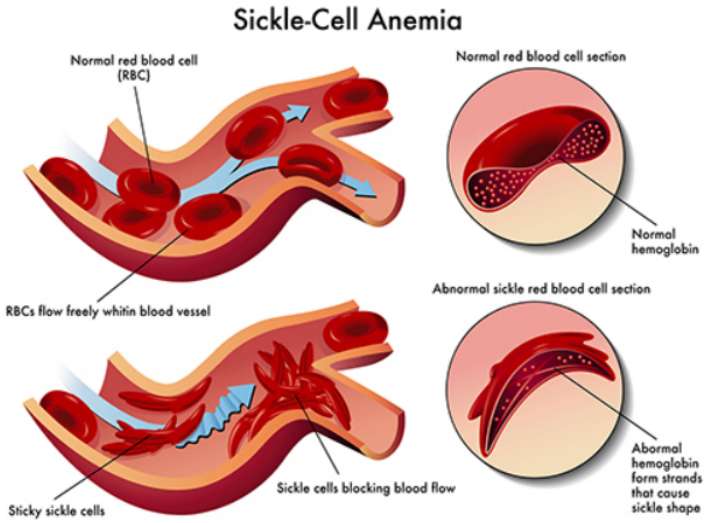

Figure 1: Sickle Cell Anemia (Cyro Cell International).

This image shows a normal red blood cell flowing through a blood vessel. A cross section of this blood cell is also shown, with normal hemoglobin. Below, sickle cells are shown blocking the blood flow within the blood vessel. Abnormal hemoglobin in the cross section of a cell is also shown.

Introduction to Gene Editing:

Genomic medicine is a constantly developing field of medicine. Advances in cell therapy and gene therapy have paved the way for gene editing. Gene editing, also referred to as genome editing, gives scientists the ability to change an organism's DNA. Gene editing technologies facilitate the alteration of genetic material at a particular location in the genome. This alternation can be done by modifying the existing DNA sequence and cutting out DNA and inserting replacement DNA.

There are several types of gene editing technologies. They include Transcription Activator-Like Effector Nucleases (TALENs), Zinc-finger nucleases (ZFNs), and meganucleases. Restriction enzymes were the original genome editors. Restriction enzymes identify patterns of

nucleotide sequences and cut at that site, allowing for the insertion of new DNA material. Zinc-finger nucleases, made up of a nuclease and zinc finger DNA binding domain, were used to disable CCR5 on human T cells, a major receptor for the Human Immunodeficiency Virus. TALENs are similar to ZFNs, but they use transcription activator-like effectors (arrays of amino acid repeats). The amino acid repeats can recognize single-nucleotides, increasing targeting capabilities of gene editing (Gaj et al. 2016.).

The most widely used genome editor today is CRISPR-Cas 9. CRISPR-Cas 9 genome editing technology makes it significantly easier to disrupt a targeted gene or insert a new gene sequence at a specific location using a DNA template. CRISPR were first found in the sequences of DNA from E. coli bacteria and described in 1987 Japan (Gostimskaya 2022). Clustered regularly interspaced short palindromic repeats (CRISPR) are bacterial DNA sequences copied from bacterial viruses. Cas enzymes use the virus's DNA to screen all DNA within the bacterial cell. If a matching CRISPR sequence is recognized, an immune response is triggered which destroys invading DNA and prevents infection. The CRISPR-Cas9 system is used to edit portions of DNA within plants and animals as well. There are three main steps in the CRISPR-Cas9 mechanism: recognition, cleavage, and repair. Guide RNA (gRNA) and the Cas9 nuclease (protein that edits DNA) are crucial to this genome editing process. gRNA is a small RNA sequence designed to bind to the target sequence. The gRNA guides Cas9 to the target DNA sequence location to cut both DNA strands of interest. The DNA will then repair itself via two pathways: NHEJ (non-homologous end joining) which will lead to a random insertion/deletion of DNA, or HDR (homology directed repair) where a homologous piece of DNA is used as a repair template. HDR will allow precise genome editing, as precise as a single base-pair (Redman et al. 2016). As mentioned above, in CRISPR-Cas9, the genetic engineering function of Cas9 has been manipulated to precisely insert or remove specific DNA fragments from a strand of genetic material, acting as a pair of molecular scissors. Either a replacement gene can be inserted or insertions/deletions mutations can be created during the natural DNA repair process (Park et al. 2021).

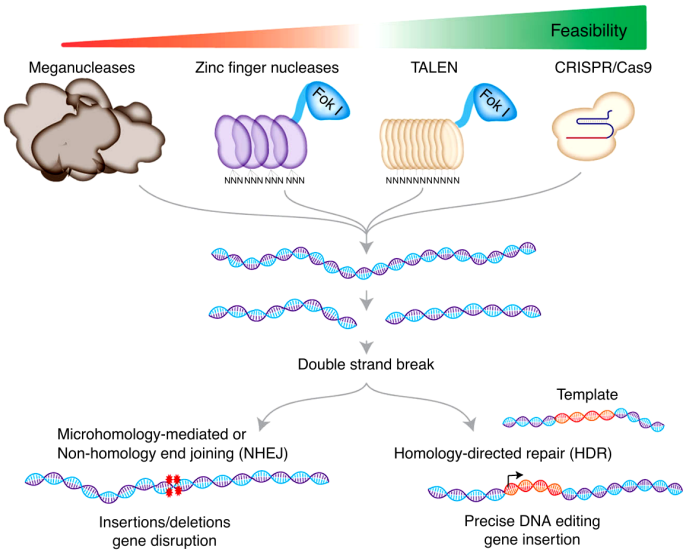

Figure 2: MEGANUCLEASE-ZFN-TALEN-CRISPR (Adli 2018).

This image shows the four most popular types of gene editing: meganucleases, zinc finger nucleases, TALENs, and CRISPR-Cas9. It shows the process the technique follows and the feasibility of use in gene editing.

Many are looking toward using CRISPR technology in treating genetic diseases because it is a powerful tool in altering genomes. CRISPR/Cas9 is an RNA-based system, so it can be efficiently modified compared to protein based approaches. It also provides the advantage of multiple sites ("Gene Editing – Digital Media Kit," NIH 2020).

Gene Editing in Genetic Disease Treatment:

Gene editing holds great promise for treating various genetic disorders, including Leber Congenital Amaurosis type 10 (LCA10), a rare inherited retinal disease that causes severe vision impairment or blindness from birth. Currently, there are no approved treatments, highlighting the considerable unmet medical need associated with LCA10 (Ruan et al. 2017). With access to precision tools like CRISPR-Cas9, scientists are exploring ways to correct the genetic mutations responsible for LCA10. EDIT-101 is a CRISPR/Cas9-based experimental medicine that works by deleting the IVS26 CEP290 mutant allele. EDIT-101 is administered via a subretinal injection to reach and deliver the gene editing machinery directly to photoreceptor cells. By deleting the mutant allele, normal CEP290 expression is restored. In preclinical data, researchers showed that subretinal delivery of EDIT-101 in a human CEP290 IVS26 knock-in mouse model resulted in fast and sustained CEP290 gene editing. They also demonstrated productive gene editing in a comparable surrogate nonhuman primate vector at levels meeting the target therapeutic threshold (Cohort 2022). Clinically, Editas Medicine reported that three of 14 patients (12 adults) with Leber congenital amaurosis 10 (LCA10) participating in the BRILLIANCE clinical trial for its EDIT-101 gene-editing therapy showed clinically meaningful improvements in best-corrected visual acuity (BCVA). Each of the three BCVA responders also demonstrated consistent improvements in two of the following three additional endpoints: a full field sensitivity test (FST), a visual function navigation course (VFN), or the visual function quality of life (VFQ) questionnaire. 3/14 patients is promising because it has been demonstrated that CRISPR-based gene editing can safely be administered to the retina and can result in clinically meaningful outcomes ("Editas Reports" 2023).

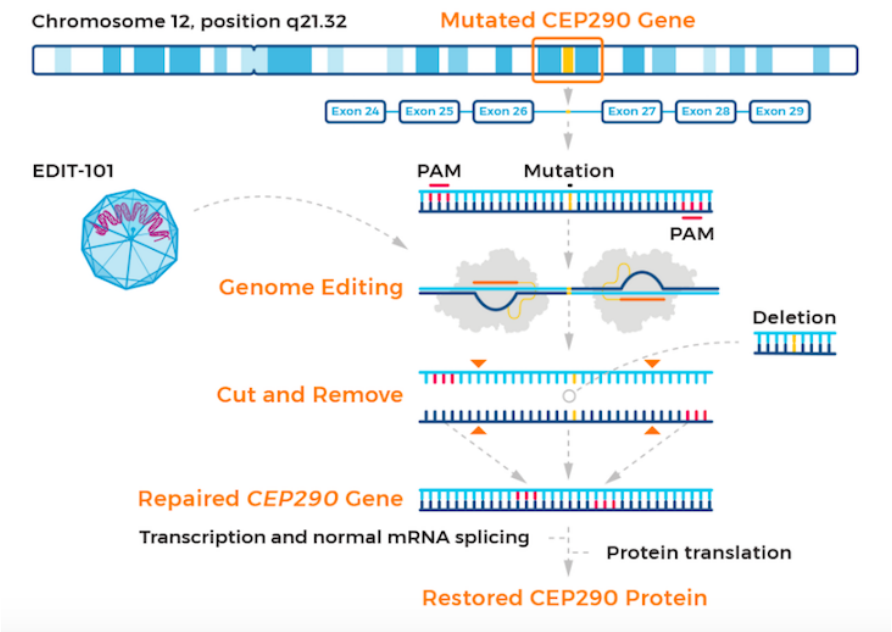

Figure 3: CRISPR-based gene-editing strategy behind EDIT-101 (EditasMedicine).

CRISPR gene editing techniques are also used in the treatment of SCD. Like mentioned earlier, SCD is caused by a genetic mutation in the HBB gene, which alters the type of hemoglobin in red blood cells. This changes red blood cells to become rigid and lack flexibility. This can block blood flow and lead to serious complications including pain and organ damage. SCD is a lifelong condition, so CRISPR gene editing holds promise in treating it. A CRISPR treatment would be performed once, whereas other treatments would be more time-consuming. Several gene editing strategies for curing SCD have shown promise in recent preclinical studies, including correction of the causative point mutation in HBB, the induction of fetal hemoglobin (HbF) via gene-disruption of γ-globin (HBG) repressors, and the induction of HbF via introducing beneficial hereditary persistence of fetal hemoglobin (HPFH) mutations on the β-globin locus (Demirci et al. 2021). BCL11A is the gene responsible for regulation of HbF levels, so HbF reactivation through BCL11A disruption is a viable option for SCD treatment. HbF inhibits sickle hemoglobin (HbS) polymerization and reduces disease symptoms. CRISPR-Cas9 editing can efficiently induce HbF by creating artificial HPFH mutations, editing transcriptional HbF silencers, and modulating epigenetic intermediates that govern HbF expression. Clinical trials investigating BCL11A enhancer editing in patients have demonstrated promising results (Demirci et al. 2021).

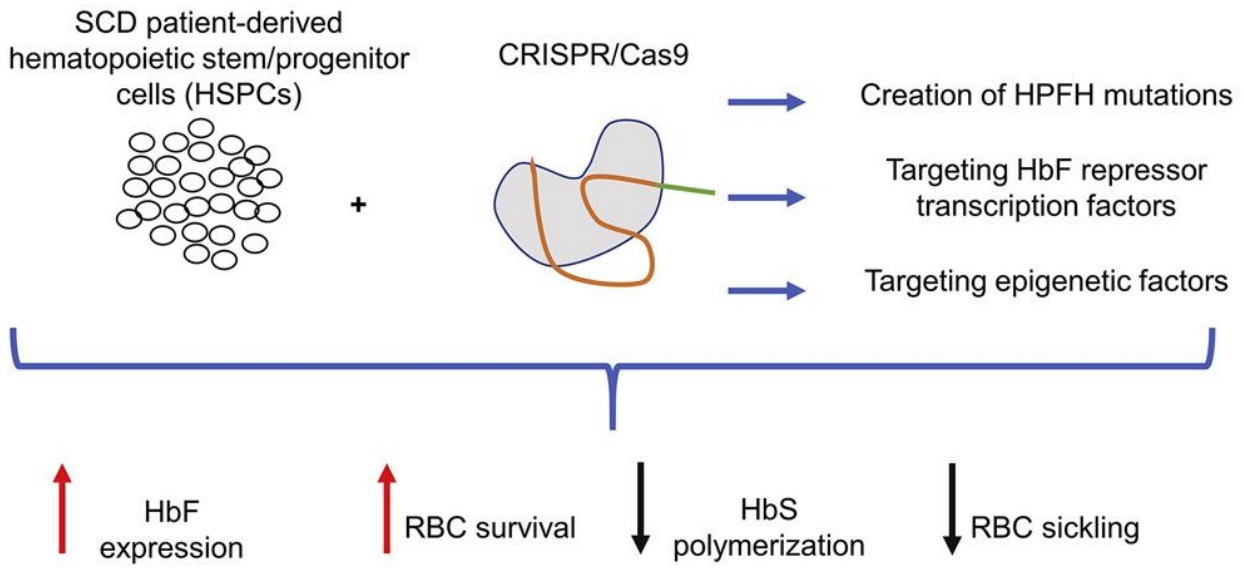

Figure 4: Graphical Abstract (Demirci, et al. 2021).

Ethical and Safety Considerations:

Genome editing introduces new challenges because its mechanism of action is different from gene therapy. Genome editing is permanent gene modification, whereas gene therapy is a one-time intravenous infusion. Due to the tolerance for nucleotide mismatches between target DNA and gRNA, the utility of CRISPR-Cas9 systems for genome editing may be compromised by their off-target activity. Off-target effects can cause disruption of normal gene function when Cas9 acts on non-targeted genomic sites and creates cleavages that may lead to adverse outcomes (Guo et al. 2023). Genomic instability or an increased likelihood of genome alteration during cell division can occur. As CRISPR/Cas9 moves forward in the clinic, patient monitoring methods need to be updated to maintain the safety of patients. CRISPR/Cas9 treatments will likely have some degree of off-target edits that will require careful monitoring over time to ensure that these events do not have a proliferative effect or cause any type of immune response. Patients should be aware of the risks genome editing holds.

Ethical concerns also arise when it comes to genome editing. Most of the changes from genome editing are completed in somatic cells (non-sex cells). These changes are isolated in certain tissues and are not passed on to the next generation. However, germline editing presents an ethical dilemma. Changes made to egg or sperm cells or to the genes of an embryo can be passed to future generations. Many people challenge this by highlighting the lack of consent. Others worry that this technology may be abused to enhance normal human traits like height. These so-called “Designer Babies” raise ethical questions about changing the genetic makeup of future generations.

With CRISPR cures within reach, patients have begun to wonder who will be able to access them. Because CRISPR technology is new, it will likely be expensive. The cost of the treatment will be a big barrier for getting treatments to underprivileged people and areas. A majority of SCD patients live in Africa. Money will be a bottleneck in new treatments reaching these areas (Molteni 2023). Many of these CRISPR therapies are also ex-vivo, meaning the edits occur outside the body. This procedure will be very involved and will require a bone marrow transplant. The US has many specialized centers where these transplants can take place. All of Africa only has three centers. Access to these procedures will be limited. Unfortunately, even though gene editing is possible and promising, it will likely still be inaccessible to the majority, especially in the beginning of its rollout (Molteni 2023).

The Future of Gene Editing:

The scientific community has almost unlocked the full potential of gene editing. Scientists can target and modify DNA with precision. In April of 2023, researchers made significant progress with correcting the mutation responsible for sickle cell disease. By effectively correcting the mutation, prime editing demonstrated remarkable efficacy in mice. A recent breakthrough has showcased the ability to eliminate antimicrobial resistance (AMR) genes from bacteria using CRISPR technology. This application of gene editing has the potential to revolutionize healthcare by addressing the threat posed by AMR or antimicrobial resistance which makes it more difficult to treat diseases (Fletcher 2023). Gene editing is not just limited to healthcare. It can be used to alter crop genomes and help conservation efforts to protect endangered species. Researchers from University of California, Davis, discovered that CRISPR could be used to create screens that allow for the identification of endangered fish (Fletcher 2023).

Conclusion:

Gene editing technologies have revolutionized the approach to genetic diseases, offering precision and hope for patients. The applications of gene editing like the CRISPR-Cas9 system have demonstrated impressive potential in case studies such as Leber Congenital Amaurosis type 10 and Sickle Cell Disease. However, ethical dilemmas and safety concerns must not be ignored. Balance between technological advancement and ethical responsibility is essential to ensure the proper use of gene editing, avoiding unintended consequences and ensuring the long-term safety of patients. As the scientific community continues its research on the uses of gene editing, it is imperative to uphold transparency and act ethically. Gene editing has the power to reshape healthcare, but its journey forward should be guided by the bioethical principles.

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