An Overview of Gene Therapy and Editing and the Differences between Them

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Abstract

This review paper provides an overview of gene therapy and gene editing, highlighting their differences, methodologies, historical developments, and ethical considerations. Gene therapy involves the use of viral vectors to introduce therapeutic copies of genes, while gene editing utilizes tools like CRISPR-Cas9 to modify existing genes. This paper explores the history of these approaches and their potential for treating genetic diseases. Ethical considerations in gene therapy and gene editing, including informed consent, safety, equity, and societal implications, are discussed.

Introduction

In recent years, the field of genetic medicine has witnessed remarkable advancements in the treatment of genetic diseases. Two significant approaches, gene therapy, and gene editing, have emerged as promising strategies for addressing inherited disorders and have shown promising results in scientific studies. However, it is important to understand how gene therapy and gene editing differ from each other to appreciate their unique benefits and challenges.

Gene therapy involves the use of genetic material to treat or prevent specific diseases. Scientists use special carriers called viral vectors or other methods to introduce therapeutic genes into a person's cells. By doing this, they hope to compensate for faulty genes, or even add new genes to help the body function properly. Gene therapy has shown great potential in clinical trials for the treatment of rare diseases, immune deficiencies, and some types of cancer (Lundstrom, 2018).

On the other hand, gene editing is a technique that allows scientists to make precise changes to a person's existing genetic material. Using tools like CRISPR-Cas9, scientists can target specific parts of the DNA and modify them with incredible accuracy. This technology holds immense promise for correcting disease-causing mutations directly within the genes themselves. It could potentially provide cures for inherited disorders and even make people more resistant to certain infections (Redman et al., 2016).

While both gene therapy and gene editing have the common goal of treating diseases by utilizing genetics, they have important differences. In this literature review, I aim to provide a basic overview of the differences between gene therapy and gene editing, as well as a brief history of each approach.

How Gene Therapy Works

Gene therapy is an innovative approach that holds great promise for the treatment of various diseases. One of the key components of gene therapy is the use of viral vectors to deliver therapeutic genes into target cells. These viral vectors are modified versions of natural viruses that scientists have sought to engineer to be safe and effective in order to deliver genetic material without causing disease. Viral vectors are capable of entering cells and transferring genetic material into the cell's nucleus (Ghosh et al., 2020). Unlike natural viruses, viral vectors

used in gene therapy have been modified to remove genes involved in replication, making them unable to cause disease (Ghosh et al., 2020). Instead, these genes are replaced with therapeutic genes that are intended to treat specific diseases.




	ADENOVIRUS	AAV	γ-RETROVIRUS	LENTIVIRUS
SIZE	~90-100 nm	~25 nm	~80-100 nm	~80-100 nm
GENOME	dsDNA	ssDNA	ssRNA	ssRNA
PACKAGING CAPACITY	~8 kb – 36 kb	~4.7 kb	10 kb	8 kb
TRANSDUCTION	Dividing and non-dividing cells	Dividing and non-dividing cells	Dividing cells	Dividing and non-dividing cells
TRANSDUCTION EFFICIENCY	High	Moderate	Moderate	Moderate
INTEGRATION	Non-integrating	Non-integrating	Integrating	Integrating
EXPRESSION	Transient	Transient or stable	Stable	Stable
BIOSAFETY LEVEL	BSL-2	BSL-1	BSL-2	BSL-2
IMMUNOGENICITY	High	Low	Moderate-High	Moderate-High
GENE THERAPY STRATEGY	*In vivo*	*In vivo*	*Ex vivo*	*Ex vivo*

Figure 1: Different types of Viral Vectors (*Viral Vector and Gene Therapy Basics Summarized*, 2021: online)

One type of viral vector is that of Adenovirus vectors, derived from Adenoviruses, which are known to cause respiratory tract infections in humans (McConnell & Imperiale, 2004). These vectors are created by removing the genes responsible for viral replication and replacing them with therapeutic genes. Adenovirus vectors have a high packaging capacity of approximately 8 kb, allowing them to accommodate larger therapeutic genes (McConnell & Imperiale, 2004).

However, one limitation of Adenovirus vectors is the pre-existing immunity in patients, as many individuals have been exposed to Adenoviruses before. To illustrate, adenoviruses are a group of viruses that cause respiratory illnesses, such as the common cold, bronchitis, pneumonia, conjunctivitis, and etc (Shieh, 2022). This pre-existing immunity can recognize and destroy Adenovirus vectors, limiting their effectiveness (McConnell & Imperiale, 2004). Strategies such as using non-human Adenovirus vectors, derived from non-human subjects like dogs or monkeys, can help overcome this limitation (Thacker et al., 2009).

AAV vectors are derived from Adeno-Associated Viruses, which are non-pathogenic and prompt a mild immune response in humans. AAV vectors are attractive for gene therapy due to their high safety profile, low immunogenicity, and episomal nature, meaning they do not integrate into the host genome (American Society for Microbiology, n.d.).

However, AAV vectors have a limited packaging capacity of about 4.7 kb, which can be challenging when targeting larger genes or specific diseases (McClements, 2017). Research is ongoing to develop strategies to overcome this size limitation, such as dual vectors, which use two separate vectors to introduce different genetic components, or minigenes, compact versions of genes that retain essential functional elements (Croze et al., 2020).

y-Retrovirus vectors and Lentivirus vectors are both integrating viral vectors. They can fuse a fragment of their genetic material into the host cell genome, resulting in stable and long-term expression of the transgene (Dufait et al., 2011). y-retrovirus vectors have been used in ex vivo gene therapy of transplantable stem cells, while lentivirus vectors have been used in CAR-T therapies and for treating certain types of blood cancers (Lana & Strauss, 2019). The use of integrating vectors raises safety concerns, such as the potential activation of oncogenesis in some applications due to disruption of normal gene regulation (Schlimgen et al., 2016).

History of Gene Therapy

Gene therapy, as a field, traces its roots back to the 1960s when the potential of manipulating genetic material for therapeutic purposes first captured the imagination of scientists (Tamura & Toda, 2019). However, it wasn't until the 1990s that the first successful clinical trials of gene therapy were conducted, marking a significant milestone in the history of this revolutionary approach (Tamura & Toda, 2019).

The first gene therapy trial took place in 1990 when a four-year-old girl with a rare genetic disorder called adenosine deaminase (ADA) deficiency became the first patient to receive gene therapy (Tamura & Toda, 2019). Scientists aimed to restore the missing ADA enzyme by introducing the ADA gene into her cells using a retroviral vector. While the initial results were promising, with a temporary improvement in the girl's immune function, the effects were not long-lasting (Tamura & Toda, 2019).

Despite the initial setbacks, the field of gene therapy continued to evolve and gain momentum. In the mid-1990s, significant progress was made in developing more efficient gene delivery methods and enhancing the safety of the techniques (Tamura & Toda, 2019). Advances in viral vectors, such as adenoviruses and lentiviruses, allowed for better targeting and delivery of therapeutic genes into the cells (Ghosh et al., 2020).

However, gene therapy also faced significant challenges along the way. In 1999, a tragic incident occurred during a clinical trial for the treatment of a rare genetic disorder called ornithine transcarbamylase (OTC) deficiency. A participant, Jesse Gelsinger, experienced a severe immune response to the viral vector used in the treatment, leading to his tragic death (Marshall, 1999). This event highlighted the importance of rigorous safety measures and sparked a reassessment of the ethical and regulatory considerations surrounding gene therapy research. Since then, the field of gene therapy has made considerable strides, with numerous ongoing clinical trials and a growing understanding of its potential applications. Researchers have successfully employed gene therapy to address a wide range of genetic disorders, including hemophilia, certain types of inherited blindness, and inherited immune deficiencies (Kumar et al., 2015).

Ethics in Gene Therapy

Gene therapy, a revolutionary field of medicine, brings hope for treating genetic diseases and improving patient outcomes. However, with its immense potential, gene therapy also presents ethical considerations that must be carefully addressed to ensure responsible and equitable practices.

One critical ethical concern revolves around informed consent. Patients must have a comprehensive understanding of the potential risks, benefits, and limitations of gene therapy to

make autonomous decisions about their treatment. As gene therapy involves introducing genetic material into a patient's cells, safety and efficacy are paramount. Rigorous preclinical studies and well-designed clinical trials are necessary to establish the therapy's safety profile. Furthermore, ongoing monitoring and long-term follow-up are essential to identify and address any adverse effects that may arise.

Equity and access to gene therapy are also pressing ethical issues. Efforts should be made to prevent disparities in access based on socioeconomic status, geography, or insurance coverage. Ensuring affordability and accessibility for all who could benefit from gene therapy aligns with principles of justice and fairness (Santa Clara University, n.d.).

How Gene Editing Works

CRISPR-Cas9, short for Clustered Regularly Interspaced Short Palindromic Repeats and CRISPR-associated protein 9, is a groundbreaking gene-editing tool derived from the bacterial immune system (Ma et al., 2014). It provides researchers with an astonishingly efficient and precise method to edit genes in various organisms, including humans. This revolutionary technology allows scientists to target specific DNA sequences, introduce modifications, or replace faulty genes accurately (Ma et al., 2014).

The CRISPR-Cas9 system consists of two main components: the Cas9 enzyme and a guide RNA (gRNA). The Cas9 enzyme acts as a pair of molecular scissors, capable of cutting the DNA strands at a specific location. The gRNA is a short RNA sequence that is designed to bind to a specific target DNA sequence. Scientists design a gRNA that matches the target DNA sequence they want to modify and attach it to the Cas9 enzyme and then this complex is introduced to the target cells. The Cas9-gRNA complex searches the genome for a matching sequence and when found it creates a double-strand break (Ma et al., 2014). Once broken, scientists can then exploit and introduce changes in the DNA. This allows scientists to insert, delete, or replace specific DNA sequences, effectively modifying the gene (Ma et al., 2014).

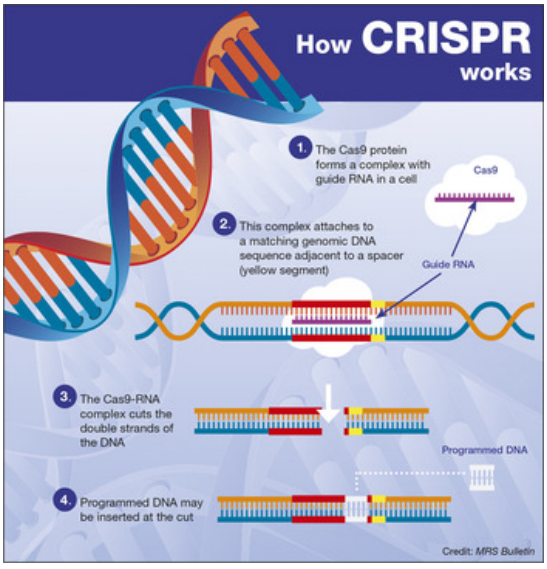

Figure 2: How CRIPSR Works (MRS Bulletin, November 2016:online)

History of Gene Editing

The history of gene editing is a relatively more recent but rapidly advancing chapter in the fascinating realm of genetic medicine. Unlike gene therapy, which primarily focuses on introducing or replacing genes, gene editing aims to precisely alter existing genes within the DNA, offering a level of precision and versatility previously unimaginable.

The foundations of gene editing were laid in the 1970s when scientists discovered restriction enzymes, also known as molecular scissors (Maguin & Marraffini, 2021). These enzymes could cut DNA at specific sites, allowing researchers to manipulate genes in a controlled manner. However, the true revolution in gene editing began in 2012 with the discovery of CRISPR-Cas9 (Ma et al., 2014).

The history of gene editing has witnessed remarkable breakthroughs in recent years, showcasing its potential to revolutionize medical treatments. One prominent example is the case of Victoria Gray, a 37-year-old woman who was born with sickle cell disease, a group of inherited blood cell disorders that affect hemoglobin, turning round flexible blood cells into stiff “sickle”-shaped discs. While normal blood cells are round and flexible, being able to move freely throughout the bloodstream, the sickle-shaped blood cells caused by sickle cell disease are not able to move as easily and can block blood flow from the rest of the body (*What Is Sickle Cell Disease? | NHLBI, NIH*, 2022). The blocked blood flow can thus lead to serious problems, including strokes, eye problems, infections, and episodes of pain (*What Is Sickle Cell Disease? | NHLBI, NIH*, 2022).

As a victim of the disorder, Gray decided to take her life back by becoming the first person to undergo experimental gene editing therapy using the CRISPR system (Frangoul & Davies, 2023). The procedure involved extracting her blood stem cells, genetically modifying them using CRISPR to compensate for the sickle cell mutation, and then reintroducing them into her body (Frangoul & Davies, 2023). The results were astounding: Gray experienced a transformation, producing fewer abnormally shaped red blood cells that cause intense pain and complications associated with sickle cell disease; although, she was left with 20% of her hemoglobin being fetal hemoglobin (Stein, 2020).

Gray's success story highlights the rapid progress of gene editing therapies, where changes are made to a person's DNA that are not heritable. Clinical trials are underway to evaluate the efficacy of CRISPR and related methods in treating various conditions, including blood disorders, cancers, diabetes, and blindness (Rafii et al., 2022). The CRISPR technique used in Gray's therapy has already been tested in more than 75 individuals and could receive approval for use in the United States in the near future (*Gene-editing Summit Touts Sickle Cell Success, While Questions on Embryo Editing Linger*, 2023). However, further research and deliberation are necessary to ensure responsible and informed decisions about the application of gene editing technologies.

Ethics in Gene Editing

Gene editing technologies, exemplified by CRISPR-Cas9, have brought unprecedented capabilities to modify the human genome. Alongside these advancements, crucial ethical considerations arise.

One significant concern is germline editing, which involves making heritable changes to the genome, potentially impacting future generations. Ethical deliberations are necessary to address the risks, unintended consequences, and responsible use of germline editing. Consent and

voluntary participation are critical in gene editing as well. Individuals must be fully informed about the potential implications, risks, and benefits of gene editing interventions. Participation should be voluntary, free from coercion or undue influence.

The precision of gene editing techniques notwithstanding, the risk of off-target effects and unintended changes to the genome remains. Ethical considerations mandate rigorous testing, transparency, and ongoing monitoring to minimize risks and ensure the safety of individuals undergoing gene editing procedures.

Moreover, gene editing raises broader societal and ethical implications. These include concerns about creating genetic disparities, exacerbating existing inequalities, and the potential for designer babies or the commodification of human traits. Ethical discussions and public engagement play a pivotal role in exploring these implications, establishing appropriate guidelines, and implementing regulations that balance scientific progress with societal values.

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