An Examination of Gene Editing and Gene Therapy in Genetic Diseases

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Abstract

The field of gene editing and therapy has helped the scientific community understand treatment options related to different genetic diseases such as ADA-SCID, Huntington's disease, and cystic fibrosis. Research has shown that CRISPR, a gene editing tool, has helped in treating genetic diseases that currently do not have conventional treatments available. Although being a greatly beneficial tool, CRISPR comes with its limitations such as leading to a possible risk of cancer through off-target edits. Gene therapy is a direct approach to treating genetic diseases, such as ADA-SCID, through either in-vivo or ex-vivo applications. This paper analyzes the application of gene editing and gene therapy for ADA-SCID, Huntington's disease, and cystic fibrosis, while also considering the ethical implications.

Introduction

Gene editing is a new type of technology that continues to evolve and produce breakthrough results. The study of genetics involves the various mutations and their effects on the genome. In the 1970s, the first targeted changes in the genome were done in yeast and mice, where a method of homologous recombination was used to change genes in the yeast and mice (Carroll, 2017). Homologous recombination is where nucleotide sequences are interchanged between two molecules of similar DNA during the process of meiosis (NIH, 2023). To carry out this process in a lab setting, the DNA fragments with similar sequences to the edited part of the genome need to be isolated (NIH, 2017). Chemicals are then used to inject the isolated fragments into single cells, which then replace the part of the genome targeted (NIH, 2017). Although the process of homologous recombination was precise, it was also highly inefficient (Carroll, 2017). This made gene targeting limited and inflexible to use on other species (Carroll, 2017). However, recent advances in genetic technology bring potential solutions to this problem. Technology for gene editing such as CRISPR-Cas9 and other nucleases can make a specific cut in the DNA, creating a double-stranded break using the Cas9 enzyme which is used in the lab to cut the strand of DNA at a particular site (Bio-Rad, 2023). As the technology of gene editing progresses, it is also important to consider the ethical implications of its use. Despite having limitations, gene editing is a beneficial tool to aid in treating genetic diseases for which traditional treatments are unavailable.

Gene Editing using CRISPR-Cas9

CRISPR-Cas9 is a new technology that allows for genes to be manipulated and edited. It is an example of gene editing which directly targets a gene that is present in a cell. Emmanuelle Charpentier and Jennifer Doudna were awarded the 2020 Nobel Prize in Chemistry for their work on and development of CRISPR-Cas9 genome editing (Westermann et al., 2020). The name CRISPR is the shortened form for “Clustered Regularly Interspaced Short Palindromic

Repeats," which are sequences in the genome of microscopic organisms, or microbes, used for protection against various viral attacks (Bio-Rad, 2023). As CRISPR-Cas9 works as a bacterial immune system, it is also used to disrupt a specific gene in cells through gene editing (MedlinePlus, 2022). Cas9, the most common enzyme used in labs, allows both DNA strands to be cut at a specific site (Bio-Rad, 2023). For the Cas9 enzyme to be directed in the right direction for editing, the guide RNA, which is a specific RNA sequence, recognizes the DNA region of interest and directs the Cas9 enzyme to that region for editing (Bio-Rad, 2023). In addition, the PAM (Protospacer Adjacent Motif) is required for the Cas9 enzyme to function, because Cas9 binds to the PAM which causes the DNA strands to separate, allowing the single guide RNA (sgRNA) to bind (Bio-Rad, 2023). The sgRNA is an RNA that is engineered to form a complex with the Cas9 enzyme (Bio-Rad, 2023). The Cas9 only cuts the DNA strands if the sgRNA is complementary to the specific sequence (Wu et al., 2014). A limitation of using CRISPR-Cas9 is the unexpected harmful mutations that could occur (Ayanoğlu et al., 2020). Despite the limitation, CRISPR-Cas9 targets specific DNA mutations and is cheaper, more precise, and more efficient (Westermann et al., 2020).

Gene Therapy

Gene therapy is a direct approach to treating various genetic conditions by inserting a healthy version of a defective gene in a patient's cells (Solomon, 2023). Although gene therapy is moderately new, it is considered a precise and targeted form of gene treatment (Chaney & Helmer, 2018). Since gene therapies need to be delivered to cells, a common example of a package that is used to reach the cells is a virus that is used as a vector to deliver the genetic material to cells (Chaney & Helmer, 2018). Gene therapy can be delivered in-vivo or ex-vivo. In-vivo gene therapy involves an injection of the packaged gene directly into a patient (Fliesler, 2020). Ex-vivo gene therapy involves the removal of stem cells from a patient, transduction of stem cells in a lab, and re-administration of treated cells back into the patient (Fliesler, 2020). There are limitations and risks when it comes to gene therapy. This includes dangerous immune responses that can occur from a triggered virus and cancer which comes from errors that could arise when the vectors insert the genetic material into a chromosome (MedlinePlus 2022).

Gene Therapy Case Study - ADA-SCID

Adenosine Deaminase Severe Combined Immunodeficiency (ADA-SCID) is an autosomal recessive disorder caused by mutations in the ADA gene (MedlinePlus 2013). This mutation leads to a weakened immune system and repeated infections which can be life-threatening (MedlinePlus 2013). A 4-year-old patient by the name of Ashanthi de Silva was born with ADA-SCID and was treated using gene therapy (Fliesler, 2020). First, Ashanthi was injected with synthetic ADA enzymes, but this only helped temporarily until doctors used a disabled virus that could not spread in the body to deliver a healthy ADA gene to Ashanthi's cells (Fliesler, 2020). After the gene therapy trials, Ashanthi showed no significant side effects, and the trial was pushed to other patients due to its success (Moritz et al., 2018).

Gene Therapy Usage in Huntington's Disease

Huntington's disease is an autosomal dominant mutation found in the Huntingtin gene (HTT) (Sharman, 2021). Those with the mutation have repeated sequences within the HTT gene many times than usual (MedlinePlus, 2020). Huntington's disease is rare, with approximately 3-7 people out of 10,000 people getting Huntington's disease (MedlinePlus, 2020). This brain disorder can cause loss of cognition and uncontrolled movement (MedlinePlus, 2020). Researchers created a model organism of a pig with Huntington's disease by inserting an elongated human CAG repeat into the pig HTT gene since pigs do not typically present with this disease (Yan et al., 2018). After developing this model, researchers could now test potential treatments. Researchers injected an adenoviral vector that contained CRISPR-Ca9 into the affected adult pigs which caused the repeated sequence to be replaced with a 20-CAG sequence (ALZForum, 2023). All of the injected pigs experienced a reduction in the amount of mutant HTT protein present (ALZForum, 2023). Researchers also saw that the injected pigs walked in a straight line and lived for a longer amount of time (ALZForum, 2023). The injected pigs that walked in a straight line showed improved coordination as compared to the untreated pigs, which is a description of Huntington's disease (MedlinePlus, 2020). However, researchers thought that CRISPR did not prove a high amount of therapeutic efficacy due to the off-target changes in the genome (ALZForum, 2023). The researchers working with CRISPR and Huntington's disease found that the gene editing had made a deletion or insertion in the huntingtin gene which disrupted the protein expression (AlZForum, 2023).

Potential For Gene Editing Treatment: Cystic Fibrosis

An example of a disease caused by genetic mutations is cystic fibrosis. Cystic fibrosis is an autosomal recessive genetic disease that affects the lungs and the digestive system (CDC, 2022). Cystic fibrosis affects how salt moves in and out of the cell, causing an increase in the production of mucus and digestive juices, which can then block airways and passageways (CDC, 2022). Every person inherits a gene for Cystic Fibrosis Transmembrane Conductance Regulator protein (CFTR) from each parent, so those who inherit two copies of the CFTR mutated gene will have cystic fibrosis (NIH, 2023). CFTR regulates salt in and out of the cell, so a mutated CFTR gene would cause a change in the protein that regulates salt in the cell (NIH, 2023). For cystic fibrosis disease to be inherited, the offspring must inherit a mutated CFTR gene from both parents. When a mutated CFTR gene and a normal CFTR gene are inherited, the offspring will be a carrier for cystic fibrosis disease (NIH, 2023). This means that to inherit the cystic fibrosis disease, both parents must pass down the mutated CFTR gene to the offspring. Scientists are still in the process of evaluating gene editing technology to fix the mutations of cystic fibrosis. Since cystic fibrosis contains mutations of both copies of the CFTR gene, the gene editing tool would need to find the exact location of the CFTR mutation out of three billion bases present in the human genome (CFF, 2023). In theory, CRISPR would be able to find the mutated sequence and cut the DNA at the targeted site (CFF, 2023). When the cut has been made, the DNA repair machinery of the cell will fix the cut, resulting in a corrected

genome (CFF, 2023). While gene editing is a precise and versatile editing tool, the risk of off-target edits is still present (CFF, 2023). Research on using gene editing to treat cystic fibrosis is ongoing, for it is still being tested on animals and would take many years to safely test on humans (CFF, 2023).

Ethical Considerations in Gene Therapy and Editing

When considering whether or not to pursue gene editing to treat devastating diseases like Huntington's disease, the ethical and safety implications of gene editing are important to consider. Firstly, injecting a virus that is used as a vector to deliver genetic material to the cell can set off a threatening immune response (MedlinePlus, 2022). An example of a tragic case involving an immune response to a viral vector is the death of Jesse Gelsinger, an 18-year-old, who went through gene therapy for a metabolic disorder (Fliesler, 2020). Secondly, using CRISPR comes with the risk of cancer through off-target editing (NCI, 2020). Off-target editing is where CRISPR cuts the DNA outside of the targeted gene, which causes unintended edits and could turn cells cancerous (NCI, 2020). Other ethical considerations to take into account are clear communication and informed consent when dealing with clinical trials for gene therapy. As seen in the case of Jesse Gelsinger, the fundamental ethical issue was that Gelsinger was told that former subjects who had received the adenovirus vector for ornithine transcarbamylase (OTC) did not suffer serious complications, but four days later, Gelsinger died due to a severe immune response (NYU, 2023). More investigation of this case showed that Gelsinger, other volunteers, and Gelsinger's family were not sufficiently informed of the negative reactions that other previous subjects and animals had to the adenovirus vector (NYU, 2023). This shows the different ethical considerations researchers must contemplate when dealing with clinical trials associated with gene therapy and editing.

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