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Genetics

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GENE THERAPY – THE FUTURE OF MEDICINE

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ABSTRACT

As one of the most hotly debated topics of the twenty-first century, 'Gene Therapy' holds the promise of a solution for most diseases, the controversy surrounding the alteration of human imperfection, and the possibility of a sort of medical care that most of us would never consider conceivable. Pharmaceuticals aren't always able to treat human disorders. So, meddling with a person's genes, the blueprints for how biological systems are constructed and operate, is the only way to fix the majority of diseases. The gene-editing method is being used by some researchers to accurately alter DNA sequences. Others are changing immune cells genetically to give them the ability to fight diseases. And, in the last few years, the development of a wide spectrum of therapies in which disease-causing genes are completely changed has accelerated dramatically. Gene therapy is defined as the treatment of disease by the transfer of genetic material into cells. This review will look at gene transfer technologies as well as present and potential applications, with a focus on future development and design.

1. INTRODUCTION

Gene therapy is a therapeutic approach to treating disease that involves delivering or modifying genetic material inside the cell [1]. It tries to fix faulty genes that cause disease development and effectively prevents or slows the advancement of the disease. Gene therapy consists of three basic intervention techniques: a) introducing a new gene into the body, b) replacing faulty genes with functional genes, and c) inactivating defective genes that cause disease [1,2]. There are two common types of gene therapy, namely somatic & germline gene therapy. Somatic gene therapy, as the name suggests, targets somatic cells, whereas germline gene therapy targets reproductive cells to avoid disease development in the following generations [2]. Gene therapy has emerged as one of the current therapeutic trends due to its potential to treat a variety of diseases such as autoimmune disorders, diabetes, malignancies, and cardiac ailments that are resistant to traditional treatments [3]. Genes are particular nucleotide sequences that include instructions for making proteins. Despite the fact that genes receive a lot of attention, proteins are responsible for the majority of life processes and even make up a large portion of cellular structures. Genetic abnormalities can occur when genes are changed in such a way that the encoded proteins are unable to perform their usual tasks [4]. A human cell's chromosomes contain around three billion pairs of nucleotides. The sequence of nucleotides in each person's genetic makeup is unique, and this is what distinguishes us from one another. Scientists estimate that each human cell contains roughly 30,000 genes. A disease, physical impairment, or shorter life span can all be caused by a mutation or flaw in any of these genes. These mutations are capable of being transferred from one generation to the next. Treatment or elimination of genetic diseases or physical ailments caused by these mutations could become a reality with gene therapy [5].

2. Gene Therapy

The ability to make local alterations in the human genome has been an aim of medicine since the discovery of DNA as the basic unit of heredity. Genes are particular nucleotide sequences that include instructions for making proteins. Genetic abnormalities can occur when genes are changed in such a way that the encoded proteins are unable to perform their usual tasks. Gene therapy is a technique for repairing faulty genes that cause diseases. Currently, gene therapy is primarily practised in research facilities, and its applicability is still in its early stages [6]. The technique is comprehensive, with the potential to treat diseases caused by recessive gene disorders (cystic fibrosis, haemophilia, muscular dystrophy, and sickle cell anaemia), acquired genetic diseases like cancer, and viral infections like AIDS [7]. Recombinant DNA technology is one of the most widely used procedures in which a healthy gene is introduced into a vector, which can be viral or plasmid; the latter is the most commonly utilized owing to its effectiveness in infiltrating cells and introducing genetic material [7]. Depending on the treatment period, type and location of target cells, and whether they divide or are quiescent, various vectors, including nonviral methods, non-integrating viral vectors, and integrating viral vectors, may be used. [8].

Table 1. Gene therapy protocols

Disease	Objective	Stem cells	Release mode	Countries with the protocol
Adenosine deaminase deficiency	Substitution of the adenosine deaminase deficiency	Blood	Retrovirus	Italy, Holland, and the United States
α 1-antitrypsin deficiency	Substitution of a 1-antitrypsin	Respiratory epithelium	Liposome	United States
AIDS	Inactivation of the HIV-presenting antigen	Blood and bone marrow	Retrovirus	United States
Cancer	Improvement of immune function	Blood, bone marrow, and tumor	Retrovirus, liposome, electroporation, and cell-mediated transfer	Austria, China, France, Germany, Italy, Holland (Netherlands), and the United States
Cancer	Tumor removal	Tumor	Retrovirus, non-complexed DNA, cell-mediated transfer	United States
Cancer	Chemoprotection	Blood and bone marrow	Retrovirus	United States
Cancer	Stem cell marking	Blood, bone marrow, and tumor	Retrovirus	Canada, France, Sweden and United States
Cystic fibrosis	Enzymatic substitution	Respiratory epithelium	Adenovirus and liposome	England and the United States
Familial hypercholesterolemia	Substitution of low-density lipoprotein receptors	Liver	Retrovirus	United States
Fanconi anemia	Complement C gene release	Blood and bone marrow	Retrovirus	United States
Gaucher Disease	Glucocerebrosidase substitution	Blood and bone marrow	Retrovirus	United States
Hemophilia B	Factor IX substitution	Skin fibroblasts	Retrovirus	China
Rheumatoid arthritis	Cytokine release	Synovial membrane	Retrovirus	United States

Source: Adapted from Misra S. Human gene therapy: a brief overview of the genetic revolution. J Assoc Physicians India. 2013;61(2):127-33. Review [7].

3. Vectors In Gene Therapy

Gene therapy relies on the transport of DNA into cells, which can be done in a variety of ways. The two major methods use recombinant viruses (viral vectors) and non-viral vectors. The therapeutic gene is delivered to the patient's target cells via a vector. The most frequent vector currently used is a virus that has been genetically modified to carry normal human DNA. Viruses have developed a harmful method of encapsulating and delivering their genomes to human cells. Scientists have attempted to exploit this potential by manipulating the virus genome to delete disease-causing genes and replace them with therapeutic genes [9] (shown in figure 1).

Viruses used as gene therapy vectors include the following [10,11]:

A. Retroviruses

Retrovirus is a type of virus that can replicate its RNA genomes in double-stranded DNA. These copies of its genome can be inserted into host cells' chromosomes. HIV is a retrovirus that causes human immunodeficiency.

B. Adenoviruses

Adenovirus are a type of virus with a double-stranded DNA genome that causes infections in the lungs, intestines, and eyes in humans. The common cold is caused by the adenovirus virus.

C. Adeno-associated Viruses (AAVs)

AAVs are a type of single-stranded DNA virus that may insert its genetic material into a specific location on chromosome 19.

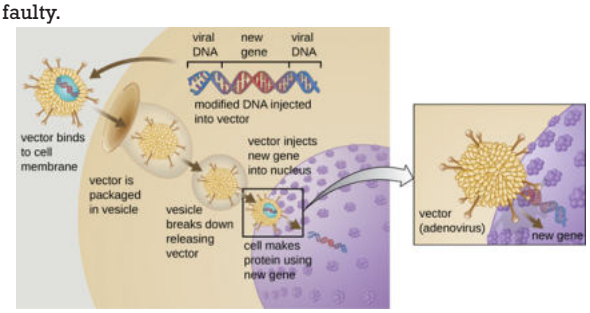
D. Herpes Simplex Viruses

HS Viruses are a type of double-stranded DNA virus that infects neurons, a specific cell type. The type 1 herpes simplex virus is a frequent human disease.

4. Viral Gene Delivery

Viruses have evolved over millions of years and are a species-specific method of transferring DNA to cells. Scientists are currently working to improve these gene delivery vehicles so that they may be used to cure human diseases and abnormalities. There are three universal needs for viral gene therapy vectors, regardless of approach. First and foremost, the delivery technique must be both safe and immune-inactive. Second, it must safeguard the genetic material against deterioration. Third, the vector must carry an effective therapeutic gene that can be expressed for a long time at a specific target location. For actual commercial use, the packed vector must be simple to generate and process, as well as have a long shelf life [12]. New goals have evolved, including tissue-specific targeting, site-specific chromo somal integration, and controlled infection of both dividing and non-dividing cells. Despite the fact that viral gene therapy has received a lot of negative attention, it still has a lot of stigmas associated with it.

Figure 1. Gene therapy with an adenovirus vector can be used to treat or cure some genetic diseases when a patient's gene is faulty.

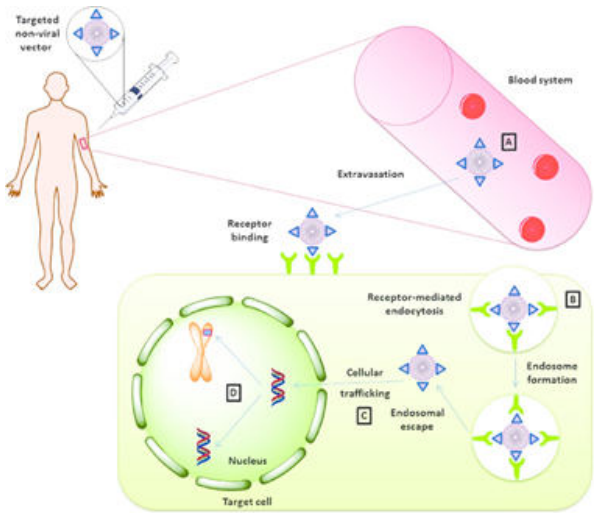


(Credit: modification of work by National Institutes of Health) [13].

5. Non-viral Gene Delivery

However, everyone focus will be on viral methods of gene delivery, it is important to note that non-viral gene therapy has made significant progress. Because of its relative safety, minimal immunogenicity and toxicity, simplicity of administration and production, and absence of DNA insert size constraint, polymeric gene delivery is preferred. The key problem is that due to the necessity for post-uptake endosomal escape and nuclear translocation of the DNA complex, gene transfer efficiency is low (Park et al., 2006). Clinical efficiency and specificity thresholds have not yet been satisfied in this regard [12].

Naked DNA and liposomes are instances of nonviral vectors. They are based on a plasmid, which is a DNA strand that is closed and circular. Direct insertion of therapeutic genes is possible. then this recombinant plasmid enters the plasmid may be injected into cells in a number of different methods. For It can, for example, be injected directly into certain tissues. as though it were bare DNA [15]. Nonviral vectors are substantially less expensive and easy to manufacture in huge quantities. These vectors have low immunogenicity, allowing for possible redosing, and they are considered safe because no recombination could result in a competent virus that could cause disease. The "gene gun," a less efficient gene transfer rate, does not need the existence of complex and potentially dangerous delivery mechanisms. The DNA is linked to microscopic gold particles, which facilitate gene material transfer. These particles are then delivered into the cell at high pressure and speed (with the aid of compressed helium) and pass through the cell membrane [9].



Therapeutic gene delivery mediated by non-viral vectors. Successful gene delivery mediated by non-viral vectors [14].

Nonviral vectors provide various advantages over virus-derived vectors, including the safety of administration without immunogenicity, as well as nearly infinite transgene size and the ability to administer several times. The therapeutic gene can be delivered to the target cell as a plasmid insert containing regulatory sequences, allowing for expression regulation control (inducible promoter), or as a PCR product. The simplest method of gene delivery is to inject naked DNA into target cells.

6. Alternative Gene Therapy

Another approach is to use bacteria for gene therapy. Bacterial protein delivery is another name for it. It's true based on the transfer of therapeutics synthesized by bacteria utilizing genetically engineered proteins to deliver proteins to the host organism bacteria that have been transformed. AGT, like Bactofection (use of bacteria as a vector for delivering therapeutic genes to target cells), is mostly used to treat malignancies and makes use of bacterial strains that are

particularly oncolytic and tumour colonising Clostridia, Bifidobacterial, and Salmonellae are all types of bacteria [16].

7. Diseases Treated By Gene Therapy

Gene therapy was originally intended to directly transfer genes into human cells, focusing on disorders characterised by single-gene abnormalities, such as haemophilia, cystic fibrosis, muscular dystrophy and sickle cell anaemia. There are three categories of disorders that can be treated by gene therapy:

Monogenic disorders are heritable diseases in which a single locus (gene) is faulty and accountable for the condition. Sickle cell anaemia, Severe Combined Immunodeficiency (ADA-SCID / X-SCID), muscular dystrophy, Cystic fibrosis, Haemophilia, Duchenne, Huntington's disease, Parkinson's disease, Hypercholesterolemia, Fanconi Anaemia, Alpha-1 antitrypsin, Chronic Granulomatous Disease and Gaucher Disease are examples of diseases. Multiple genes are implicated in polygenic illnesses, and the condition may be influenced by environmental factors and lifestyle. Heart disease, cancer, diabetes, schizophrenia, and Alzheimer's disease are just a few examples. Infectious diseases, such as HIV [24].

a. Combined Immune Deficiency (Severe Combined Immune Deficiency) (ADA-SCID)

The bubble boy sickness is another name for ADA-SCID. Without bone marrow transplantation from matched donors, affected infants are born without a functioning immune system and would succumb to illnesses outside of the bubble. Investigators in Italy completed a historic trial that represents the first example of gene therapy "cure," or at least long-term correction, for individuals with a fatal genetic condition. The therapeutic gene ADA was inserted into the bone marrow cells of these patients in the laboratory, and the genetically corrected cells were then transplanted again into the same patients, followed by the genetically repaired cells being transplanted back into the same patients. All six patients had their immune systems reconstructed without any visible adverse effects, and they are now able to live normal lives with their families without the need for medication.

b. Haemophilia

Patients born with Haemophilia are unable to form blood clots, resulting in life-threatening external and internal bleeding. The therapeutic gene was put into the liver of patients in a clinical experiment done in the United States, and they gained the capacity to have normal blood clotting time as a result. However, because the genetically repaired liver cells were detected as strange and rejected by the patients' healthy immune systems, the therapeutic benefit was only temporary. Immunosuppression or other gene delivery tactics are now being investigated in preclinical animal models of this condition, and a curative result via gene therapy may be possible with immune-suppression or alternative gene delivery systems.

c. Blindness

Leber's congenital amaurosis (LCA) is a rare genetic eye illness that affects around 1 in 80,000 people. It develops at birth or within the first few months of life. Theodore Leber was the first to describe it in the nineteenth century. Nystagmus, slow or absent pupillary responses, and severe vision loss or blindness are all symptoms of LCA. The first gene therapy clinical study for individuals with RPE65 LCA was undertaken by researchers at Moorfields Eye Hospital and University College London in London. Early in 2007, the first patient was operated on. Six young individuals were treated with gene therapy by researchers at Children's Hospital of Philadelphia and the University of Pennsylvania. Dr. Al Maguire, an ophthalmologist, and Dr. Jean Bennett, a gene therapy expert, came up with the idea.

d. Cancer

Suicide gene therapy, oncolytic virotherapy, anti-angiogenesis, and therapeutic gene vaccines are just a few of the gene therapy treatments that have been developed to treat a range of tumours. Two-thirds of all gene therapy studies are for cancer, and several are already at advanced stages, including a Phase III study of Ad.p53 for head and neck cancer and two Phase III gene vaccine trials for prostate and pancreas cancer. In addition, university medical institutions and biotechnology businesses are conducting several Phase I and Phase II clinical studies for malignancies of the brain, skin, liver, colon, breast, and kidney, among others, employing breakthrough technologies and medicines created on-site.

e. Neurodegenerative Diseases

Recent advancements in gene therapy have enabled for innovative therapies of neurodegenerative disorders such as Parkinson's disease and Huntington's disease, with promising outcomes in animal models of the corresponding human diseases. These neurodegenerative illnesses are now undergoing or will shortly undergo Phase I clinical studies[24].

8. Advanced Gene Editing: CRISPR Cas9

CRISPR/Cas9 technology, which is a genetic modification tool developed from the defence mechanism of some bacteria against viruses and plasmids, reactivated genome editing in 2012. This approach is simple to use and has been employed in a number of experimental contexts, including cell lines, laboratory animals, plants, and even clinical trials in humans. The CRISPR/Cas9 method involves utilising a short RNA molecule as a guide to lead the Cas9 nuclease to construct a site-directed double-strand DNA break [17].

9. Is Gene Therapy Safe?

The possibility of using gene therapy to treat disease is being investigated. Gene therapy is now being studied to see if it is safe, and future studies will see if it is a viable treatment option. Several studies have already demonstrated that this strategy can result in major health consequences such as toxicity, inflammation, and cancer. Because the procedures are so new, some hazards are unknown; however, medical researchers, institutions, and regulatory bodies are attempting to make gene therapy research as safe as possible [18].

10. Applications Of Gene Therapy

Gene therapy is more likely to be successful in treating diseases caused by single gene abnormalities. Gene therapy had been approved for disorders such as severe combined immunological deficiency, familial hypercholesterolemia, cystic fibrosis, and Gaucher's disease by the end of 1993. To date, the majority of protocols have been developed to treat cancer; a few have also been developed to treat AIDS. Gene therapy is being considered for a variety of conditions, including Parkinson's and Alzheimer's diseases, arthritis, and heart disease. The Human Genome Project, which is attempting to locate all of the genes in the human genome, continues to uncover inherited diseases [19,20].

11. Advantages Of Gene Therapy

- Germ-line gene therapy provides an actual cure, not only palliative or symptomatic relief.
- For some hereditary illnesses, germ-line gene therapy could be the sole viable treatment option.
- The cost and risk of somatic cell treatment for several generations are avoided by stopping the spread of disease genes.
- Medicine should address the reproductive health requirements of prospective parents who are at risk of passing on major hereditary illnesses to their children.
- Within the limitations of ethical human study, the scientific world has the freedom to unfettered inquiry[21].

12. DISADVANTAGES OF GENE THERAPY

- There is too much scientific ambiguity and clinical danger

in gene therapy research, and the long-term ramifications of such therapy are unclear.
- Such gene therapy will unlock the door to attempts to change human features that aren't linked to sickness, thereby exacerbating societal discrimination issues.
- Because germ-line gene therapy research includes early embryos and their children, it basically generates generations of unwitting study volunteers.
- Gene therapy is incredibly expensive and it will never be cost-effective enough to be considered a significant societal priority.
- Germ-line gene therapy will interfere with future generations' rights to inherit a genetic inheritance that has not been tampered with [22].

13. Ethical Issues

Because gene therapy involves changing the body's basic instructions, it raises a number of ethical concerns. Some of the ethical difficulties surrounding gene therapy are as follows:

I. Who determines which characteristics are normal and which are signs of a disability or disorder?
II. How do "moral" and "immoral" gene therapy usage be distinguished?
III. Is it possible that the widespread use of gene therapy will lead to a decrease in society's acceptance of persons who are different?
IV. Will gene treatment only be offered to the rich due to its hefty costs?
V. humans be able to employ gene therapy to improve fundamental human characteristics like height, intellect, or athletic ability?
VI. Are future generations containing "Superhumans"? [23].

14. FUTURE PERSPECTIVES AND CONCLUSION

According to me, Gene therapy will bring a revolution in medicine near future. There are a lot of ongoing projects, one of them is HGP (Human genome project) has recently completed, by using advanced techniques completed sequencing and mapping of all 30,000 genes in the human cell. Now it is easier to bring a new strategy to treat and cure diseases. Some prominent testing of genes like whole-genome sequencing can be able to diagnose several genetic associated diseases. Thereby we can prevent them precisely. Gene therapy is an exciting thing for future generations. It has enormous ability to prevent "Childhood" diseases and even stop diseases that imprint from generation to generation. By using advanced techniques now researchers possibly treat the genetic diseases for the unborn child in the uterus itself.

Current research and clinical trials create a bunch of opportunities and even challenges too. However, Gene therapy is going to bring incredible change in the world of future generations.

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