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GENE THERAPY, FUTURE APPROACH FOR TREATMENT: A REVIEW

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Abstract

Gene therapy is a novel technique that uses genes from several sources to treat or prevent different diseases. This technique allows treating a disorder (including inherited disorders, some types of cancer, and certain viral infections) by inserting a gene into a patient's cells instead of using drugs or surgery. The principle of this technique includes replacing, inactivation or introducing a new gene in to the body which is use for metabolic manipulation, gene augmentation or surgical approaches. Gene therapy using several techniques in inserting a new gene which either by using viral vectors like (Retroviruses, Adenoviruses) or non-viral vectors likes (injection of naked DNA, physical methods to enhance delivery gene like (electroporation, sonoporation) or chemical methods to enhance delivery gene like (oligonucleotides, hybrid methods). This new technique has many advantages and disadvantages besides the presence of many ethical and social considerations that make it practically difficult to apply. The conclusion scientists believe that gene therapy is the most promising application for treatment different types of diseases. Gene therapy is uprising in the field of medicine; scientists believe that after 20 years, this will be the last cure of every genetic disease.

Key words: Chemical methods, DNA, Gene, Gene therapy, Physical methods

Introduction

Each human being carries normal as well as some defective genes. Usually, the individual does not become aware of the presence of defective gene until a disease associated with the gene is manifested in him or her or in a relative (Maria *et al*., 2018). More than 4,000 medical disorders caused by defective genes have been identified, each with varying degrees of seriousness. About 10% of the human population will evidence, sooner or later, some type of disorder (Aunan *et al.*, 2017). Although genes are responsible for predisposition to disease, the environment, diet, and lifestyle can affect the onset of the illness (*Keane et al.,* 2008).

An example of a genetic disease is the cystic fibrosis, which frequently becomes evident in the first years of life for the child carrying the defective gene; where, the mutant gene can cause the development of cysts and fibrous tissue in the patient's pancreas and the production of thick and viscous lung mucous, which can make breathing very difficult and, in many cases, is fatal (Gershman *et al.,* 2006).

One possibility of treatment is a genetically engineered virus, carrying the corrective gene, which after being introduced into the patient's lung cells would allow the lungs to function properly and to produce cystic fibrosis transmembrane conductance regulator protein (CFTR protein) that eliminates the mucus (Gardner 2007).

Some genetic diseases have some benefits, classic example of a genetic disease that has a beneficial effect on human survival disease is a sickle cell anemia; where, individuals carrying two copies of the defective hemoglobin (Hb) gene and a blood problem caused by the defective Hb and consequently misshapen red blood cells (RBCs). The mutant Hb has less affinity to oxygen, and becoming a poor oxygen transporter in the blood (MacKenzie *et al.,* 2014). However, carriers of a single copy of the defective allele do not have the disease and they are also resistant to malaria. There is an obvious advantage of carrying a single allele of the defective Hb gene, especially in regions where malaria is endemic, as in tropical regions of Africa (*Bartoloni et al.,* 2012). Gene therapy is a technique for correcting defective genes responsible for the development of disease. Researchers may use one of several approaches for correcting faulty genes (Patil *et al.,* 2012).

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Principles of Gene Therapy (Kohn, 2010)

A normal gene may be inserted into a nonspecific location within the genome to replace a nonfunctional gene. This approach is the most common.

An abnormal gene could be swapped for a normal gene through homologous recombination.

The abnormal gene could be repaired through selective reverse mutation, which can return the gene to its normal function.

Gene therapy is the insertion, alteration, or removal of genes within an individual's cells and biological tissues to treat diseases. It is a technique for correcting defective genes that are responsible for disease development (Kohn, 2010). Fig. 1.

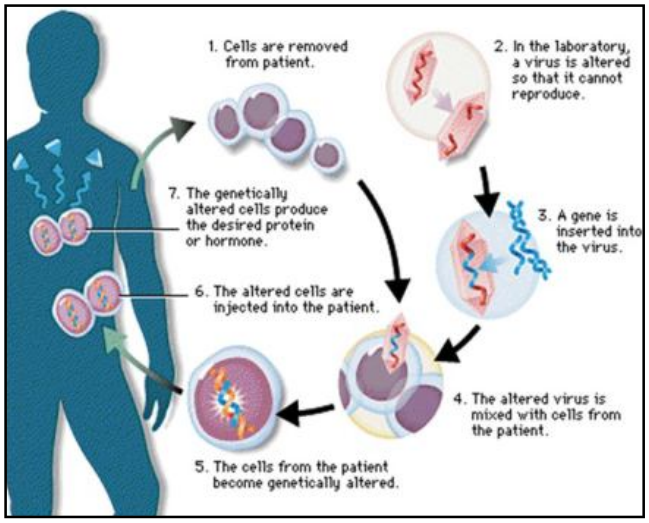

Fig. 1: Flow chart shows gene therapy (Kohn , 2010).

Approaches of Gene Therapy

There are several applications for gene therapy (Randy *et al.,* 2016; Moore *et al.,* 2014).

Metabolic Manipulation

Physicians have developed approaches to regulate the metabolic pathways associated with a number of disorders, like phenylketonuria (PKU) (Grisch-Chan *et al.,* 2019), hereditary angioedema (Rohan *et al.,* 2016), familial hypercholesterolemia (Van *et al.,* 2011) and many others. In other cases, metabolic manipulation may involve the use of small molecules or drugs to target the activity of proteins linked to disease. For instance, familial hypercholesterolemia is associated with high levels of low density lipoprotein-cholesterol (LDL-C) and early heart disease; in this case, treatment can include both dietary modifications (a diet low in cholesterol) and the administration of a class of drugs called statins; where, these medications can inhibit the activity of an enzyme 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMGR) that involved in a rate-limiting step of cholesterol biosynthesis (Ezim *et al.,* 2016).

Gene Augmentation

In another approach called gene augmentation, physicians can treat patients by providing them with a purified form of the missing, defective, or depleted gene (Eyal *et al.,* 2015). This gene -add-back approach has been used to successfully treat patients suffering from a wide range of diseases, including various membrane transport disorders (cystic fibrosis) (Iwona *et al.,* 2019), coagulation disorders (hemophilia A, hemophilia B and Von Willebrand disease) (Manno, 2002), emphysema (α1-antitrypsin deficiency) (Maria *et al.,* 2016) and many other diseases. Fig. 2.

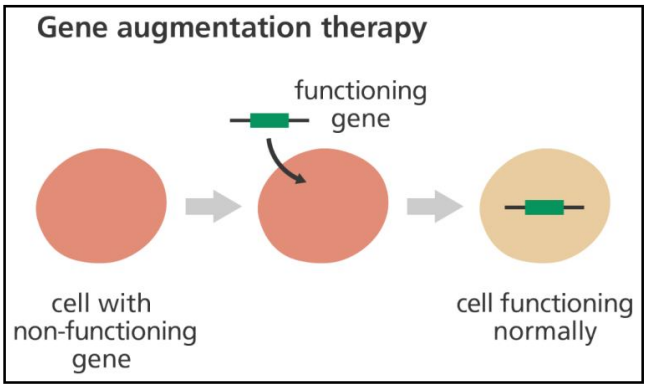

Fig. 2: Gene Augmentation Therapy (Eyal *et al,* 2015).

Surgical Approaches

Although more invasive, organ transplantation is also used to treat certain genetic diseases that affect particular organs (Timothy *et al.,* 2011). Unless the organ donor and the organ recipient are monozygotic twins, the chromosomal DNA sequence of the donor will be different from that of the recipient (Blodi , 2004). Despite these differences, organ transplantation remains a viable therapy that continues to be used widely to this day. The example for this type is Intravitreal (IVT) injection is a widely-used technique to deliver therapeutic agents; the most common being drugs inhibiting vascular endothelial growth factors, antibiotics and glucocorticoids. IVT injections are one of the most commonly performed ocular surgery procedures in the developed world, second only to cataract surgery (Birch *et al.,* 2007). Fig. 3.

Vectors in Gene Therapy

There are several vectors for gene (Rochat *et al.,* 2002)

Viral vectors

Some of different types of viruses can be used as gene therapy vectors Fig. 4:

Retroviruses: A class of viruses that can create

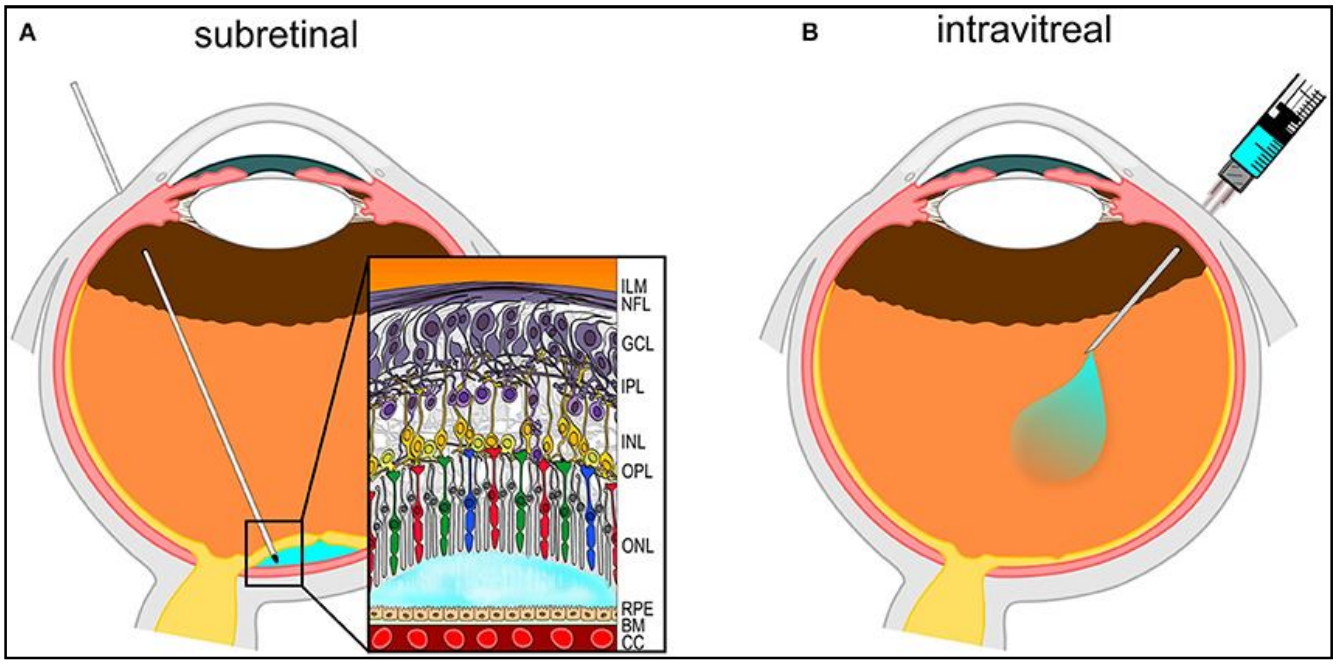

Fig. 3: surgical approach for gene therapy (Birch *et al,* 2007).

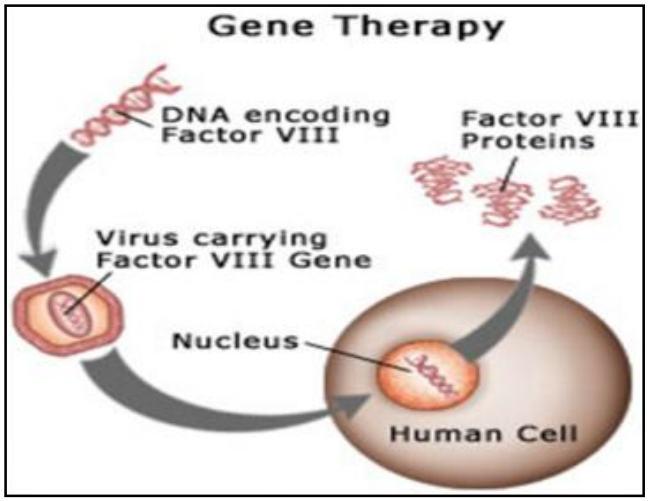

Fig. 4: Viral method of gene therapy (Flavia *et al,* 2006).

double-stranded DNA copies of their RNA genomes. These copies of its genome can be integrated into the chromosomes of host cells. An example of a retrovirus is the Human immunodeficiency virus (HIV) (Flavia *et al.,* 2006). One of the problems of gene therapy using retroviruses is that, the integrase enzyme can insert the genetic material of the virus into any arbitrary position in the genome of the host; it may randomly insert the genetic material into a chromosome; thus, if the genetic material happens to be inserted in the middle of one of the original genes of the host cell, this gene will be disrupted (insertion mutagenesis); furthermore, if the gene happens to be one regulating cell division, uncontrolled cell division (*i.e.* cancer) can occur. This problem has begun to be addressed by utilizing zinc finger nucleases or by including certain sequences such as the beta-globin locus control region to direct the site of integration to specific chromosomal sites (Youngsuk *et al.,* 2011).

Adenoviruses: A class of viruses with double-stranded DNA genomes that cause respiratory, intestinal, and eye infections in humans. The virus that causes the common cold is an adenovirus (Wold *et al.,* 2013).

Herpes simplex viruses: A class of double-stranded DNA viruses that can infect a particular cell type, neurons. The herpes simplex virus type 1 is a common human pathogen that causes cold sores (Veijo, 2010).

Non-Viral Methods

Non-viral vectors are simple in theory but complex in practice. Apart from intracellular and extracellular barriers, number of other challenges also needs to be overcome in order to increase the effectiveness of non-viral gene transfer. These barriers are categorized as production, formulation and storage. No one-size-fits-all solution to gene delivery, which is why in spite of various developments in liposome, polymer formulation and optimization, new compounds are constantly being proposed and investigated (Murali *et al.,* 2015).

Injection of Naked DNA

This is the simplest method of non-viral transfection. Clinical trials carried out of intramuscular (I.M.) injection of a naked DNA plasmid have occurred with some success; however, the expression has been very low in comparison to other methods of transfection (Audouny *et al.,* 2002, Varga, 2001).

Physical Methods to Enhance Delivery Gene

Electroporation

The other terms used for electroporation are gene electro injection, gene electro transfer, electrically mediated gene therapy, electro gene transfer. Applying an electric field that is greater than the membrane capacitance will cause charges of opposite polarity to line up on either side of cell membrane thus forming a potential difference at a specific point on the cell surface. As a result membrane breakdown form a pore and allows the molecule to pass. Pore formation occurs in approximately 10 nanoseconds. The pore of the membrane can be reversible based on the field strength and pulse duration. If it is reversible cells remain viable, otherwise cell death results as shown in Fig. 5 (Heller *et al.,* 2010).

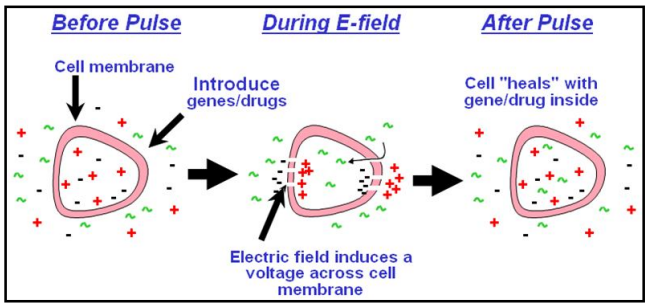

Fig. 5: Principle of electroporation (Heller *et al,* 2010).

Gene Gun

The use of particle bombardment, or the gene gun, is another physical method of DNA transfection. In this technique, DNA is coated with gold particles and loaded into a device which generates a force to achieve penetration of DNA/gold into the cells. Furthermore, researchers reported that if the DNA is integrated in the wrong place in the genome, for example in a tumor suppressor gene, it could induce a tumor. This has occurred in clinical trials for X-linked severe combined immunodeficiency (X-SCID) patients, in which hematopoietic stem cells were transduced with a corrective transgene using a retrovirus, and this led to the development of T cell leukemia in 3 of 20 patients(Yang *et al.,* 2004).

Sonoporation

Sonoporation is a method that uses ultrasonic frequencies to deliver DNA into cells. The process of acoustic cavitation is thought to disrupt the cell membrane and allow DNA to move into cells (Liang *et al.,* 2010) as shown in Fig. 6.

Magnetofection

In a method termed magnetofection, the DNA is complexed to magnetic particles, and a magnet is placed underneath the tissue culture dish to bring DNA complexes into contact with a cell monolayer (Holzbach *et al.,* 2010). Fig. 7.

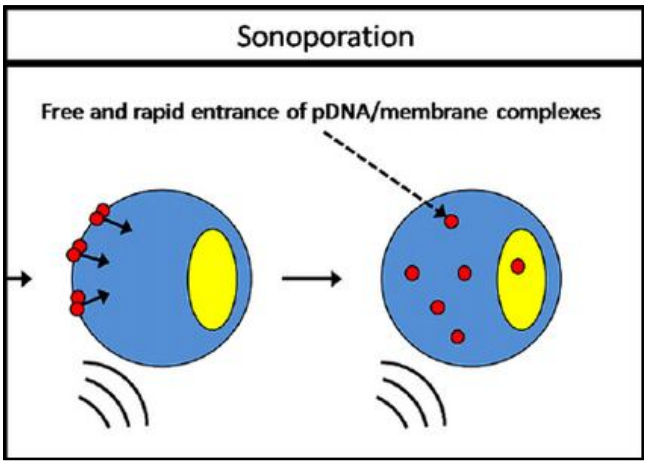

Fig. 6: Principle of sonoporation (Liang *et al,* 2010).

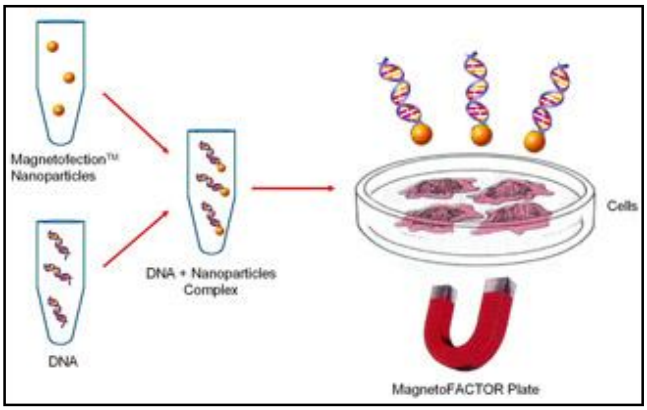

Fig. 7: Principle of magnetofection (Holzbach *et al,* 2010).

Chemical Methods to Enhance Delivery of Gene

Oligonucleotides

The use of synthetic oligonucleotides in gene therapy is to inactivate the genes involved in the disease process. There are several methods by which this is achieved (Karin *et al.,* 2015). One strategy uses antisense specific to the target gene to disrupt the transcription of the faulty gene (Joshua *et al.,* 2013). Another uses small molecules of RNA called siRNA to signal the cell to cleave specific unique sequences in the mRNA transcript of the faulty gene, disrupting translation of the faulty mRNA and therefore expression of the gene (Elizabeth *et al.,* 2008). A further strategy uses double stranded oligodeoxynucleotides as a decoy for the transcription factors that are required to activate the transcription of the target gene. The transcription factors bind to the decoys instead of the promoter of the faulty gene, which reduces the transcription of the target gene, lowering expression (Mahato *et al.,* 2005).

Hybrid methods

The development of novel and safer vector tools for stable maintenance of therapeutic DNA and transgene products within a cell, especially in rapidly dividing cells (*e.g.,* bone marrow derived cells), is of great interest to the research community(Huang *et al.,* 2013). For instance therapies for these genetic diseases would benefit from such tools because they rely on stable production of the defective gene product and subsequent long-term phenotypic correction (Jager *et al.,* 2009). Furthermore, these vectors would circumvent repeated vector administration necessary for life-long correction. Moreover, inconveniences for the patient due to repeated drug administration could be avoided (Nadine *et al.,* 2009). Virosomes are one example; they combine liposomes with an inactivated HIV or influenza virus. This has been shown to have more efficient gene transfer in respiratory epithelial cells than either viral or liposomal methods alone. Other methods involve mixing other viral vectors with cationic lipids or hybridising viruses (Kaneda, 2012).

Advantages of Gene Therapy

There are several advantages of gene therapy (Jafarlou *et al.,* 2016).

1. It offers hope.
2. Genetic disorders can be treated.
3. It may treat more than just disease.
4. It would create a new field of medicine.
5. Gene therapies aren't limited to humans.
6. Gene therapy is based on technology.

Disadvantages of Gene Therapy

There are several disadvantages of gene therapy (Cotrim *et al.,* 2008).

1. It is a costly treatment option.
2. Nature is adaptable.
3. It may unlock unethical forms of science.
4. Gene therapies have been stuck in trials for a generation for a good reason.
5. It may encourage gene doping.
6. It can provide a false hope.

Ethical and Social considerations

The concept of changing a person's DNA, even to cure a fatal genetic disease, differs from more traditional remedies like surgery, pharmaceuticals, and physical therapy, and it is frightening to some people (Isaac , 2003). Successful treatment approaches are available for a handful of single-gene disorders, most of which are enzyme deficiencies, like Gaucher's disease (a lysosomal storage disorder) (Fabrega *et al.,* 2002). Other disorders, including Duchenne muscular dystrophy (DMD), are the result of the complete loss of a functional protein. DMD is an X-linked recessive disorder that is caused by a mutation in the dystrophin gene. Mutation of this gene results in failure of muscle regeneration, leading to progressive muscle weakness and eventually causing fibrosis in essential organs (*i.e.,* the heart and diaphragm). At this time, no medical, surgical, or other option exists to correct the underlying genetic cause of DMD and preserve muscle function in affected males. For individuals with DMD, the promise of gene therapy through one of the methods listed above means the possibility of leading a normal life (Julian *et al.,* 2015).

Some of the ethical considerations for gene therapy include:

- Deciding what is normal and what is a disability.
- Deciding whether disabilities are diseases and whether they should be cured.
- Deciding whether searching for a cure demeans the lives of people who have disabilities.
- Deciding whether somatic gene therapy is more or less ethical than germ line gene therapy Initial experiments using gene therapy have been conducted primarily in patients for whom all other treatments have failed, so that the risks are small. Many people feel that because gene therapies use altered genes and potentially dangerous viruses, those treatments should be tested more extensively (Kasuya *et al.,* 2002).

Applications of Gene Therapy

Since 1989, the year of the first gene therapy clinical trial, over 1500 clinical studies have been conducted involving several tens of thousands of patients. If objectively evaluated, the overall clinical success of these trials has been modest so far (Ulrike *et al.,* 1996). With some remarkable exceptions, most of the trials have encountered unanticipated technological and biological problems. In this evaluation, however, it should be taken into account that the vast majority of the diseases faced by gene therapy are life-threatening conditions, for which no conventional medical therapy exists, and that gene therapy is a completely new discipline, both conceptually and technically (Marta *et al.,* 2017).

Indeed, 20 years after the first application, 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 (Cicalese *et al.,* 2015). The recent results obtained by gene therapy of inherited blindness (Dejneka *et al.,* 2004) and of some neurodegenerative disorders, as well as the progress made in several other clinical trials, now encourage informed and firm optimism on the eventual success (Sudhakar *et al.,* 2019).

List of clinical conditions that submitted to gene therapy trials;

- In Parkinson's diseases (PD) (Tobias *et al.,* 2018).
- In Alzheimer's disease (AD) (Combs *et al.,* 2016).
- In Diabetic Neuropathy(Marina *et al.,* 2008)
- In Metastatic Melanoma (Menezes *et al.,* 2018)
- Cancer. (Gordon *et al.,* 2004)
- Atherosclerosis (Kivela *et al.,* 2015)

Conclusion

Most scientists believe that the potential for gene therapy is the most exciting application of DNA science, yet under taken. How widely this therapy will be applied, this may depend on the simplification of procedure. As gene therapy is uprising in the field of medicine, scientists believe that after 20 years, this will be the last cure of every genetic disease. Genes may be used as medicine and given as simple intravenous (I.V.) injection of gene transfer vehicle that will seek the target cells for stable, site-specific chromosomal integration and subsequent gene expression. Moreover a draft of the human genome map is now complete; thus, research is focusing on the function of each gene and the role of the faulty gene play in disease.

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