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Gene Therapy: The Future Of Medicine

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Abstract :

Genetic diseases present a major threat to human health and have long been difficult to tackle. Fortunately, there are promising gene therapy strategies, such as siRNA, shRNA, antisense oligonucleotides, CRISPR/Cas9, plasmid DNA, and miRNA, which hold significant potential in biomedical applications. However, to maximize the effectiveness of these therapies, it is essential to create advanced drug delivery systems that can protect these therapeutic agents from degradation in the body and accurately target specific tissues, cells, and even organelles. In conclusion, developing efficient drug delivery vehicles is vital for progressing gene therapy and addressing genetic disorders.

As one of the most significant topics of the 21st century, gene therapy offers the promise of cures for many diseases, raises ethical debates about altering human traits, and introduces a form of medical treatment that many once deemed unimaginable. However, conducting human gene therapy comes with various concerns. This review article explores the background of gene therapy, including its introduction, the nature of genetic diseases, gene function, germline gene therapy, the challenges faced, and the various approaches employed. It covers ex vivo, in vitro, and in vivo gene therapies, along with the associated risks. The review addresses a wide range of topics, from the historical context and societal relevance of gene therapy to the latest advancements in the field, including research and developments in India.

Keywords: Gene therapy, CRISPR/Cas9, drug delivery systems, genetic disorders, ex vivo gene therapy.

1.Introduction :

Human genetic have long explored gene transfer as a potential treatment for inherited diseases. Recent advancements in recombinant DNA technology and cell biology have significantly increased the feasibility of this once far-fetched concept. Techniques such as CRISPR and viral vectors are now being developed to deliver therapeutic genes directly to patients' cells, offering hope for conditions previously deemed untreatable. Moreover, the scope of gene therapy is expanding beyond single-gene disorders to include a wider range of medical applications, such as cancer treatment, autoimmune diseases, and even regenerative medicine. As research continues and clinical trials progress, gene therapy holds the promise of transforming modern medicine, providing innovative solutions to some of the most challenging health issues we face today.[1]

Gene therapy represents a groundbreaking approach in medicine, offering potential treatments for a range of genetic disorders. The European Medicines Agency (EMA) defines a gene therapy medicinal product as a biological product that satisfies two primary criteria. First, it must contain an active substance composed of recombinant nucleic acid, which is intended for use in humans to regulate, repair, replace, add, or remove genetic sequences. Second, the therapeutic, preventive, or diagnostic effects of the product must be directly associated with the recombinant nucleic acid sequence or its resultant genetic expression. Notably, gene therapy medicinal products do not encompass vaccines designed to combat infectious diseases.[1]

Similarly, the U.S. Food and Drug Administration (FDA) provides a comprehensive definition of gene therapy, describing it as products that achieve their effects through the transcription and/or translation of transferred genetic material and/or by integrating this material into the host genome. These products can be delivered in various forms, including nucleic acids, viruses, or genetically engineered microorganisms. They may also modify cells in vivo or be transferred ex vivo before administration to the recipient. This versatility allows for targeted approaches in treating a variety of conditions, highlighting the innovative potential of gene therapy.[2]

Gene therapy can generally be classified into two main categories: germ line gene therapy and somatic gene therapy. The primary distinction between these two approaches lies in their inheritance patterns. Somatic gene therapy involves the insertion of genetic material into specific target cells, with the crucial aspect being that any changes made do not pass on to future generations. In contrast, germ line gene therapy permits the modified or therapeutic gene to be inherited by subsequent generations. This difference is of great significance, particularly regarding ethical considerations and regulatory frameworks, as current legislation primarily allows gene therapy to be performed only on somatic cells.[2]

As the field of gene therapy continues to evolve, understanding these definitions and classifications is essential. The potential to treat previously untreatable genetic disorders and improve patient outcomes is immense. Figure 1 in related literature illustrates some of the key milestones in the history of gene therapy, marking significant advancements and achievements that have paved the way for current and future innovations in this exciting area of medicine. The ongoing research and development in gene therapy hold promise for transforming the landscape of medical treatment and genetic disease management.[2]

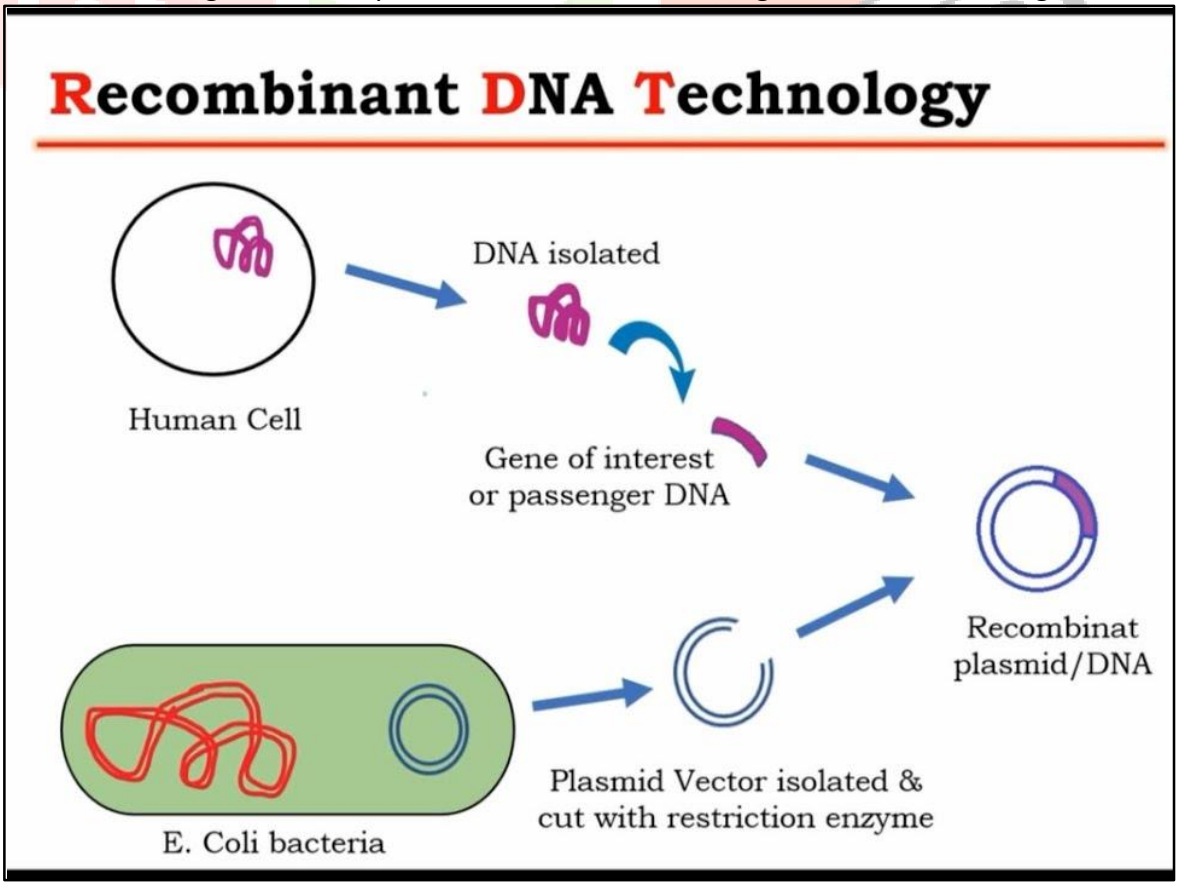


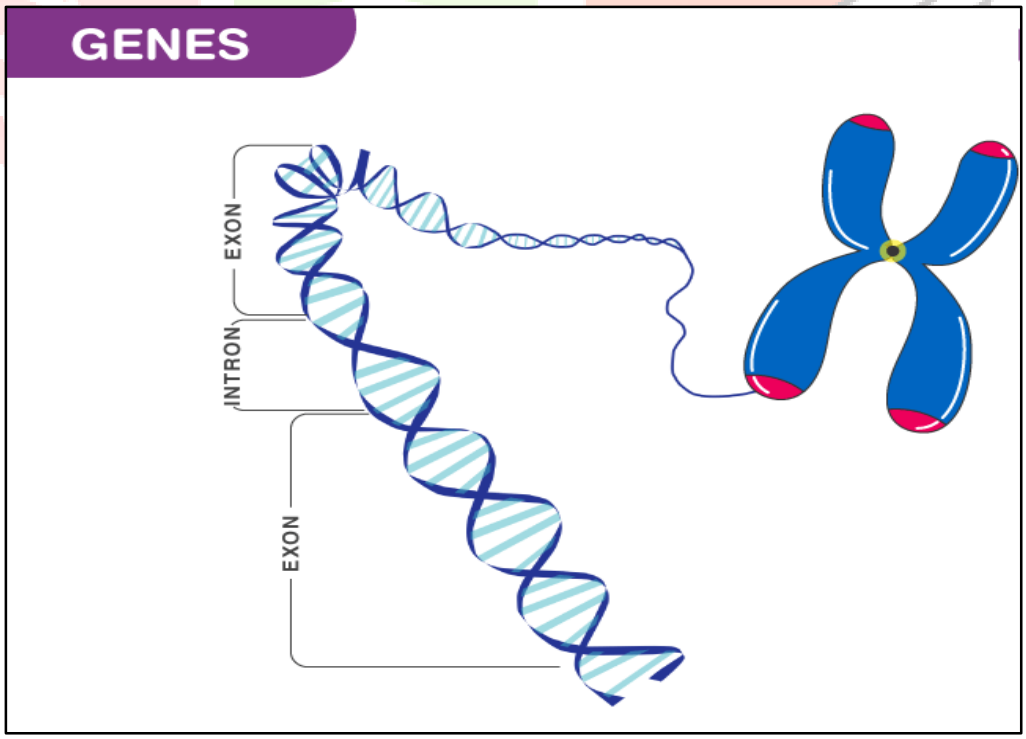
Fig (1): Recombinant DNA Technology [3]

2. What Are Gene?

Genes are the essential physical and functional units of heredity, consisting of ordered nucleotide sequences found at specific locations on chromosomes. They encode functional products, such as proteins or RNA molecules, and are often referred to as "biological units of heredity." Inherited from parents, genes determine unique traits, including eye color, hair color and texture, and even characteristics like sex, blood oxygen capacity, and IQ.[4]

Genes are made up of long strands of DNA, organized linearly within chromosomes. The genetic information is encoded in subunits known as nucleotides. Human cells contain approximately three billion pairs of nucleotides in their chromosomes, and the unique sequence of these nucleotides in each individual is what contributes to our distinct genetic identity.

Scientists estimate that each human cell contains approximately 30,000 genes. A mutation or flaw in any of these genes can lead to diseases, physical disabilities, or a reduced lifespan. These mutations can be inherited from one generation to the next, similar to how traits like a mother's blonde hair or a father's brown eyes are passed down. However, with advancements in gene therapy, there is potential for treating or even eliminating inherited diseases and physical conditions caused by these mutations.[5]



Fig(2):Genes [6]

1. Gene Therapy

Gene therapy is an experimental approach aimed at treating or preventing diseases by replacing, altering, or adding healthy genes to address nonfunctional or malfunctioning ones. Genes are specific sequences of nucleotides that encode instructions for synthesizing proteins, which carry out essential functions in living organisms and contribute to cellular structures. While genes are often the focus of medical research, it is ultimately the proteins they produce that are critical for maintaining life processes.

When genes are modified in ways that impair the function of the resulting proteins, genetic disorders can develop. These disorders can lead to a variety of health issues, ranging from mild to severe. Gene therapy seeks to correct these underlying genetic problems by introducing functional genes into cells, thereby restoring the normal production of proteins. This innovative approach has the potential to provide long-term solutions for genetic conditions, offering hope for patients who currently have limited treatment options. As research in this field advances, gene therapy holds promise for transforming the landscape of medicine and improving the quality of life for individuals affected by genetic disorders.[7]

2.Gene therapy history and future

On September 14, 1990, a girl received treatment at the Clinical Center of the National Institutes of Health in Bethesda, Maryland. Dr. W. French Anderson and his team conducted the procedure, which involved extracting white blood cells from the patient. They then implanted genes responsible for producing adenosine deaminase (ADA) into these cells before reintroducing them into her body. This innovative gene therapy resulted in significant improvements in her immune system. Following this groundbreaking treatment, research into gene therapy expanded to address a variety of diseases. Notably, patients suffering from skin cancer, including melanoma, also began receiving gene therapy as part of clinical trials. These efforts marked a pivotal moment in medical research, showcasing the potential of gene therapy to treat genetic disorders and cancers by correcting underlying issues at the cellular level. The success of the girl's treatment provided hope for many others, leading to further investigations into the efficacy of gene therapy in different medical conditions. As research progressed, the focus remained on developing targeted therapies that could improve patient outcomes and pave the way for more advanced treatments in the future.[8]

3.Principle of gene therapy

An abnormal gene can be replaced with a normal one using homologous recombination. Alternatively, selective reverse mutation can repair the abnormal gene, restoring its normal function. Additionally, the regulation of a specific gene—the extent to which it is activated or deactivated—can also be modified. This ability to alter genes and their regulation opens new possibilities for addressing genetic disorders and enhancing gene function.[8]

3.Vectors in gene therapy

Some of the different types of viruses used as gene therapy vectors:

1.Retroviruses-A group of viruses capable of producing double-stranded DNA versions of their RNA genomes. These DNA copies can be integrated into the chromosomes of host cells. An example of a retrovirus is the human immunodeficiency virus (HIV).

One issue with using retroviruses for gene therapy is that the integrase enzyme can randomly insert the viral genetic material anywhere in the host's genome. If this insertion occurs within one of the host's existing genes, it can disrupt that gene, a phenomenon known as insertional mutagenesis. If the disrupted gene regulates cell division, this could lead to uncontrolled cell growth, or cancer. Recently, this problem has been addressed by using zinc finger nucleases or by incorporating specific sequences, like the beta-globin locus control region, to guide the integration to particular chromosomal locations.[9]

2.Adenoviruses-A group of viruses with double-stranded DNA genomes that can lead to respiratory, intestinal, and eye infections in humans. An example of an adenovirus is the virus responsible for the common cold.

3.Adeno -associated viruses-A group of small, single-stranded DNA viruses that can integrate their genetic material at a specific location on chromosome 19.

4.Cis and trans-acting elements-Replication-defective vectors always include a "transfer construct" that contains the gene to be delivered, known as the transgene. This transfer construct also includes sequences essential for the proper functioning of the viral genome, such as packaging sequences, replication repeats, and, when necessary, elements for priming reverse transcription. These components are referred to as cis-acting elements because they must be present on the same DNA molecule as both the viral genome and the gene of interest.[10]

5.Herpes simplex viruses-A group of double-stranded DNA viruses that specifically target neurons. Herpes simplex virus type 1 is a well-known human pathogen responsible for cold sores.[11]

4.Approaches of gene therapy

1.Gene modification encompasses various techniques, including replacement therapy and corrective gene therapy. Replacement therapy aims to substitute defective genes with functional ones, while corrective gene therapy focuses on fixing the underlying genetic defects.

2.Gene transfer methods can be categorized into three main types: physical, chemical, and biological. Physical methods often involve direct delivery techniques, chemical methods use compounds to facilitate gene uptake, and biological methods utilize vectors, such as viruses, to transport genetic material into cells.

3.When it comes to gene transfer in specific cell lines, there are two primary approaches: somatic gene therapy, which targets non-reproductive cells, and germ line gene therapy, which affects reproductive cells and can be passed on to future generations.

4.Additionally, the eugenic approach involves gene insertion to enhance certain traits. Other forms of genetic engineering include gene targeting and the knockout of specific genes using engineered nucleases like zinc finger nucleases, I-CreI homing endonucleases, and nucleases derived from TAL effectors. These advanced techniques are currently being explored in several human clinical trials, aiming to develop effective treatments for various genetic disorders.[12]

5.Advantages of gene therapy

1.In the case of HIV that hasn't progressed to AIDS, gene therapy could potentially "silence" the virus, preventing the onset of the disease and alleviating suffering.

2.Gene therapy holds promise for eradicating hereditary conditions like cystic fibrosis and may offer cures for heart disease, AIDS, and cancer.

3. Skeptics would likely opt for gene therapy, particularly if it represented their last chance for recovery or for a loved one, as seen with many patients seeking this treatment.[8]

6. Disadvantages of Gene Therapy

1.The temporary effects of gene therapy are a significant limitation.

2.An immune response may occur when genes delivered via a virus trigger the body's defenses against the virus. Additionally, there are concerns that viral vectors could regain their disease-causing abilities after entering the patient.

3.For disorders involving multiple genes, the genetic material may not be delivered to the correct cells or positioned accurately within the cell's DNA.[8]

7.Physical Methods to Enhance Delivery

1.Electroporation-Electroporation is a technique that employs brief bursts of high voltage to facilitate the entry of DNA into cells by creating temporary pores in the cell membrane. This method is effective and applicable to various cell types. However, the high rate of cell death that often follows electroporation has restricted its use, particularly in clinical settings.

2.Gene Gun-Particle bombardment, also known as the gene gun, is another physical method for introducing DNA into cells. In this technique, DNA is coated with gold particles and propelled into the cells using a force generated by a device. However, if the DNA integrates incorrectly within the genome—such as inserting into a tumor suppressor gene—it could potentially trigger tumor formation. This issue was observed in clinical trials for patients with X-linked severe combined immunodeficiency (X-SCID), where hematopoietic stem cells were modified with a corrective transgene via a retrovirus, resulting in T cell leukemia in 3 out of 20 patients.

3.Sonoporation-Sonoporation employs ultrasonic frequencies to introduce DNA into cells. The mechanism involves acoustic cavitation, which disrupts the cell membrane and facilitates the entry of DNA into the cells.

4.Magnetofection-In a technique known as magnetofection, DNA is attached to magnetic particles, and a magnet is positioned beneath the tissue culture dish to draw the DNA complexes into proximity with a layer of cells.[13]

8.Chemical Methods to Enhance Delivery

1.Oligonucleotides-Synthetic oligonucleotides are employed in gene therapy to inactivate genes linked to disease processes, using various approaches. One strategy involves antisense sequences designed to target the faulty gene, thereby hindering its transcription. Another approach utilizes small RNA molecules, known as siRNA, which instruct the cell to cut specific sequences in the mRNA of the defective gene, disrupting its translation and subsequently preventing the expression of that gene**.**

2.Lipoplexes and polyplexes-To enhance the delivery of new DNA into cells, it is essential to safeguard the DNA from damage and ensure it carries a positive charge. Initially, anionic and neutral lipids were utilized to create lipoplexes for synthetic vectors.

3.Dendrimers-A dendrimer is a highly branched macromolecule with a spherical structure. Its surface can be modified in various ways, influencing many of the properties of the resulting construct. Notably, a cationic dendrimer can be created, which has a positive surface charge. When exposed to genetic material like DNA or RNA, the complementary charges allow for a temporary association between the nucleic acid and the cationic dendrimer. Upon reaching its target, this dendrimer-nucleic acid complex is taken up by the cell through endocytosis.

4.Hybrid methods-Because each gene transfer method has its limitations, hybrid approaches have been developed that integrate two or more techniques. One such example is virosomes, which combine liposomes with inactivated HIV or influenza viruses. Research indicates that virosomes facilitate more effective gene transfer in respiratory epithelial cells compared to using either viral or liposomal methods alone. Additionally, other strategies include mixing different viral vectors with cationic lipids or creating hybrid viruses.[14]

9.Application of gene therapy

1. In case of Parkinson's diseases

Recent reports from The Independent and other outlets highlight promising advancements in gene therapy for Parkinson's disease. This innovative approach aims to enhance levels of GABA, a brain chemical that is deficient in individuals with Parkinson's. In a small clinical trial involving 45 participants with advanced disease, researchers implanted tubes in their brains targeting movement-related areas. Half of the participants received injections of a virus designed to boost GABA production, while the other half received a harmless saline solution. After six months, those treated with gene therapy experienced a 23% improvement in movement, compared to just half of that in the control group.

This trial served as a "proof of concept," assessing whether gene therapy could alleviate some symptoms of advanced Parkinson's. Specifically, it involved introducing a gene for glutamic acid decarboxylase (GAD) into the basal ganglia, which play a crucial role in movement control. In Parkinson's patients, GABA levels are often reduced in certain areas of the basal ganglia. The study employed a double-blind design, ensuring that neither the participants nor the researchers knew who received the gene therapy versus the sham treatment.

One patient, Sharon Jokela from San Francisco, shared her life-changing experience after participating in the trial. She described how the treatment stopped her severe tremors, allowing her to return to her active lifestyle. Previously, her shaking had severely limited her daily activities, making even simple tasks like eating or driving difficult. After finding little relief from medications, Sharon volunteered for the clinical trial at Stanford University, ultimately regaining a sense of normalcy and hope for the future.

2. In case of Alzheimer's disease

Scientists have made significant strides in Alzheimer's research by successfully deactivating a gene believed to contribute to the disease, as reported by the Daily Mirror. They utilized tiny particles known as exosomes, which are naturally released by cells, to deliver drugs directly into the brains of mice. This study highlights the potential of exosomes to transport gene therapies targeting specific genes, such as BACE1, which produces a protein linked to Alzheimer's.

These findings open new avenues for research and are particularly exciting for the scientific community. Exosomes show promise in delivering targeted therapeutic agents to brain cells, indicating various possible applications. However, it's important to note that this is preliminary research, and the technology has yet to be tested in humans. Additionally, there are numerous technical and ethical considerations surrounding gene therapy for human applications.

Many neurological diseases involve cell degeneration and loss, making neurotrophic factors—proteins that support cell growth and have neuroprotective properties—ideal candidates for gene therapy. In 1988, nerve growth factor (NGF) was the first therapeutic molecule delivered via ex vivo gene therapy in an animal model of a neurological disorder, and clinical trials for NGF are currently underway in Alzheimer's disease.

Another neurotrophic factor, glial cell-derived neurotrophic factor (GDNF), has gained attention for its neuroprotective effects on certain dopaminergic neurons in the midbrain, which degenerate in Parkinson's disease. Studies have shown that administering GDNF into the brain can eliminate behavioral symptoms of Parkinson's and prevent the degeneration of critical neuronal pathways. The authors of this research suggest that gene therapy may be more effective, as their administration method resulted in localized delivery, potentially preserving other areas from degeneration.

Additionally, a lentiviral vector system based on equine infectious anemia virus (EIAV) has been developed, effectively transfecting brain and spinal cord cells. This method has shown success in delivering GDNF in animal models of Parkinson's disease.

Research on pain management has focused on herpes simplex virus (HSV) vectors, which can be applied peripherally and transported to the dorsal root ganglia. A study using HSV-mediated GDNF administration successfully alleviated pain and induced neurochemical changes. While this delivery method is minimally invasive—allowing for transfection through a simple skin scratch—it also has several drawbacks.

1) Viral toxicity and immune reactions are concerns; however, advancements in viral development techniques allow for the removal of the toxic and immune-triggering components of the virus.

2) The virus can replicate within the transfected cells, but this leads to a relatively brief duration of expression.
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Virally mediated spinal administration of various substances has shown success in some pain models. Eaton et al. have reported the neuroprotective effects of AAV-BDNF administration. However, GDNF has not yet been tested through intraspinal administration in models of peripheral neuropathy. So far, only a few studies have examined the intraspinal injection of GDNF-expressing viral vectors (such as Lv and AAV) in models of ALS and ventral root avulsion.

4.In case of cyctic fibrosis

In therapy, the focus is on addressing the underlying causes of cystic fibrosis rather than merely alleviating its symptoms. While initial gene therapy efforts have centered on lung cells, researchers are optimistic that these techniques can be adapted to target other organs affected by cystic fibrosis.

- Overview of Cystic Fibrosis
- Causes of Cystic Fibrosis
- Cystic Fibrosis Gene
- Early Symptoms of Cystic Fibrosis
- General Symptoms of Cystic Fibrosis
- Diagnosis of Cystic Fibrosis
- Sweat Test for Cystic Fibrosis
- Prenatal Testing for Cystic Fibrosis
- Genetic Testing for Cystic Fibrosis
- Treatment Options for Cystic Fibrosis
- Living with Cystic Fibrosis
- Cystic Fibrosis and Its Impact on Individuals

The history of gene therapy for cystic fibrosis dates back to 1990, when scientists successfully corrected defective cystic fibrosis transmembrane conductance regulator (CFTR) genes by introducing normal gene copies into laboratory cell cultures. In 1993, the first experimental gene therapy for cystic fibrosis was administered to a patient. Researchers utilized a modified common cold virus as a delivery vehicle to transport normal genes to the CFTR cells in the lung airways. Since then, various gene delivery methods have been explored, including lipid capsules, synthetic vectors, nasal drops, and the use of a flexible tube to deliver cells directly to the CFTR cells lining the airways. Currently, researchers are investigating aerosol delivery through nebulizers.

Challenges in developing this type of therapy include identifying the most effective delivery system for normal CFTR genes. Additionally, scientists need to:

- Assess the lifespan of the lung cells affected by cystic fibrosis
- Identify the "parent cells" responsible for producing CFTR cells
- Determine the duration of treatment and the frequency of necessary repetitions.

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The initial gene therapy experiments for cystic fibrosis focused on lung cells due to their accessibility and because lung damage is the most prevalent and serious issue for CF patients. Nonetheless, scientists are optimistic that the technologies developed for lung treatment can be adapted to address other organs affected by cystic fibrosis.

5. In case of Diabetic Neuropathy

Gene therapy appears to be a promising treatment for diabetic polyneuropathy, a condition that often affects long-term diabetics, according to a recent study. Researchers in Boston found that intramuscular injections of the vascular endothelial growth factor (VEGF) gene may benefit patients with this disorder. The study involved 39 patients who received three sets of VEGF gene injections in one leg, while 11 patients received a placebo.

Diabetic neuropathy is characterized by symptoms such as loss of sensation and pain in the legs and feet, weakness, and balance issues. The loss of sensation can lead to undetected ulcers on the feet, which may result in amputations. "Most patients had quite severe neuropathy, so expectations for improvement were not particularly high," stated Dr. Allan Ropper, executive vice chair of the neurology department at Brigham and Women's Hospital in Boston.

The VEGF gene used in this study can function without being packaged in a virus, providing a significant safety advantage, according to the researchers. "This study demonstrates that this type of gene transfer therapy can be conducted relatively safely, but further research with a larger group of participants is necessary before it can be adopted as a standard treatment," Ropper added.

6. In case of Metastatic Melanoma

Since the discovery of DNA and the understanding that genetic defects—whether inherited or acquired—can lead to various diseases, the idea of gene therapy has been highly attractive. However, despite significant efforts in this area of medicine, gene therapy has not yet made a substantial impact on patient treatment. Numerous technical challenges remain that need to be addressed before gene therapy can be widely implemented.

The gene delivery system, or vector, faces both extracellular and intracellular barriers. It must be non-toxic, non-immunogenic, and capable of facilitating adequate expression of the target gene. Although many vectors have been developed to tackle these challenges, an ideal expression vector for human use has yet to be discovered. Both inherited and acquired diseases could potentially benefit from gene therapy. For instance, X-linked severe combined immunodeficiency-X1, an inherited disorder, is a focus of active research in gene therapy, and promising advancements have been made in recent years.

10.PERSPECTIVES

The emerging field of gene therapy holds the promise of significant medical advancements in treating a wide range of human diseases, including immunological disorders, heart disease, and cancer, generating both high hopes and expectations. The concept of using genetic information from the human genome sequencing to develop treatments is compelling. However, for gene therapy to become a standard part of medical practice, collaboration among scientists from various disciplines is essential. Geneticists need to identify specific target genes linked to diseases or their progression. Virologists must create efficient and safe vectors to deliver these genes to the appropriate cells and ensure proper expression of the genetic material. Cell biologists will explore methods to enhance gene transfer and identify stem cells that can be used for organ regeneration. Bioengineers will play a critical role in demonstrating how to create three-dimensional tissues and even entire organs in the lab. Clinicians will conduct clinical trials with vectors tailored to the disease and the needs of patients.

Gene therapy has faced intense scrutiny in recent years. It is crucial to reassure the public that the health and welfare of patients are the top priority. Strict adherence to established guidelines is essential for all scientists and researchers involved in clinical trials. The gene therapy community must adapt to new regulations and guidelines from the NIH and FDA to ensure the quality of clinical trials and safeguard the volunteers participating in them. We aim to continue leading the nation in leveraging the benefits of this unprecedented era of biomedical research.[15]

11.Conclusions

In this review, we examined the most commonly used gene therapy medications and highlighted recent research developments. We summarized delivery methods for both viral and non-viral vectors, which open new possibilities for gene therapy drug administration. Additionally, we discussed the diseases targeted by gene therapy, providing insights into its future directions.

A critical aspect of gene therapy is identifying specific genes that can therapeutically address diseases, based on an understanding of their molecular mechanisms. The choice of gene carrier is vital in this process. Advancements in gene therapy will occur only when these medications are effectively used to address their limitations. Currently, the main challenge facing gene therapy is the delivery mechanism, which acts as a bottleneck in its application.

Our review focuses on the latest delivery strategies and aims to develop feasible gene editing techniques. With the emergence of new technologies such as second-generation sequencing and gene editing, gene therapy has become more advanced. However, the genetic diversity of inherited diseases, the complexity of their causes, and individual differences complicate clinical trials and practical applications.

The use of viral vectors in gene therapy remains a contentious issue regarding safety. While these vectors are highly effective at integrating into host cells, concerns about their infectivity and potential off-target effects continue to pose challenges. Furthermore, addressing the cost and reimbursement of gene therapy is crucial for making these treatments widely available.

Given the intricacies of human genetics, the outcomes of gene therapy can be unpredictable. There is no guarantee that genetic modifications will not inadvertently disrupt unknown functions. Consequently, gene therapy raises not only medical questions but also ethical considerations.

Challenges such as inefficient delivery systems, the need for sustained expression, and immune responses to the vectors still exist. Future research in gene therapy will likely focus on two main areas: deepening fundamental research to identify more effective therapeutic targets and solving delivery challenges, while also refining clinical trial processes to establish objective evaluation standards and improve treatment precision.

In summary, as fields like molecular biology, molecular genetics, clinical medicine, and new nanomaterials progress, gene therapy will continue to evolve and find more applications in clinical settings. We believe that as research advances and new vectors are developed, gene therapy will significantly impact the future treatment of genetic disorders.

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