A Review On: Principle, Types, Method and Application of Gene Therapy

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Abstract - Gene therapy is a novel approach to treat, cure or prevent disease by changing the expression of a person's genes. For the disease which do not curve by medicine or treatment that is curved by gene therapy which produce excitement, the controversy surrounding the altering of human imperfection, and the promise of a type of medical treatment most of us would never imagine possible. This is one of the best way to treat hereditary diseases by altering and correcting the genes. Nucleic acids is introduced into cells has as a purpose of medical condition or disease. Currently, gene therapy studies a broad range of potential therapeutic interventions, including the body's immune reaction to tumors, new blood vessels in the heart to alleviate heart attacks and to stop HIV-replication in patients with AIDS. There is also renewed emphasis on the gene therapy of genetic diseases, such as haemophilia A and B, and cystic fibrosis. Human gene therapy experimentation raises many issues. In this review article, background of gene therapy, introduction, genetic diseases, gene function, germ line gene therapy, hurdles in gene therapy, methods for gene therapy, ex vivo, in vitro and in vivo-gene therapy, risks associated with gene therapy, have been given. One way gene therapy works is to turn viruses into genetic vectors that carry the gene of interest to the target cells. Based on the genome's nature, these vectors are divided into RNA-based or DNA-based viral vectors. Most RNA-based vectors are derived from simple retroviruses, such as the murine leukaemia virus. One major drawback of these viruses is that they are not transferred to non-dividing cells (post-mitotic cells). This problem can be solved by using new retroviral vectors derived from lentiviruses, such as the human immunodeficiency virus (HIV). DNA-based vectors originate from adeno-viruses and adeno-associated viruses (AAVs). In this report included history, types of gene therapy, vectors & applications.

Keywords: Gene, gene therapy, treatment, viruses, gene types, genetic diseases.

INTRODUCTION

A gene is a DNA segment. discovered to be a functional and physical component of heredity. Each gene is inherited in two copies by each individual, one from each parent. They hold the blueprints for our unique features, such as the color of our eyes and hair. The genetic code of an individual codes for the proteins that make up our physical selves. [1] Gene therapy is an attempt to treat diseases by either repairing or replacing faulty genes with healthy ones in order to enhance gene function. When Blaese et al. reported the first trial results of T lymphocyte directed gene therapy in Adenosine Deaminase (ADA) deficiency related Combined Immunodeficiency (SCID) in 1995, the world was first introduced to gene therapy about two decades ago.[2]

The concept of gene transfer for the treatment of hereditary disorders has been explored by many human geneticists. Recent significant developments in cell biology and recombinant DNA technology have raised the likelihood that this pipe dream will come true. In addition, gene therapy will likely find use in many areas of medicine rather than just treating single-gene disorders. [3]

The National Institutes of Health (NIH) conducted the first approved gene-transfer study in 1989.Tumor-infiltrating lymphocytes were isolated for this marker study (see Genetic Markers), tagged with a retroviral vector for genetic expression, and then rein fused to test the cells' ability to locate tumors. The first concrete proof that genetically altered human cells may be

restored to a patient without causing harm was given by this seminal work. [4]

GENE THERAPY

The basic structural and functional building block of heredity is the gene. A gene is an organized sequence of nucleotides that codes for a particular functional product (a protein or RNA molecule) and is found at a specific location on a certain chromosome. The term "biological units of heredity" refers to genes. Unique characteristics, such as the color and texture of the hair and eyes, are inherited from the parents. These also establish the child's gender, the blood's oxygen carrying capacity, and it's IQ. [5]

Usually, gene therapy needs two basic parts. A therapeutic gene comes first, followed by a vector that enables the gene to be delivered to the right cells. In order to guarantee that the therapeutic gene continues to act within the target cells, strategies are also needed. [6]

PRINCIPLE OF GENE THERAPY:

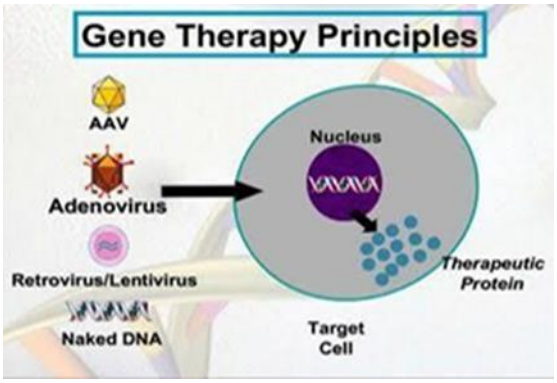

Figure 1. Gene Therapy Principle

1. Selective reverse mutation can be used to restore an aberrant gene to its normal function.
2. Homologous recombination can be used to exchange an aberrant gene for a normal gene.
3. It is possible to modify the regulation, or the extent to which a gene is activated or inactive.[7]

GENE MODIFICATION

• Replacement therapy: homologous recombination is used to swap out a natural gene for a non-natural gene.
• Modifier gene therapy: Using selective reverse mutation, a damaged gene can be made to operate normally again.
• There are three different methods for transferring genes: chemical, biological, and physical. Gene transfer to a particular cell line Somatic gene therapy and sex cell gene therapy fall under this category.[8]

HISTORY OF GENE THERAPY

In order to treat genetic disorders, researchers hypothesized in the middle of the 1960s that DNA groups may be inserted into patients' cells. Martin Cline was the one who first attempted to modify human DNA in 1980. Nevertheless, it took a while until the first successful outcome of atomic quality. was observed, finally appearing in May 1989.[9] French Anderson achieved the first direct insertion of human DNA into the atomic genome in September 1990, which was also its primary useful use. Ashanthi de Silva, then 4 years old, became the main example of a great treatment patient overcoming adversity in 1990. Because she was born without the protein adenosine deaminase (ADA), she was born with an extreme combined immunodeficiency (SCID). In absence of ADA, her T cells died off, making her inadequate to battle contaminations. Infusions of an engineered ADA compound aided, however just immediately. The doctors decided to use an impaired virus that is unable of spreading across the body to instill a comparatively strong ADA quality into her platelets. The success they achieved encouraged more preliminary work for a related SCID in the 1990s. Despite having a rare illness, de Silva, who is currently in her 30s, is full of life. Over 2,900 clinical preliminary cases were conducted between the hours of 1989 and December 2018, with the majority of those cases falling into stage 1. The first high-quality medicines to hit the market with FDA approval were Spark Therapeutics' Luxturna (for visual impairment caused by RPE65 mutation) and Novartis' Kymriah (antigen T cell therapy of chimeric receptor). [10]

The 2002s The modified cancer gene therapy was approved, and according to the Wiley gene therapy clinical trial, the method has demonstrated encouraging outcomes when treating six distinct malignant tumors: glioblastoma, as well as ovarian, colon, prostate, liver, and uterine malignancies. In mice, sickle-cell disease is treatable.

For the first time, brain gene therapy was used in China in 2003 to treat squamous cell cancer of the head and neck.

2006s the successful use of gene therapy to treat two adult patients for X-linked chronic granulomatous disease, 2008s A clinical trial done to cure an inherited blinding disease caused by mutations in the RPE65 gene. Total three trials done in all clinical trials, patient's recovered functional vision without apparent side-effects.

2010: 18-year-old male patient in France with beta-thalassemia major had been successfully treated

In 2011 Neovasculgen was registered in Russia as the first-in-class gene-therapy drug for treatment of peripheral artery disease, including critical limb ischemia;

2013: Researchers reported that two children born with adenosine deaminase severe combined immunodeficiency disease (ADA-SCID) had been treated with genetically engineered stem cells

2014: Clinical trials of gene therapy for sickle cell disease were started on human sample.

2017: The FDA approved „Tisagenlecleucel" a modified genetic material for acute lymphoblastic leukemia.

2019: The first ever "in body" human gene editing therapy to permanently alter DNA - in a patient with Hunter Syndrome. [1]

Since that time, medications like Alnylam's Patisiran and Novartis' Zolgensma have likewise gotten the backing of the FDA, notwithstanding other organizations' quality treatment drugs. The vast majority of these techniques use Adeno Associated Virus (AAVs) and lentivirus for executing quality inclusions, ex-vivo and in-vivo individually. [11]

TYPES OF GENE THERAPY

Understanding some of the jargon and different forms of gene therapy becomes crucial. Both biologists and laypeople frequently employ alternative forms of therapy such as molecular therapy and DNA-based therapy. One genetic engineering technique used to lessen the effects of inherited disorders is gene therapy. [12] Broadly speaking, though, engineering may also involve manipulating genes to improve an organism's capabilities beyond what is normally expected, in addition to trying to change genes to fix genetic flaws. The latter genetic engineering concept is risky. A. Depending on the kind of cells that the therapeutic genes alter, gene therapy can be divided into two categories: somatic cell therapy and germ cell therapy. [7, 13]

1. Somatic Gene Therapy- In order to treat a genetic condition, a functional and expressible gene must be inserted into a target somatic cell. In terms of contemporary scientific and clinical research, it symbolizes the mainstream line where any alterations and consequences are not passed down to the patient's progeny or subsequent generations. Since somatic gene therapy only affects the patient's targeted cells and is not inherited by future generations, it is seen to be a more cautious and safe technique. On the other hand, somatic cell treatment has a limited lifespan because most tissues' cells eventually die and are replaced by new ones. Furthermore, there are issues with getting the gene to the intended cells or tissue. Regardless of these difficulties, however, somatic cell gene therapy is appropriate and acceptable for many disorders. [14]

2. Germline Gene Therapy: - This method involves introducing a functioning gene into germ cells, either sperm or egg. As a result, the effects of therapy would be inherited. While germline gene therapy is a highly effective strategy for treating genetic and hereditary disorders, it is not currently being tried for safety, ethical, or technical reasons. The genetic changes that occur in somatic cells do not pass down to the following generations. As a result, somatic gene therapy is favored and being researched thoroughly with the ultimate goal of curing human diseases. [15]

METHOD FOR GENE THERAPY

1. Physical
1.1 Direct injection of DNA
1.2 Liposome-mediated DNA transfer
1.3 Calcium phosphate transfection-
1.4 Electroporation
2. Retrovirus vectors
3. Other viral vectors
4. Targeted gene transfer via receptors
5. Artificial chromosomes
6. Site-directed recombination
7. Activation of genes of related function.

a "normal" gene is put into the genome to replace a "abnormal," disease-causing gene in the majority of gene therapy research. Target cells in the patient are given the therapeutic gene via a carrier molecule known as a vector. At the moment, a virus that has

undergone genetic modification to transport normal human DNA is the most prevalent vector. Viruses have developed a means of encasing and transferring their genes in a harmful fashion into human cells. Utilizing this ability, scientists have attempted to alter the virus genome in order to eliminate genes that cause sickness and add genes that promote healing. [16]

SOME OF THE DIFFERENT TYPES OF VECTORS USED AS GENE THERAPY

VIRAL VECTORS

1. Retroviruses: - A class of viruses that can create double-stranded DNA copies of their RNA genomes. These copies of its genome can be integrated into the chromosomes of host cells. Human immunodeficiency virus (HIV) is a retrovirus.
2. Adenovirus: - a group of viruses that infect humans' eyes, intestines, and respiratory systems. Their genomes are made of double-stranded DNA. An adenovirus is the type of virus that causes the common cold.
3. Adeno-associated viruses: - A group of tiny, single-stranded DNA viruses that have the ability to implant their genetic material into a particular location on chromosome 19.
4. Herpes simplex viruses: - a family of double-stranded DNA viruses that target neurons as their target cell type. Cold sores are commonly caused by the human disease Herpes simplex virus type 1. [17, 18]

NONVIRAL VECTORS

Therapeutic genes can also be transferred using nonviral vectors; liposomes are the most well-known example. The spherical lipid vesicles known as liposomes encapsulate DNA and facilitate its passage through the cell membrane. Liposomes don't have the same transfer efficiency as viruses, but they still avoid a lot of the problems associated with viral vectors. Additionally, 47th (artificial) chromosome introduction is being experimented with by researchers in hopes of using it as a vector to transfer massive amounts of genetic code.

DIFFERENT NONVIRAL VECTOR

- Liposome
- DNA–polymer conjugates
- Naked DNA [6]

APPLICATION OF GENE THERAPY:

1. Cancer: - Gene therapy-related research and its clinical application have been mostly utilized in the field of malignancy. By the end of 2009, nearly two third of gene therapy-related research was concentrated on cancers. [14] Oncolytic viruses are used to introduce genes into malignant cells, thereby causing death of Gene Therapy in India- Current Status malignant cells. Another approach is to deliver p53 gene (tumour suppressor gene) and thereby induce onycholysis. Gendicine that was first approved anticancer drug which was based on this gene therapy principle. Suicide gene therapy is another attempt to treat tumour by delivering of gene coding for enzyme that metabolizes prodrugs into locally active chemotherapeutic drug moiety.

The idea of malignancy has been widely applied to research on gene therapy and its clinical presentation. Approximately two thirds of all gene therapy-related research is being done on cancer. By introducing genes into cancerous cells, oncolytic viruses cause the cancerous cells in question to die. Using the p53 gene, a tumor suppressor gene that causes onycholysis, is an alternative tactic. Genedicine was the first approved anticancer medication developed using this gene therapy concept. One further attempt to treat cancer is suicide gene therapy, which involves giving the gene coding for the enzyme that converts prodrugs into locally active chemotherapeutic drug moiety. [19]

2. Immunodeficiency: - Although gene therapy has been improving for years, the first notable advancement since the initial study was observed in the early 1990s. Despite clinical trials demonstrating the potent therapeutic benefits of gene therapy in the management of both X-linked severe combined immunodeficiency (X-SCID) and SCID caused by adenosine deaminase (ADA) deficiency, there was a preliminary setback during which two patients died after receiving treatment for X-linked SCID using retroviral vectors due to leukemia. In addition to primary immunodeficiency infection, secondary immunodeficiency states such as HIV have also become potential candidates for gene therapy. For specific defence against HIV infection to these cells, Transgenes can be transported into haematopoietic stem cells or into T- cells. They make the milieu unsuitable for HIV-1replication or disable the HIV-1 protein. [20]
3. Eye Diseases: - Following the initial setback observed in SCID, leber's congenital amaurosis led to

a resurgence of faith in gene therapy. Because the eye is a tiny organ, a large number of ocular cells may become transfectable. Clinical ophthalmologic diseases for gene therapy include Leber's hereditary optic neuropathy, glaucoma, macular degeneration, and red-green color blindness. A phase I trial examining the effects of the antiangiogenic cytokine Pigment Epithelium-derived Factor (PEDF) is currently treating age-related macular degeneration. In adult red-green colorblind monkeys, the creation of trichromatic color vision is significantly improved by subreticular injections of an adeno-associated virus encoding an L-opsin gene. [21]

4. Cardiac Diseases: - Because cardiac disorders have multiple etiologies, they are challenging to treat. Research is underway to test strategies that scientists have devised to transfer genes encoding distinct growth factors, such as VEGF and FGF, in order to promote the formation of new blood vessels. Their findings, however, did not show a discernible improvement in stress-induced myocardial perfusion; instead, improved regional wall motion suggested a beneficial anti-ischemic effect, prompting further study in this area.

5. Central Nervous System: - In contrast to heart ailments, gene therapy has shown promise in treating neurological conditions like Parkinson's and Alzheimer's disease. Currently in phase 1 or phase 2, a number of trials are being carried out to investigate the potential, safety, and tolerability of gene therapy in Parkinsonism for in vivo investigations. Several techniques are employed, such as transferring the gene into the subthalamic nucleus of glutamic acid decarboxylase or delivering the gene in putamen cell bodies for nurturing. Gene therapy is being used to try and send nerve growth factor into the central nervous system in cases of Alzheimer's disease.

6. Parkinson's Disease: - Independent investigations have demonstrated the efficacy of gene therapy in treating Parkinson's disease (PD). For instance, one of the suggested approaches involves raising the brain's concentration of GABA, a neurotransmitter whose deficiency results in Parkinson's disease. Tubes were inserted into the brain regions related to movement in 45 volunteers with severe Parkinson's disease as part of an investigation. A control group of half the participants received an innocuous saline solution, while the other half received injections of viruses with the gene that enhances GABA synthesis. After six months, the movement capacity of individuals who received gene therapy improved by 23%, more than twice as much as that of the control group. The study under discussion was a randomized controlled trial to look into the use of gene therapy to treat advanced Parkinson's disease symptoms. In the study, basal ganglia cells—a group of brain regions that regulate movement—were given genes that produce the chemical agent glutamic acid carboxylase (GAD). The GABA chemical messenger was elevated in response to the transplanted GAD gene. In certain regions of the basal ganglia, Parkinson's disease patients have lower levels of GABA. [22]

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