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A REVIEW ON GENE THERAPY FOR THE GENETIC REVOLUTION

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Abstract:

The ability to make site-specific modifications to the human genome has been an objective in medicine since the recognition of the gene as the basic unit of heredity. Thus, gene therapy is understood as the ability of genetic improvement through the correction of altered (mutated) genes or site-specific modifications that target therapeutic treatment. It is the concept of gene therapy involves the transfer of genetic material in to a cell, tissue, or whole organism to cure a disease and improve the Patient compliance. Gene therapy is to transform viruses into genetic material, which will deliver the gene of interest into the target cells. Based on the nature of the viral genome, these gene therapy vectors can be divided into RNA and DNA viral vectors or non viral vectors. The majority of virus-based vectors have been derived from simple retroviruses adenoviruses, Adeno-associated viruses, herpes simplex viruses and non viral based vectors are naked DNA, electroporation, gene gun method etc. Non-viral vectors are far less efficient than viral vectors, but they have advantages due to their low immunogenicity and their large capacity for therapeutic DNA. Disease potentially treated by gene therapy is cancer, neurological disorder, infectious diseases, inherited disease cardiovascular disease.

Key words: *Ex vivo, In vivo methods of gene therapy, Lentivirus vector system Adeno-associated viruses, Electroporation, gene gun method.*

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INTRODUCTION:

Gene therapy is a technique that uses genetic material (a piece of DNA) for the long-term treatment of genetic or hereditary disorders. It is a technique delivering a copy of a healthy or correct or therapeutic gene for repairing a faulty gene. Simply gene therapy involves inserting a normal gene to replace an abnormal gene. Other approaches used in gene therapy are as follows:

- Replacement of mutated gene that causes disease with the healthy copy or corrective gene of the gene.
- Inactivation of mutated genes that are functioning improperly.
- Introducing a new or corrective gene into the body to help cure a disease
- Change the regulation of gene pairs

Heredity functional unit are the Genes, that are specific sequences bases that encode instructions to make proteins as well as genes get a lot of attentions, it is the proteins that perform most functions of life. If genes are altered then, encoded proteins are not carry out their normal functions and causes genetic disorders. Gene therapy (use of genes as medicines) is basically to correct defective genes responsible for genetic disorder by one of the following approaches [1,2].

Gene therapy has been defined as 'nucleic-acid based treatment, or transfer of DNA/RNA to somatic target cells in the intention to treat serious illness'. In somatic gene therapy, new genes are introduced into body (somatic) cells. The therapy will only affect the treated person, and not future generations. In germ cell therapy, the human germline is modified, and the genetic alterations would be transferred to offspring. The latter is not considered ethical, and is not permitted in any country in the world.

Advantages of gene therapy

1. Targate the specific types of cells.
2. Gene therapy can potentially be used to treat genetic disorders with single or few administrations.
3. Reducing frequent dosing, improving quality of life and reducing the need for visits to hospital..
4. Gene therapy also offers the potential to specifically target the affected tissues within the body.
5. Gene therapy prevent and eliminate hereditary diseases such as **cystic fibrosis** and is a possible cure for **heart disease**, **AIDS** and **cancer.**
6. Gene therapy certainly choose especially if it was the last hope for them or one of their loved ones as is the case for many gene therapy patients.
7. Gene expression can be controlled

Disadvantages of Gene Therapy

1. Short-lived nature of gene therapy:

DNA introduced into target cells must remain functional and cells containing the therapeutic DNA must be long-lived and stable. Problems with integrate therapeutic DNA molecule into the genome and the rapidly dividing nature of many cells prevent gene therapy from achieving any long-term benefits.

2. Immune response:

Any time a foreign object is introduced into human tissues, the immune system get activated and produce immune responses, that reduces gene therapy effectiveness is always a possibility. Further rmore, the immune system's enhanced response and difficulty for gene therapy to be repeated in patient.

3. Problem with viral vectors:

Viruses transporters are choice in most gene therapy studies, present a variety of possible problems to the patients toxicity, immune and inflammatory responses and gene manage and targeting issues. In addition, there is always the fear that viral vector, once inside the patient, may recover or its ability to cause disease.

4. Multigenic disorders:

Conditions or disorders that arise from mutation in a single gene are best candidates for gene therapy. Unfortunately, some of the most commonly occurring disorders, such as **heart disease, high blood pressure, Alzheimer's disease, arthritis and diabetes,** are caused by the combined effects of variations in many genes. Multigenic disorders would be especially difficult to treat effectively using gene therapy.

5. Insertional mutagenesis:

The main problem that geneticists are encountering is the virus may target the incorrect cells. If the DNA is incorporated in the wrong place in the genome, for example in a tumor suppressor gene, it could induce a tumor.

Approaches of gene therapy

1. Gene modification
   - Replacement therapy
   - Corrective Gene therapy
2. Gene transfer
   - Physical
   - Chemical
   - Biological
3. Gene transfer in specific cell line
   - Somatic gene therapy
   - Germ line gene therapy

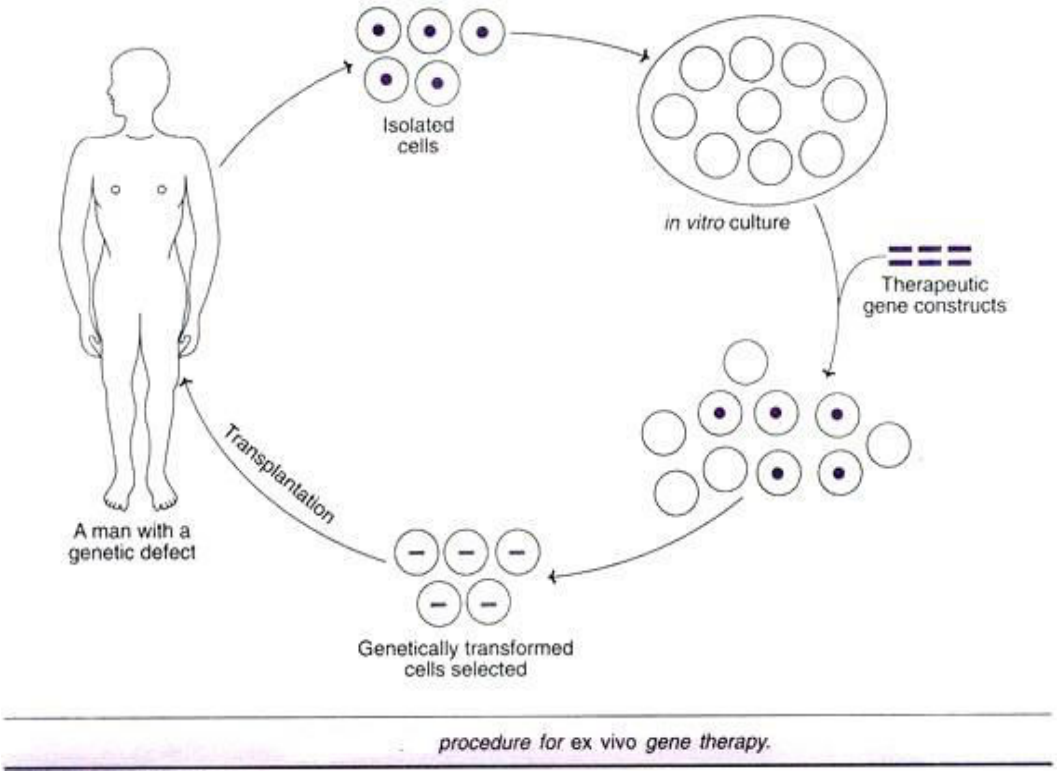
The basic concept of gene therapy is simple:

introduce into target cells a piece of genetic material that will result in either a cure for the disease or a slowdown in the progression of the disease. To achieve this goal, gene therapy requires technologies capable of gene transfer into a wide variety of cells, tissues, and organs. The process of gene delivery and expression is known as transduction. Successful transduction requires overcoming a number of obstacles that are common to all vector systems .Researchers are studying gene therapy for a number of diseases, such as Severe combined immuno-deficiencies (SCID) Parkinsons disease Cancer Hemophilia HIV etc.

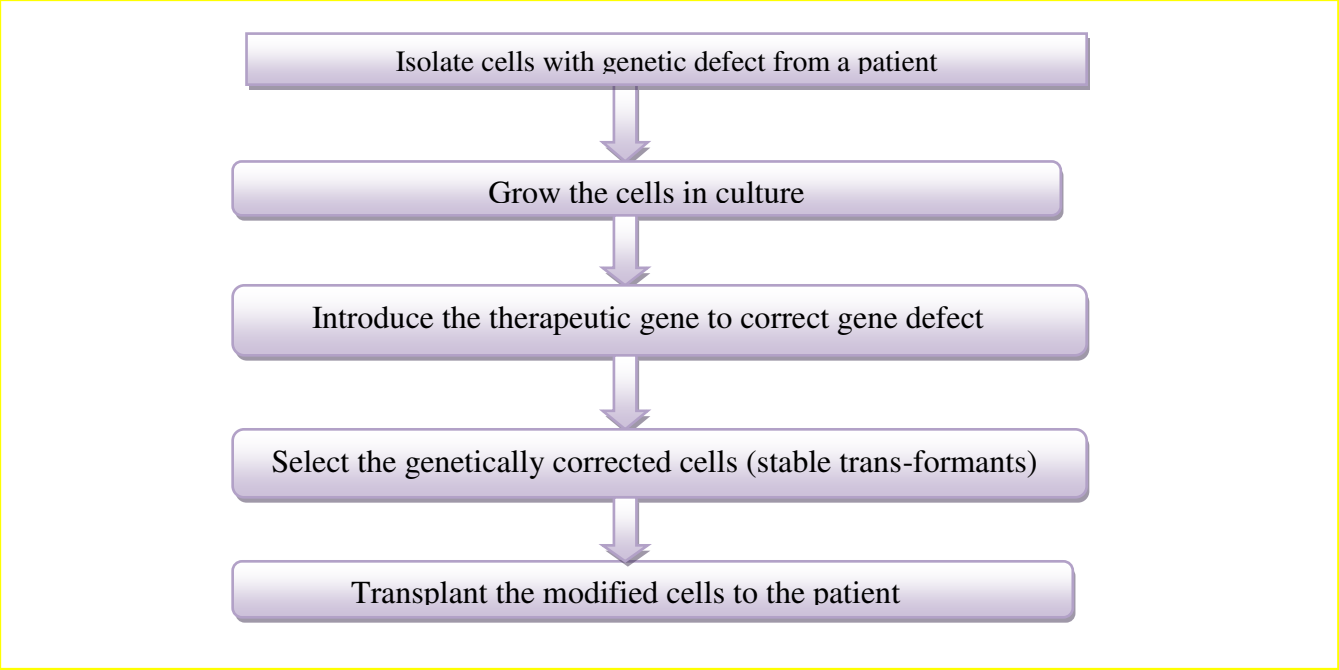
Methods of gene therapy: there are mainly two methods

1. Ex vivo method
2. In vivo method

I) Ex vivo method The ex vivo gene therapy can be applied to only selected tissues (e.g., bone marrow) whose cell cultured in the laboratory.The technique of ex vivo gene therapy involves the following steps (**Figure-1**:Ex vivo method)



procedure for ex vivo *gene therapy.*



The procedure basically involves the use of the patient's own cell for culture and genetic correction, and then their return back to the patient.This technique is therefore, not associated with adverse immunological responses after transplanting the cells. Ex vivo gene therapy is efficient only, if the therapeutic gene (remedial gene) is stably incorporated and continuously expressed. This can be a achieved by use of vectors [3].

II) *In vivo* gene therapy: The direct delivery of the therapeutic gene (DNA) into the target cells of a particular tissue of a patient constitutes in vivo gene therapy (fig). Many tissues are the potential candidates for this approach.These include liver, muscle, skin, spleen, lung, brain and blood cells. Gene delivery can be carried out by viral and non viral vector systems. These success of invivo gene therapy mostly depends on the following parameters

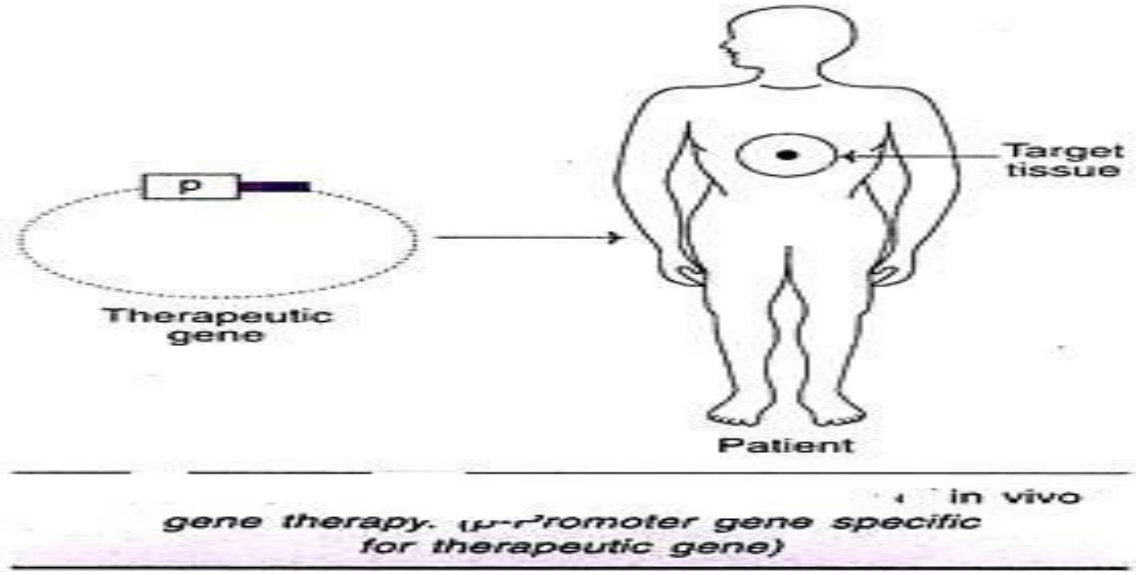


Figure- 2*: In vivo* method of gene therapy

The efficiency of the uptake of the remedial (therapeutic) gene by the target cells

↓

Intracellular degradation of the gene and its uptake by nucleus

↓

The expression capability of the gene

In vivo gene therapy with special reference to gene delivery systems (viral, non-viral) with suitable examples is described[3,4].

Difference between invivo and exvivo gene delivery system

Invivo	Exvivo
Less invasive	More invasive
Technically simple	Technically complex
Vector introduced directly	No vector introduced directly
Safety check not possible	Safety check possible
Decrease control towards target cell	Close control possible

Gene transfer techniques:

Vectors in gene therapy: To transfer the desired gene into a target cell, a carrier is required such vehicles of gene delivery are known as vectors. Mainly two classes, Viral & Non-viral methods.

1. Viral Vectors: Uses recombinant Viruses.
2. Non Viral Vectors: Uses naked DNA or DNA complexes.

It is recognized as powerful strategy for gene therapy. Some of the important viral vectors include,

1. Retrovirus
2. Adenovirus
3. Adenoassociated virus
4. Herpes simplex virus

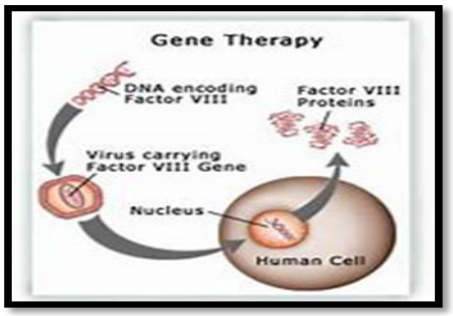


Figure 3- Viral method of gene therapy

Viral vectors viruses

Introduced their genetic material into the host cell as part of their replication cycle. Remove the viral DNA and using the virus as a vehicle to deliver the therapeutic DNA. The viruses used are altered to make them safe although some risks still exist with gene therapy.

Basic concept of viral vectors is to control the natural ability of viruses to deliver genetic material into the infected cell. In general, the viruses are replicate and produce copious amounts of progeny. Most viruses gain little by killing the host, but unfortunately many viral infections lead to toxic effects on the host, accompanied by damage of infected host cells. Damaging effects can be caused by induction of genes whose products are dangerous to the host or by acquiring host genomic material that can lead to pathogenesis. The basic principle of turning these pathogens into delivery systems relies on the ability to separate the components needed for replication from those are responsible for causing disease . The first step of viral vector design is to identify the viral sequences required for replication, assembly of viral particles, packaging of the viral genome, and delivery of the transgene into the target cells. Next, not necessary genes are deleted from the viral genome to reduce replication and pathogenicity, as well as expression of immunogenic viral antigens.

The gene of interest together with transcriptional regulatory elements (referred to as the transgene) is inserted into the vector construct, and a recombinant virus is generated by supplying the missing gene products required for replication and virion production.

The more genes that are removed from the virus, the more replication defective the vector will be, and there is less ability of recombination to generate the infectious parental virus. The nature of the virus biology will usually determine the means of production. For example, retroviruses are produced in packaging cell lines, and vector particles collect in the culture medium.

In difference, adenovirus and adeno-associated virus (AAV) vectors are generally produced from transfections, and cells must be lysed to liberate the viral particles.

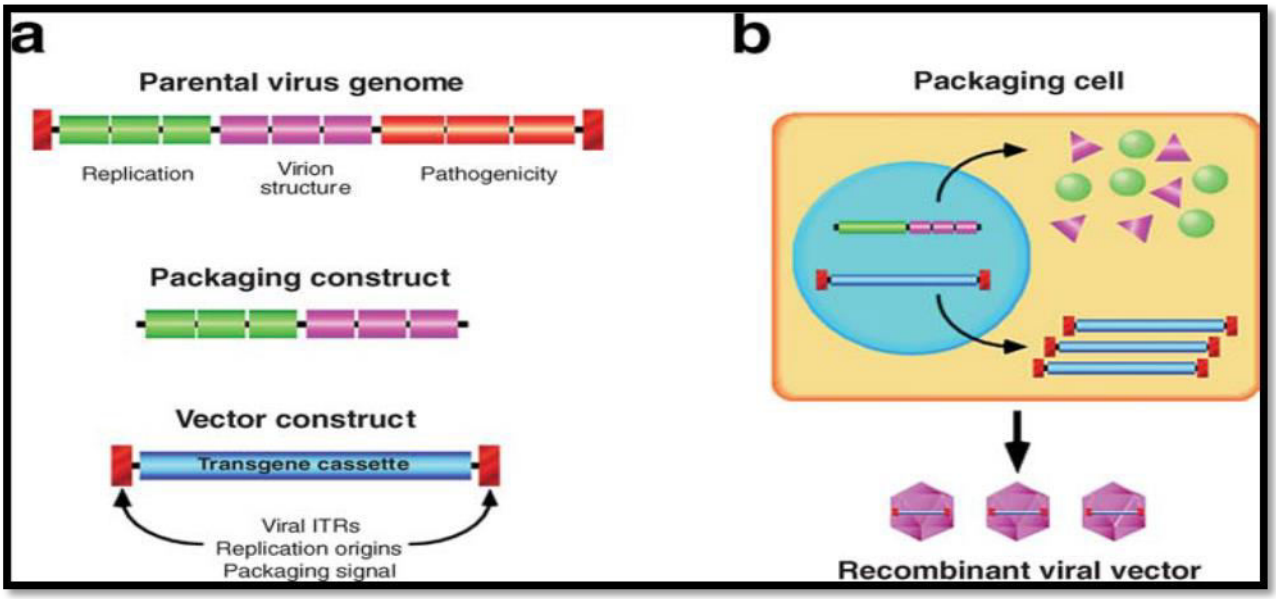


Figure -4: Principle of generating a viral vector. (*a*) Converting a virus into a recombinant vector. Schematic of a generic viral genome is shown with genes that are involved in replication, production of the virion, and pathogenicity of the virus. The genome is flanked by *cis*acting sequences that provide the viral origin of replication and the signal for encapsidation. The packaging construct contains only genes that encode functions required for replication and structural proteins. The vector construct contains the essential *cis*-acting sequences and the transgene cassette that contains the required transcriptional regulatory elements. (*b*) The packaging and vector constructs are introduced into the packaging cell by transfection, by infection with helper virus, or by generating stable cell lines. Proteins required for replication and assembly of the virion are expressed from the packaging construct, and the replicated vector genomes are encapsulated into virus particles to generate the recombinant viral vector.

Ideal Vector

- Targate the right cell.
- Integrate the gene in the cells.
- Activate the gene.
- Avoid harmful side effects.
- No universal vector exists.

Some different types of viruses used as viral vectores:

1. **Retroviruses**-

The recombinant retroviruses have the ability to integrate into host genome. Can carry a DNA of size-less than 3.4kb.and it is mainly Targate to the dividing cell. A class of viruses that can create double-stranded DNA copies of their RNA genomes. These copies of its genome can be included into the chromosomes of host cells. Human immunodeficiency virus (HIV) is a retrovirus. Eg: One of the problems of gene therapy using retroviruses is that the integrase enzyme can insert the genetic material of the virus into any random position in the genome of the host, it randomly inserts the genetic material into a chromosome. If genetic material happens to be inserted in the middle of one of the original genes of the host cell, this gene will be disrupted (insertional mutagenesis). If the gene happens to be one regulating cell division, uncontrolled cell division results in cancer can occur[5].

Lentivirus vector system-

Subclass of retroviruses. the viral genome in the form of rna is reverse transcribed when the virus enters the cell to produce DNA, Which is then inserted into the genome at a random position via viral integrase enzyme. Targate cell dividing, non-dividing.

2. Adenoviruses-

Adenoviral DNA does not integrate into the genome and is not replicated during cell division.humans commonly come in contact with adenovirus, majority of patients have already developed neutralizing antibodies which can activate the virus.targate to the non dividing and dividing cells.

The Ad genome consists of a double-stranded linear DNA molecule with overlapping transcription units onboth strands. Contains a linear double stranded DNA genome. The inserted DNA is not incorporate into genome.Has to be reinserted when more cells divide. Common cold.

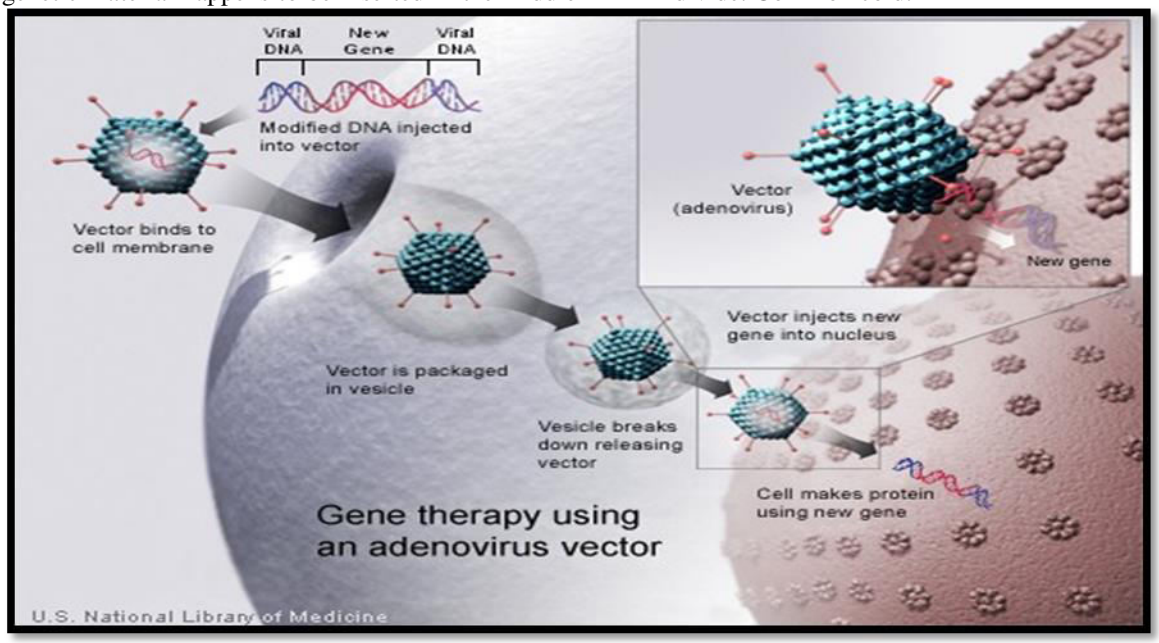


Figure -5: Gene therapy using an adenovirus vector

3. Adeno-associated viruses-It is a human virus. A class of small, single-stranded DNA viruses that can insert their genetic material at a specific site on chromosome. AAV enters host cell become double stranded and gets integrated into chromosome.AAV is not currently know to cause disease and consequently the virus cause a very mild immune response.targate to the both dividing and non dividing cells. AAVs are highly safe as the recombinant Adeno associated vectors contains only gene of interest and 96% viral genes are deleted [6].

5. Herpes simplex viruses- viruses which have natural tendency to infect a particular type of cell. The herpes simplex virus is a human neurotropic virus. This is mostly examined for gene transfer in the nervous system. A class of double-stranded DNA viruses that infect a particular cell type, neurons. Herpes simplex virus type 1 is a common human pathogen that causes cold sores [7].

Non-viral methods

1. Naked DNA.
2. Oligonucleotides.
3. Liposomes.
4. Microinjection.
5. Gene Gun.
6. Chemical Methods.
7. Hybrid Methods

Non-viral methods present certain advantages over viral methods,

1. with simple large scale production
2. Require low host immunogenicity.

1. Naked DNA-Naked DNA method are simplest method of non-viral transfection. Clinical trials carried out of intramuscular injection of a naked DNA plasmid have occurred with some success however, the expression has been very low in comparison to other methods of transfect ion.

2. Electroporation- In Electroporation method using the short pulses of high voltage to carry DNA across the cell membrane.This shock is thought to cause temporary formation of pores in the cell membrane, allowing DNA molecules to pass through. Electroporation is efficient and works across a broad range of cell types. However, drawback of this method is a high rate of cell death subsequent electroporation has limited use, in clinical applications.

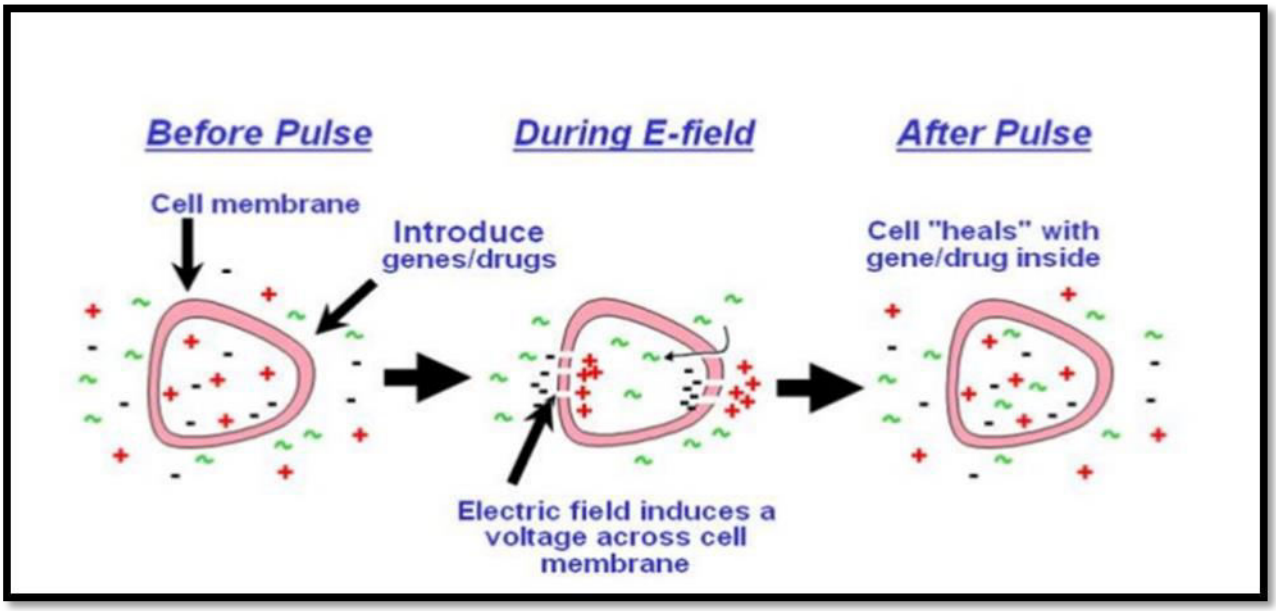


Figure-6: Electroporation method

3. Gen gun method This method also called as particle bombardment. The use of particle bombardment is another physical method of DNA transfection. In this technique, DNA molecules are coated with gold particles and employs a high pressure delivery system to shoot tissue with gold or tungsten particles are coated with DNA.eg:- If the DNA is integrated in the wrong place in the genome, for example in a tumor suppressor gene, i t could induce a tumor. This has occurred in clinical trials for X-linked severe combined immunodeficiency patients[8].

4. Sonoporation- Sonoporation uses ultrasonic frequencies to deliver DNA into cells. The process of sound cavitation is thought to disrupt the cell membrane and allow DNA to move into cell.

5. Microinjection-It is process of using a glass micropipette to into a single living cell. Normally performed under a specialized optical microscope set up called a micromanipulator. Involves the delivery foreign DNA, by the help of glass micropipette into a living cell. the cell held against a solid support or holding pipette and micro needle containing the desired DNA is inserted in to cell. The tip of pipette used is about 0.5 to 5um resemble as injection needle.

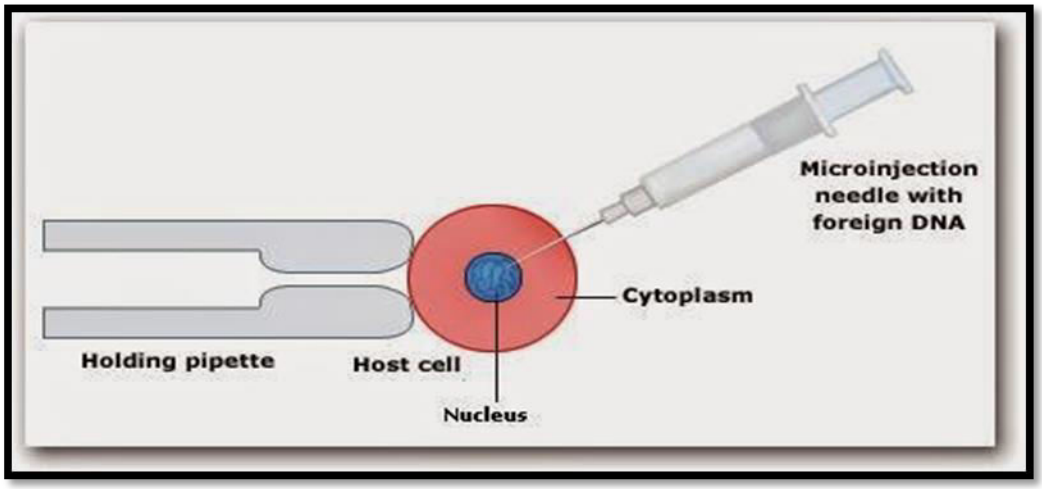


Figure-7: Microinjection

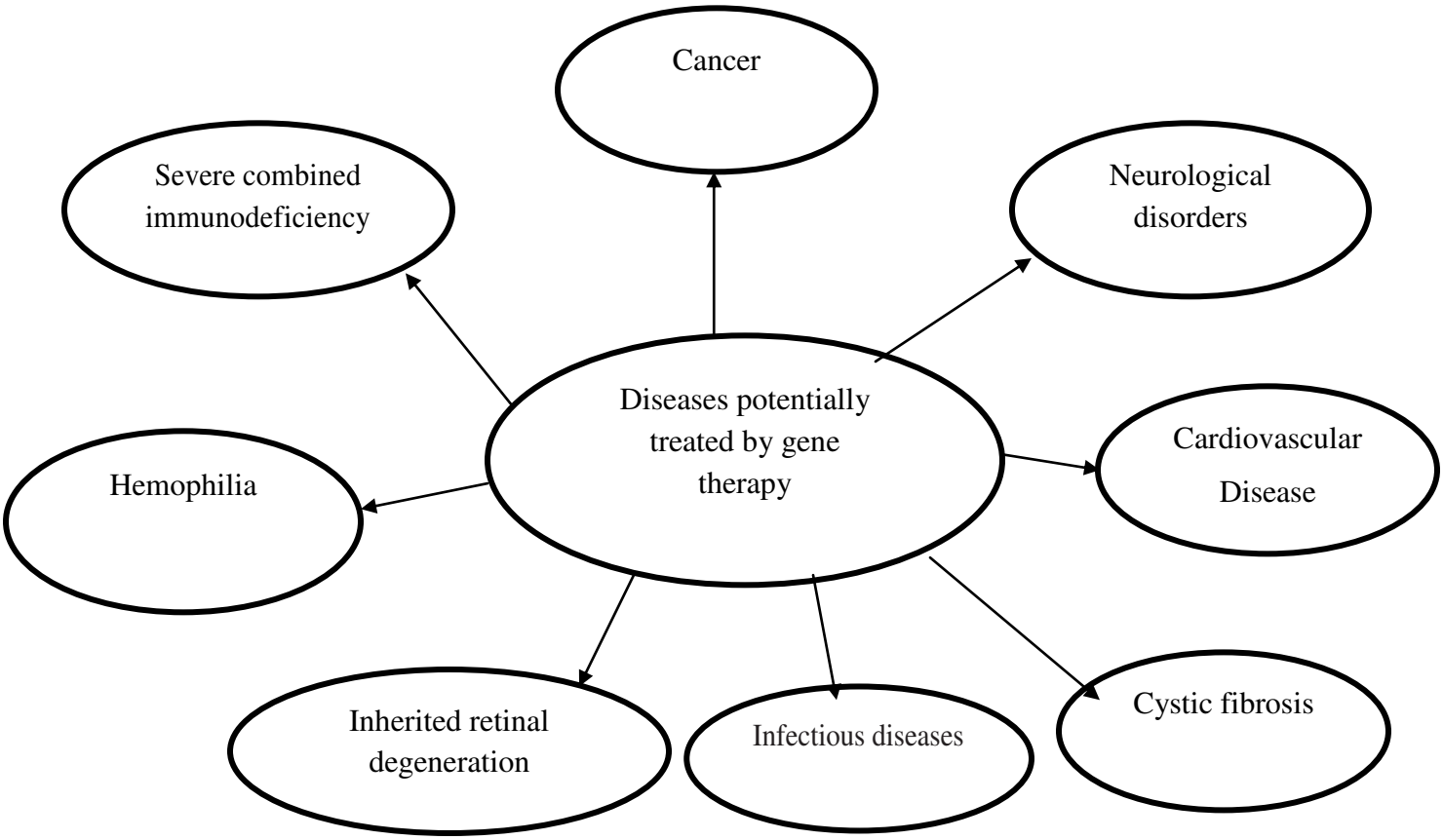
6. Chemical methods:

Chemical methods involve the use of certain chemicals which increases cellular permeability. One example is CaPO4 that increases cellular permeability by creating pores. The desired genes are then inserted into the target cell through these pores.

7. Hybrid methods:

Involves the combination of two or more techniques. An example is Virosome. It is the combination of liposome with inactivated HIV or Influenza virus. Results in more efficient gene transfer.

Diseases potentially treated by gene therapy



Applications

I) Cancer

Gene therapy strategies against cancer include the introduction of tumour suppressor genes, genes that induce apoptosis, genes that inhibit tumour angiogenesis and genes codimg for enzyme to convert prodrug to active drugs, and immunotherapy proceed. Cancersfor which gene therapy are developed include gliblastoma, metastic melanoma, head, Neck cancer, non-small cell cancer, prostate cancer, renal cell cancer, prostate colorectal cancer. Some cancer, such as malignant melanoma, are particularly sensitive to immunotherapy and gene therapy vaccines are developed against such cancers. TroVax, an experimental gene therapy, targets the tumour antigen 5T4 and is delivered by the "modified vaccinia Ankara" (MVA) vector (see accompanying article discussing vector technologies, p261). Tumour antigen 5T4 is a surface glycoprotein expressed on 80–90% of solid tumours but with low expression in normal tissue. 5T4 expression occurs throughout the whole tumour and correlates with poor prognosis. TroVax has been tested in phase I and phase II clinical trials in colorectal, renal and prostate cancer patients.

II) Neurological disorders

Gene therapy are used to treat the neurological disorder such as Parkinson's disease, Alzheimer's disease and motor neurone disease.As an example, ProSavin is currently being trialed for Parkinson's disease, which is caused by deficiency of dopamine in the brain. With help of lentivirus vector ProSavin are delivered, genes that encode for three enzymes required for dopamine synthesis.

III) Inherited disease

Gene therapy maily used in inherited disease, for people with inherited diseases, gene therapy could replace defective or missing gene or express a missing biological factor, enzyme or protein.

1. Haemophilia

For people with the hemophilia disease , gene therapy could be designed to deliver genes that express the missing factors VIII & IX and there is no longer need to inject exogenous clotting factors. For haemophilia research is being undertaken with adeno-associated virus and lentivirus vectors[10].

2. Cystic fibrosis

Gene Therapy are used to treat the Cystic fibrosis It is

caused by mutation of genes which encodes the cellular protein called "cystic fibrosis transmembrane conductance regulator" (CFTR). Abnormal ion transportation within lung and other cells due to this mutation. The CFTR gene has been cloned and researchers are now developing gene therapies to enable expression of CFTR. By using Non-viral vectors to deliver gene therapy locally to the lungs via nebulizer[11].

3. Inherited retinal degeneration disease

Ocular gene therapy can offer treatment strategy for people with retinal degeneration disease.Both viral and non-viral vectors are being researched. backgrounds of ocular diseases are genetically and biologically well defined and so gene therapy can be targeted appropriately. For example, in Leber congenital amaurosis type 2, a type of hereditary blindness, photoreceptor cells are unable to respond to light because of a defect in the RPE65 gene. An adeno-associated virus was used in a phase I clinical trial to deliver a functioning RPE65 gene to the retina. The study showed an acceptable side-effect profile for the product and patients said that their vision had improved slightly[12,13].

4. Severe combined immunodeficiencies:

"Severe combined immunodeficiencies" (SCID) is an inherited disorder that mainly occurs in children because in children immune system are not developed properly . This can lead to severe recurrent opportunistic infections, which can be fatal. The most common types of SCID are SCID-ADA (which is due to adenosine deaminase deficiency) and SCID-X1 (X-linked SCID, due to a defect on the X-chromosome). The most successful treatment is a bone marrow transplant from a related or compatible donor. However, finding a suitable donor can be difficult and graft-versus-host disease is a common complication. SCID-ADA can be treated with enzyme replacement therapy of the ADA enzyme, which is administered weekly; however, this can cause side effects.

Gene therapy was studied in humans for the first time in 1990 for children with SCID-ADA. Trials have explored the use of, for example, retroviral vectors to deliver the ADA gene to patients with SCID-ADA. There are reports of patients still being a live two to eight years after receiving gene therapy for SCID in clinical trials[14].

5. Infectious diseases

Gene therapy vaccines are being developed and trialed for tackling infectious diseases, including tuberculosis, malaria, HIV and influenza.

Tuberculosis vaccines are being studied that use genetically modified vaccinia comprising a recombinant fowl pox virus and a recombinant MVA virus, each containing a tuberculosis antigen. Malaria vaccines undergoing trials utilise chimpanzee adenovirus containing a malaria antigen[15].

A randomised, double-blind, placebo-controlled phase II study of gene therapy has been undertaken in 74 patients with HIV-1 infection. The patients received either an anti-HIV ribozyme (OZ1) or placebo. Cells from individual patients were removed and treated with the gene therapy before being infused back into the patient (ie, *ex vivo* gene therapy with autologous haematopoietic progenitor cells). Results showed the viral load was reduced in patients receiving OZ1, compared with placebo, 100 weeks after treatment. CD4+ lymphocyte counts were higher in the OZ1 group throughout the 100 weeks. Such therapeutic vaccines have the potential to preserve the immune system and reduce the need for lifelong treatment with antiretrovirals for people with HIV.

6. Cardiovascular Disease

Targeted gene therapy also be investigated for the managing of cardiovascular disease. The use of gene therapy several cardiac diseases, such as heart failure and coronary artery disease. Heart failure is one of the leading causes of morbidity and mortality in the United States. Transporting gene involved in myocardial contraction and relaxation to treat advanced heart failure[15].

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