Gene therapy: Current status and future perspectives

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Abstract

The concept of transferring genes to tissues for clinical applications has been discussed for nearly half a century. Advances in molecular biology and biotechnology have helped to understand the genetic basis of inherited diseases and have brought gene therapy to the forefront of medical research. Gene therapy is the process of introducing foreign genomic materials into host cells to elicit a therapeutic benefit. While originally conceived as a way to treat life-threatening disorders refractory to conventional treatment, gene therapy now is considered for many non-life –threatening conditions, including those adversely affecting a patient's quality of life. Although gene therapy is still at a fairly primitive stage, however, the possibility of success of gene therapy now appears much closer. This is a consequence of the significant improvements made in the development of both in vivo and ex vivo systems for gene delivery and the identification of novel classes of therapeutic genes. Although as of now, gene therapy has not offered any permanent cure to any human patients, a breakthrough may come anytime. The recent results obtained by gene therapy of inherited blindness and of some neurodegenerative disorders encourage firm optimism on the eventual success of this discipline.

Keywords: gene therapy, gene delivery, viral & non-viral vectors, pros and cons

Introduction

James Watson was quoted as saying "we used to think that our fate was in our stars, but now we know, our fate is in our genes." Genes, the basic functional unit of heredity are specific base sequences that carries information needed to make specific proteins. Variations in the DNA sequence or code of a gene are called mutations, which often are harmless but sometimes can lead to serious disease. Gene therapy is a technique in which a functional gene replaces the defective gene so that the body can make the functional protein and therefore eliminate the root cause of the disease. One of the following approaches may be used to correct the faulty genes responsible for genetic disorder [1,2]:

- A normal gene could be inserted into a nonspecific location within the genome to replace the non-functional gene (most common approach)
- An abnormal gene could be swapped for a normal gene through homologous recombination
- An abnormal gene could be repaired through selective reverse mutation
- Regulation of a particular gene could be altered

In the last few years, there have been several breakthroughs that have made it possible for clinical trials in gene therapy to begin. Current and possible candidates for gene therapy include autosomal or X-linked recessive single gene disorders (e.g. cystic fibrosis, haemophilia, muscular dystrophy, sickle cell anaemia etc.), acquired genetic diseases such as acquired immunodeficiency syndrome (AIDS) and cancer. Other conditions such as cardiovascular diseases, arthritis, diabetes mellitus, parkinson's and alzheimer's disease are also being targeted by various gene therapy trials. Table 1 shows a selected list of some genetic disorders at various stages of gene therapy trials.[3,4]

Table 1: Human gene therapy trials[4]

Disease	Gene therapy
Severe combined immunodeficiency (SCID)	Adenosine deaminase (ADA)
Cystic fibrosis	Cystic fibrosis transmembrane regulator
Familial hypercholesterolemia	Low density lipoprotein (LDL) receptor
Emphysema	Alpha-1- antitrypsin
Thalassemia	Alpha or beta globin
Sickle-cell anemia	Beta-globin
Gaucher's disease	Glucocerebrosidase
Fanconi anemia	Complement group C gene delivery
Melanoma	Tumour necrosis factor (TNF)
Glioblastoma	Thymidine kinase
Duchenne muscular dystrophy	Dystrophin
Diabetes mellitus	Glucose transporter-2 (GLUT-2)

Concept of gene therapy

Originally, the term gene therapy referred to proposed treatments of genetic disorders by replacing a defective gene with its normal counterpart. Currently, it includes all treatments in which there is an introduction of genetic material into body cells to treat a variety of diseases.

Approaches for gene therapy

There are two approaches to achieve gene therapy:

1) **Somatic gene therapy**- It involves the insertion of a functional and expressible gene into a target somatic cell to correct a genetic disease. It represents the mainstream line of current basic and clinical research where any modifications and effects will not be inherited by the patient's offspring or later generations.

Somatic gene therapy is viewed as a more conservative and safer approach because it affects only the targeted cells in the patient and is not passed on to future generations; however, somatic cell therapy is short lived because the cells of most tissues ultimately die and are replaced by new cells. In addition, transporting the gene to the target cells or tissue is also problematic. Regardless of these difficulties, however, somatic cell gene therapy is appropriate and acceptable for many disorders [5].

2) **Germline gene therapy**- In this approach, functional genes are introduced into germ cells (sperm or egg). Therefore the changes due to therapy would be heritable. Although this approach is highly effective in counteracting genetic and hereditary diseases, but for safety, ethical and technical reasons, germline gene therapy is not being attempted at present.

The genetic alterations in somatic cells are not carried to the next generations. Therefore, somatic gene therapy is preferred and extensively studied with an ultimate objective of correcting human diseases[6].

Types of gene therapy

There are two types of gene therapy as described in Fig.1[7]:-

1. **Ex vivo gene therapy** : This technique involves the following steps-

a) Isolate cells with genetic defect from a patient
b) Grow the cells in culture
c) Introduce the therapeutic gene to correct gene defect
d) Select the genetically corrected cells and grow
e) Transplant the modified cells to the patient

The procedure basically involves the use of the patient's own cells for culture and genetic correction, and then their return back to the patient.

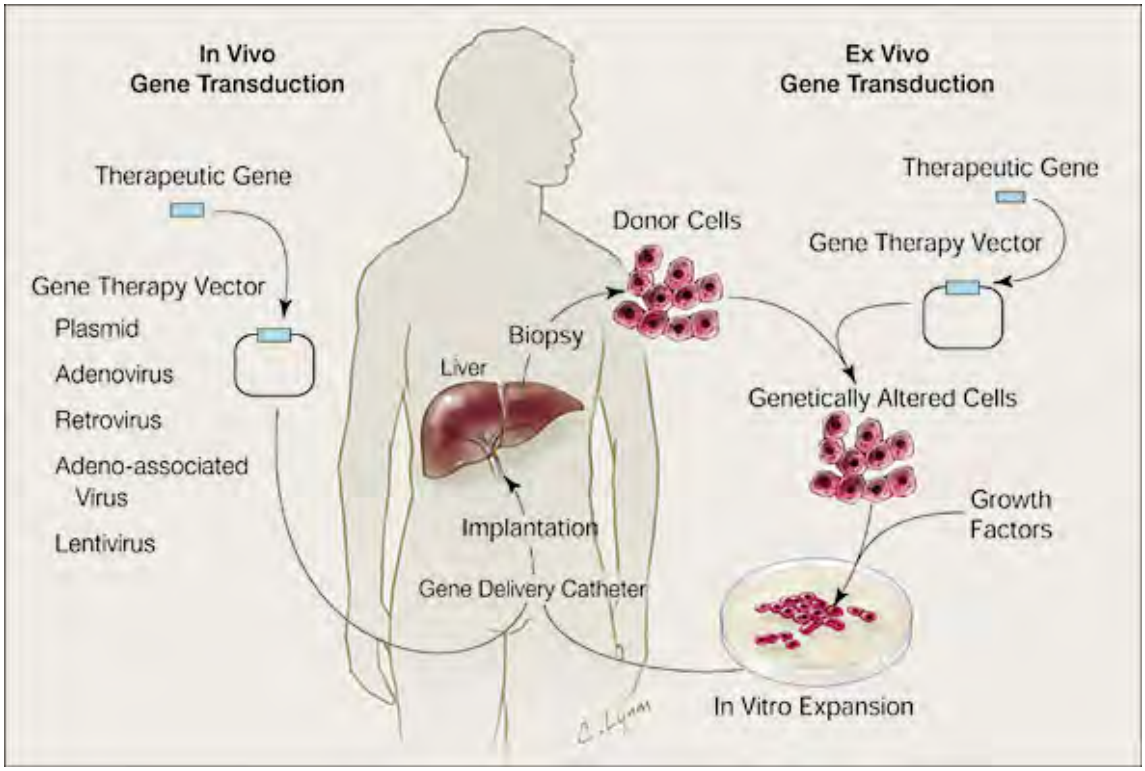

Fig. 1: In-vivo and Ex-vivo gene transduction[7]

2. **In vivo gene therapy**: The direct delivery of the therapeutic gene into the target cells of a particular tissue constitutes in vivo gene therapy. Many tissues are the potential candidates for this approach. For example liver, muscle, skin, spleen, lung, brain and blood cells etc.

The success of in vivo gene therapy mostly depends on the following parameters:

- The efficiency of the uptake of the therapeutic gene by the target cells
- Intracellular degradation of the gene and its uptake by nucleus
- The expression capability of the gene

Techniques of gene transfer (Vectors in gene therapy)

The most fundamental requirement for gene therapy to be successful is to effectively deliver the therapeutic gene to the target cell. The carrier particles or molecules used to deliver genes are referred to as vectors. There are different viral and non-viral vectors for gene delivery. The ideal gene delivery vector should be very specific, capable of efficiently delivering one or more genes of the size needed for clinical application and unrecognized by the immune system. Finally, a vector should be able to express the gene for as long as is required.

Gene delivery by viruses

Viruses are one of the most promising vectors currently being used for gene therapy. Viruses are actually genes wrapped in a protein coat. This coat contains special proteins that can bind to the surface of cells. Once bounded they either force their way in or are sucked into the cell itself. Scientists have tried to take advantage of this capability and manipulate the viral genome and replace them with working human gene. Once the transplanted gene is 'switched on' in the right location within the cells of an infected person, it can then issue instructions for making specific proteins [8].

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

- **Retroviruses**

Retroviruses were the first viruses to be used as vectors in gene therapy experiments. They contain the enzyme reverse transcriptase which can create double- stranded DNA copies of their RNA genomes. These copies of its genome can be integrated into the chromosome of host cell. Although retroviruses have been used in most gene therapy experiments, there are some drawbacks.

The main limitations of retroviral vectors are their low efficiency in vivo, immunogenic problems, the inability to transduce the non-dividing cells and the risk of insertion, which could possibly cause oncogene activation or tumour-suppressor gene inactivation [9,10] .

However, with development of our understanding of the function of nucleases such as zinc finger nucleases in humans, the genes encoding nucleases are incorporated into chromosomes; the expressed nucleases then "edit" the chromosome, disrupting genes causing disease. Treatment of the X- linked severe combined immune deficiency using retroviral vector represent the most successful application of gene therapy till date [(11)].

- **Adenoviruses**

Adenovirus type 2 and 5 can be utilized for transferring both dividing and non-dividing cells and have low host specificity so can be used for gene delivery into large range of tissues. The vectors based on adenovirus are generally used for therapeutic strategies that require the therapeutic gene to be active for only a short time.

However, it is a disadvantage where sustained gene activity is required for many months such as in the treatment of some tumours, neurodegenerative disease and HIV infection. Natural and acute immunologic responses against adenoviruses have made their clinical application limited to a few tissues, such as liver, lung (especially for Cystic Fibrosis), or localized cancer gene therapy [(12,13)].

Although the risk of serious disease following natural adenovirus infection is rare and the viral genome would not integrate into the host genome, gene therapy by adenoviral vectors has caused serious bad side effects and even death of some patients. Recently, in addition to safety of these vectors, several essential genes have been deleted so that viral replication can only occur under control and also most of the viral genome is deleted to obtain sufficient space for transgene particles, this kind of adenoviruses are called "gutless" or "pseudo" adenoviruses.

Fig.2 shows gene therapy using an adenovirus vector.[(14)]

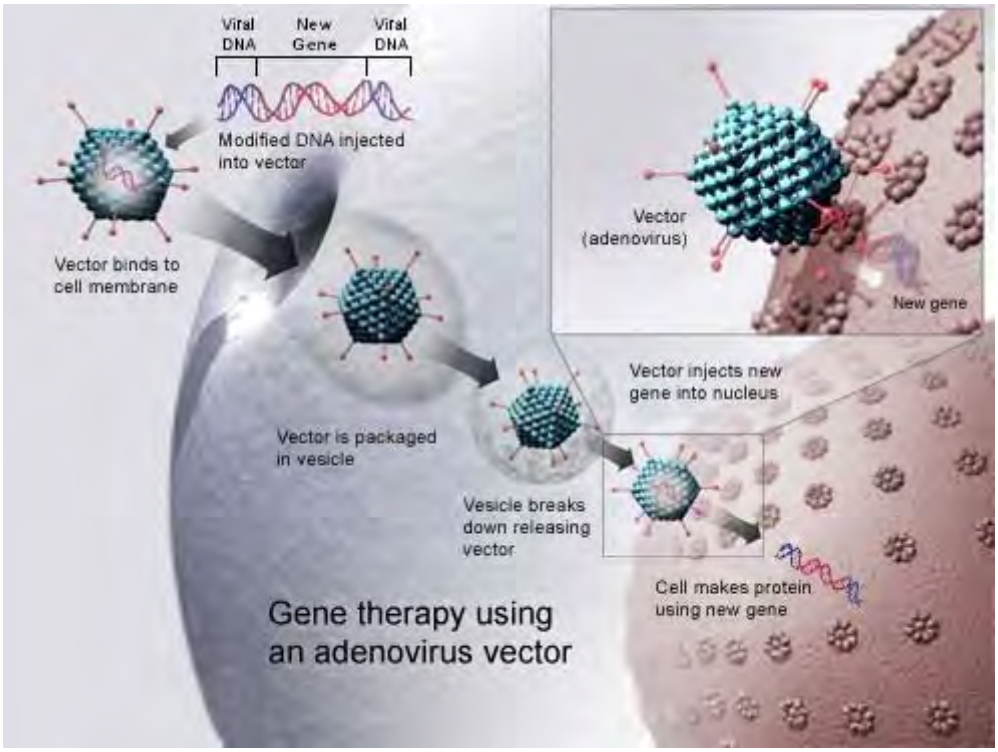

Fig. 2: Adenovirus as a gene delivery vector (14)

- **Adeno- associated viruses[AAVs]**

AAVs are small viruses (parvovirus family) with a single stranded DNA genome. These are like adenoviral vectors in their features but because of having some deficiency in their replication and pathogenicity, are safer than adenoviral vectors. In human, AAVs
are not associated with any disease. It can insert genetic material at a specific site on chromosome 19 with near 100% certainty and do not provoke an immune response. AAVs have been used in the treatment of some diseases, such as Cystic Fibrosis, haemophilia B, Leber congenital amaurosis, and Alpha 1 antitrypsin deficiency.**[(15)]**

As the virus can infect a broad range of cells including both dividing and non-dividing cells, AAV vectors are also being used in clinical trials to deliver genes to brain. The chief drawback of AAV is that its payload is relatively limited and it can also produce unintended genetic damage due to direct insertion of genes into host cell's DNA.

- **Herpes Simplex Virus (HSV)**

It is mostly used for gene transfer in nervous system. Presence of large genome compared to other viruses makes HSV suitable for the treatment of disorders caused by more than one gene defect. Ability to infect a wide range of tissues including neurons makes HSV an ideal vector for gene delivery [16].

- **Lentivirus**

Lentiviruses are a subclass of retroviruses. They have recently been used as gene delivery vectors due to their ability to naturally integrate with non-dividing cells, which is the unique feature of lentiviruses as compared with other retroviruses, which can infect only the dividing cells. Because lentiviruses have strong tropism for neural stem cells, extensively used for ex vivo gene transfer in central nervous system with no significant immune responses and no unwanted side effects. Lentiviral vectors have the advantages of high efficiency infection of dividing and non-dividing cells, long term stable expression of a transgene, low immunogenicity, and the ability to accommodate larger transgenes [17]

Gene delivery by non-viral systems

Non-viral systems comprise all the physical and chemical systems except viral systems. Efficiency of this system is less than viral systems in gene transduction, but their cost effectiveness, availability, and more importantly less induction of immune system and no limitation in size of transgenic DNA compared with viral system have made them more effective for gene delivery than non-viral delivery systems to date.[18]

Physical methods of gene delivery

Physical methods applied for in vitro and in vivo gene delivery are based on making transient penetration in cell membrane by mechanical, electrical, ultrasonic or laser based energy.

Naked DNA

Naked DNA alone is able to transfer a gene into skin, thymus, cardiac muscle, and especially skeletal muscle and liver cells when directly injected, also it has been applied directly. Long term expression has been observed in skeletal muscle following injection for more than 19 months. Although naked DNA injection is a safe and simple method, its efficiency for gene delivery is low so it is only proper for some applications, such as DNA vaccination. Clinical trials to inject naked DNA plasmids have been performed successfully, however, the expression has been very low in comparison to other methods of transfection[19,20].

Electroporation- It is temporary destabilization of the cell membrane targeted tissue by insertion of a pair of electrodes into it so that DNA molecules in the surrounding media of the destabilized membrane would be able to penetrate into cytoplasm and nucleoplasm of the cell but unfortunately the trangene can integrate only to 0.01% of the treated cells. A high rate of cell death following electroporation has limited its use.[21]

Gene guns- DNA particle bombardant by gene gun is an ideal alternative technique to injection of naked DNA. Gold or tungsten spherical particles (1–3 μm diameter) are coated with plasmid DNA and then accelerated to high speed by pressurized gas to penetrate into target tissue cells.[22]

Sonoporation- In this method, Ultrasonic frequencies are used to make nanomeric pores in membrane to facilitate intracellular delivery of DNA particles into cells of internal organs or tumors, so the size and concentration of plasmid DNA have great role in efficiency of the system.The most important limitation of the system is low efficiency.[23]

Magnetofection- In this method the magnetic fields are used to concentrate particles containing nucleic acid into the target cells. Magnetofection is a simple and efficient transfection method that has the advantages of the nonviral biochemical (cationic lipids or polymers) and physical (electroporation, gene gun) transfection systems in one system while excluding their inconveniences, such as low efficiency and toxicity.[24]

Chemical methods of gene delivery

Chemical systems are more common than physical methods and generally are nanomeric complexes, which include compaction of negatively charged nucleic acid by polycationic nanomeric particles, belonging to cationic liposome/micelle or cationic polymers.

Lipoplex and polyplex :The nanomeric complex between a cationic liposome and nucleic acids is called lipoplex; but polyplex is the nanomeric complex formed between a cationic polymer and nucleic acids.

Oligonucleotides- The aim of using synthetic oligonucleotides in gene therapy is to inactivate the genes involved in the disease. One strategy uses antisense specific to target gene to disrupt the transcription of the faulty gene. Another approach uses small molecules of RNA called as short interfering RNA or Si RNAs which signal the cell to cleave specific sequences in the mRNA transcript of the faulty gene, disrupting translation of the faulty mRNA. This RNA interference or gene silencing may be used to treat Huntington's disease.

Dendrimers-A dendrimer is a highly branched macromolecule with a spherical shape. In the presence of genetic material, charge complementarities leads to a temporary association of the nucleic acid with the cationic dendrimer which is then taken into cell via endocytosis.

Hybrid methods- Recently there have been some hybrid methods developed that combine two or more techniques. For example- Vibrosomes that combine liposome with an inactivated HIV or influenza virus. This has been shown to have more efficient gene transfer in respiratory epithelial cell than either method alone.
Human artificial chromosome- Researchers are also experimenting with introducing a 47th (artificial human) chromosome into target cells. Scientists anticipate that it would be able to carry substantial amount of genetic code and because of its autonomy and construction, would not be attacked by body's immune system.

Historical Perspectives and Current status of Clinical Trials

The first successful gene therapy trial was performed on a 4 year old girl named Ashanti Desilva, with a rare genetic immune system disorder called severe combined immune deficiency (SCID) on 14 September 1990 at the National Institute of Health, under the direction of professor William French Anderson[(25)]. In 1992, Claudio Bordignon of Italy performed the first procedure of gene therapy using hematopoietic stem cells as vectors to correct hereditary disease.[(26)]

In 2003, Los Angeles research team used liposome coated in a polymer to insert genes into brain. This method has potential for treating Parkinson's disease. The transfer of genes into brain is a significant achievement because viral vectors are too big to get across the blood brain barrier.[(27)] One of the first demonstrations of effectiveness of gene therapy in treating cancer comes from the success of the scientists at the National Institute of Health who successfully treated metastatic melanoma in two patients using killer T cells genetically retargeted to attack the cancer cells.

In 2007, Moorefield's Eye Hospital and University College London's Institute of Ophthalmology announced the world's first gene therapy trial for a type of inherited retinal disease i.e. Leber's Congenital amaurosis, which is caused by a mutation in the RPE65 gene. Sub-retinal delivery of recombinant AAV carrying RPE65 gene yielded positive results with no apparent side- effects.[(28)]

A paper by Komaromy et al. published in April 2010, deals with gene therapy for a form of achromatopsia (complete colour blindness) in dogs. It is presented as an ideal model to develop gene therapy directed to cone photoreceptors. In July 2012, the European Medicines Agency recommended approval of a gene therapy treatment called Adipogene tiparvovec (Glybera) which compensates for the lipoprotein lipase deficiency.[(29,30)]

In April 2013, researchers in the UK and the US performed gene therapy experiments to combat heart disease. The clinical trials were designed to increase the level of SERCA2a protein in the heart muscles to improve their function.[(31)] In July 2013, the Italian San Raffaele Telethon Institute for gene therapy reported that the treatment of two severe hereditary diseases i.e. Metachromatic Leukodystrophy and Wiskott- Aldrich Syndrome yielded positive results after 7-32 months of gene therapy.[(32)]

In January 2014, researchers at the University of Oxford reported an improvement in the sight of six people suffering from choroideremia, an inherited genetic eye disease. These patients had been treated with a genetically engineered AAV with a copy of REP1 gene.[(33)]

In March 2014, researchers at the University of Pennsylvania reported that 12 patients with HIV had been treated since 2009 in a trial with a genetically engineered virus with a rare mutation known to protect against HIV (CCR5 deficiency).[(34)]

Speculative uses for gene therapy

✓ **Gene doping**

A number of gene therapies have potential applications to athletic enhancement and the gene therapy technologies might be abused to improve athletic performance. This is known as gene doping.[(35)]

✓ **Human genetic engineering**

It has been speculated that genetic engineering could be used to change physical appearance, metabolism and even improve physical capabilities and mental faculties. These speculations have led to ethical concerns and claims, including the belief that every foetus has an inherent right to remain genetically unmodified.[(36)]

Pros and cons of gene therapy

The positive aspect of gene therapy is apparent. Gene therapy is a "medicine" for the future since it can wipe out genetic diseases before they can begin and eliminate suffering for future. However, no therapy is without some associated risks. Some of the problems associated with gene therapy are:

- Immune response- Genes injected with a virus may trigger an immune response in the body.
- Multigene disorders- Disorders arising from single gene mutation are best candidates for gene therapy. Unfortunately, some of the most commonly occurring diseases, such as heart disease, hypertension, Alzheimer's disease, arthritis and diabetes are multigene disorders.
- Insertional mutagenesis- If the DNA is integrated in wrong place in the genome, for example in a tumour suppressor gene, it could induce a tumour.
- Problems with viral vectors- The viral vector may recover its ability to cause disease.

- Short-lived nature

There are several ethical and legal issues associated with gene therapy. A review board, the Recombinant Advisory Committee (RAC) has been developed to address these concerns. The consequences of gene therapy are many.

The first issue targets putting human fate in our own hands. Some people are concerned that gene therapy could be used for any genetically linked trait such as eternal appearance, personality or physical enhancement.

Another great concern is religion. Some consider it sinful to manipulate DNA. If religion is a factor then somatic cell therapy should be applied which allows the next generation to make their own decision. In addition to ethical issues, one of the major concerns is the cost of gene therapy. However, scientists are optimistic that the cost will be much cheaper in future.

The last but not the least is the risk of the procedure. Since gene therapy is still in its developmental stage, finding the precise location of the gene and replacing with a normal one is definitely a challenge. But it is true that with the invention of new and advanced techniques, researchers will soon be able to achieve a great success in this applied modern science.[37]

Conclusion

Theoretically, gene therapy is the permanent solution for genetic diseases. But it is not as simple as it appears since gene therapy has several inbuilt complexities. Gene therapy is both beneficial and harmful depending on how it is applied. The advantage of gene therapy is to cure someone who is born with a genetic disorder or who develops deadly diseases like AIDS, cancer etc. The government, the public groups and the scientific society should cooperate and walk hand in hand to encourage gene therapy applications. A breakthrough may come anytime and a day may come when almost every disease will have a gene therapy, as one of the treatment modalities.

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