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Gene Therapy to Cure Genetic Disorder - An Overview

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ABSTRACT :

Gene therapy is a treatment of the genetic diseases using normal copies of the defective genes.It has the ability to recover genetic diseases with long lasting therapeutic benefits.It involves insertion, correction and inactivation of specific gene in patients who suffering from genetic disorder. The different types of gene therapy such as somatic gene therapy,germline gene therapy is discussed. The electroporation, sonoporation, genegun, magnetofection, oligonucleotide, lipoplex, dendrimers, etc techniques are used for improving the gene therapy.In gene therapy various vectors such as gene retrovirus, adenovirus,adeno associated virus herpes simplex virus are used for gene transfer.It is applicable for the diseases like Parkinson's, Alzheimer's, cystic fibrosis, diabetic neuropathy,cancer,etc.Thus the purpose of this review is to summarise the general concept of gene therapy and it's applications.

Keywords: gene therapy, cancer therapy, viral vector, adeno associated therapy.

Introduction:

Gene therapy has provided treatment for untreatable disease by correcting the underlying genetic problem.The basic concept of the gene therapy is to transform viruses into genetic shuttles which delivers the intrest into the target cell. Gene therapy cures various genetic disorders such as blood disorders,eyes problems, cancer, generation of nerve cells, immuno deficiency and metabolic disorders.Gene therapy is used to correct defective genes or treat diseases or help our body to better fight against disease.

There are several ways to do gene therapy:

1. replacing mutated gene:

Gene replacement is a technique of replacing a faulty gene by applying a small piece of DNA in its correct form through carrier molecule which is known as viral vector to the gene.This technique is best for correct the loss function of mutations that results in a non

functional gene.The inserting correct normal gene gives the missing product thus the replacing gene may help to treat certain diseases for eg.gene P53 prevent tumour growth.

2. Fixing mutated gene:

Mutated genes which causes diseases that could be turned off so they are no longer causes diseases and normal healthy gene helps to inhibit the disease. Gene therapy or gene addition are earliest methods of gene therapy are developed. Introduce a new gene into the cell to help fight against disease.eg.to fix the faulty mutated DNA is cut and replace it with healthy normal gene strand that is injected along side the crispr-cas molecule.

3. Making diseased cells more evident to the immune system:

In some cases our immunue system fails to identify the disrupting gene or element so it doesn't attack diseased cells.In such cases it is important to train our immunue system to identify the cells that are threat.

Types of gene therapy:

1. **Somatic gene therapy :**

somatic gene therapy involves introducing normal 'good' gene into target cell for treating the patient but not the patient's future children because this gene do not pass along with offspring.This is safer approach because it affects only target cells in patient and it is not passed on to future generation.This therapy is short lived because of cells of most tissues are ultimately die & replace by new cell and transporting of gene to the target cell or tissue is also difficult.

2. **Germline gene therapy :**

In germline gene therapy the therapeutic gene is transferred into the fertilized egg or in sperm producing cells.It is inheritable and passed on to future generation.It has potential for preventing inherited diseases.Germline gene therapy is extremely controversial and current very little research is done on it.For safety, ethical and technical reasons it is not being attempted at present.

Vectors used in gene therapy :

1. Retrovirus :

Retroviral vector is commonly used to modify the cells for gene therapy. They are derived from the natural retroviruses that have evolved the gene transfer mechanism.

Retroviruses such as human immuno deficiency virus(HIV) is a class of viruses that can be Convert RNA genomes into double stranded DNA and merge into host cell chromosome. If genetic material is inserted in middle one of the main gene in host cell then the function of this gene will be impaired. This is one of the problem occurs with using retrovirus.

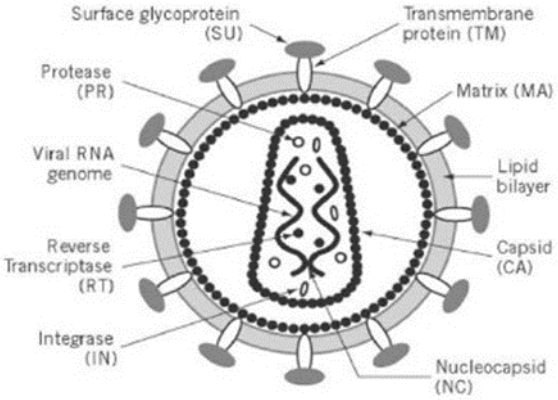


Fig. Retrovirus

2. Adenovirus :

This class of viruses has a double stranded DNA genome that cause intestinal, respiratory and ocular infections in human beings.when adenoviruses infect a cell, they infect their DNA molecules into host cell. Adenoviral vector are able to transduce into both replicating and quiescent cells.however there DNA is does not integrated into host genome but rather recides episomally in the host nucleus. This vector system has been promoted for treating the cancer of overies,liver & indeed the first gene therapy product to be licensed for treating the head and neck cancer.

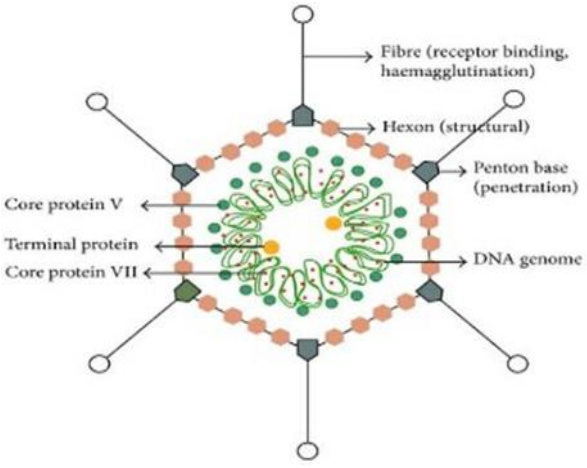


Fig. Adenovirus

3. Adeno associated virus :

It is a class of small single stranded DNA viruses that can be insert there genetic material at a specific site on chromosome 19.AAVs has a ability to infect both dividing and non dividing cells with constitutive expression.The ability of these viruses to be present in both lysogenic and lytic forms in cells,& it has made them good for gene therapy.The main drawback of AAV is that it is small & therefore it carry only two genes.

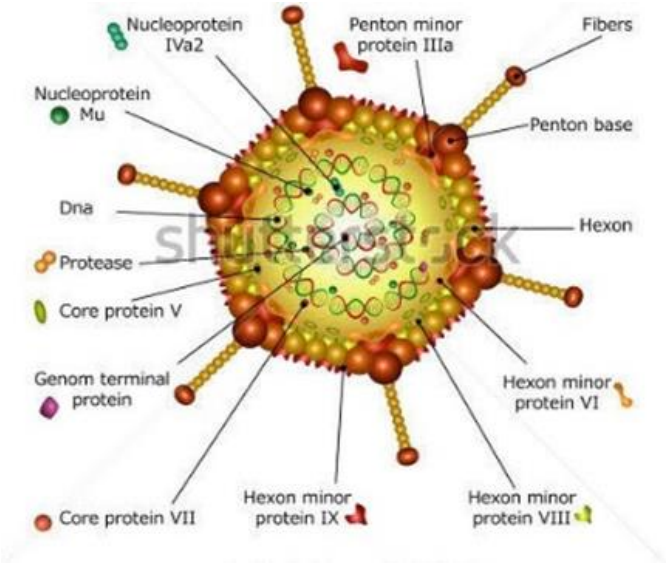


Fig. Adeno associated virus

4. Herpes simplex virus :

Is a class of double standard DNA that infect a specific type of neural cells. The type 1 herpes simplex virus is a common human pathogen that can cause fever blisters,cold sores. Herbs simplex virus is a human neurotropic virus and it is mainly used for the gene transfer in nervous system. HSV makes an ideal vector as it can infect a wide range of tissues including liver lung muscles and nerve. The antibodies of hsv 1 are commonly in humans however complications due to herbs infection is rare.

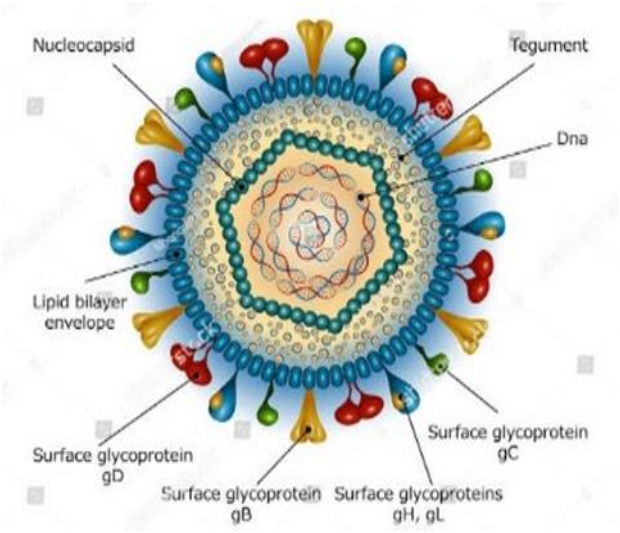


Fig. Herpes simplex virus

Target cells for gene therapy :

1. Peripheral blood lymphocytes
2. Fibroblasts
3. Keratinocytes
4. Airway epithelial cells
5. Haemopoietic stem cells
6. Vascular endothelial cells
7. Hepatocytes

8. Skeletal muscle myoblasts
9. Tumor cells

Physical methods for improving DNA transfer :

1. Electroporation :

Electroporation is a method that uses short pulses of high voltage to introduce DNA into cells. Small pores are formed by electric voltage for temporarily on the surface of cell membrane which makes permeable to nucleic acid it is a procedure that known for standard gene transfer.It can be used with many cell types,yield a high frequency of stable transformation and transient gene expression and it is easier than alternate techniques.

2. Gene gun :

The gene gun is a physical method for DNA transfer. It is a device used to transfer the gene with foreign DNA by bombarding the target cell with DNA coated microparticles. The DNA is coated with gold particles then placed inside the device which provide the required force to DNA for enter into the cell.

3. Sonoporation :

Sonoporation is a physical method which uses and ultrasonic frequency to transfer DNA into the cell. Sonoporation is a method of formation of small pores by using ultrasonic frequency or ultrasound for the transfer of the nucleic acid material.The major advantages of sonaporation is its non invasiveness and ability of transfer of gene into internal organ without surgical procedure.

4. Magnetofection :

Magnetofection is a transfection method in which magnetic field is uses to concentrate particles consisting vectors to target sale in body.In this method DNA complex is formed with magnetic particles and the gene magnetic complex is injected intravenously with the help of strong external magnet then the gene is isolated from magnetic particles This method is used to transfer gene to primary cells & other cells to which it is difficult to transfer gene by other methods.

Chemical methods for improving DNA transfer :

1. Oligonucleotides :

The chemically synthesized oliginucleotides are used in gene therapy to inactivate and disable the genes involved in disease process.These agents can inhibit gene expression and protein function by binding specific sequence of a target gene or protein. Therefore oligonucleotides are high specific and target molecules. The use of antisense for the target gene decrease the transcription of defective gene and use of siRNA that leads to breakdown the specific sequence of defective gene mRNA to stop its translation and expression.

2. Lipoplex and Polyplex :

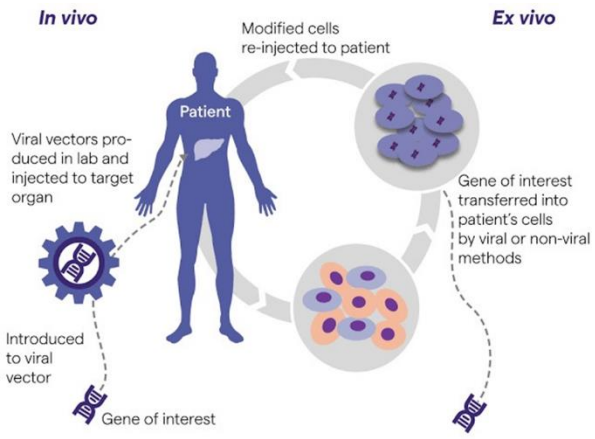
Lipoplexes and polyplex are the molecules that have the ability to protect the DNA during transfection process. The most common use of lipoplex in gene transfer into cancer cells where the transferred genes have activated tumor suppressor control gene in the cell & decrease the activity of oncogens.The complexes of polymer with DNA are known as polyplex.Most polyiplex includes cationic polymers and their production. Therefore neutral & anionic liposomes are used for the formation of lipoplexes as synthetic vectors.

3. Dendrimers :

The dendrimers are the sperical branched macromolecules.They helps in complexing gene along with providing stability and higher transfection efficiency.The dentrimer condenses nucleic acids into small nanoparticles by ionic interaction & prevent the therapeutic gene from nuclear and endosomal degradation. The dendrimer is use as a carrier in delivery of gene.

4. Hybrid methods :

The hybrid methods are the combination of several techniques are being developed.A virosome is an example of the hybrid method,it is combination of liposome with an inactive HIV virus. This method of gene delivery is more convenient than the liposome and viral method alone.This method involves the combination of various viral victors with cationic liposomes or hybrid viruses.



Advantages of gene therapy :

1. Gene therapy has ability to remove and prevent hereditary this is this like ADA-SCID, cystic fibrosis,etc.
2. Gene therapy helps to eradicate diseases from the future generation.
3. Therapy helps to treat AIDS, cancer and heart related diseases.
4. In gene therapy the faulty non functional gene is replace with the normal healthy functional gene and this gives the someone normal life.
5. Gene therapy can be modified so it can not be destroy or replicate the cells.
6. For certain diseases,which are not cure by any other treatment they can cure by gene therapy and it could save many lives.

Disadvantages of gene therapy :

1. The disorders which are caused by defect in multiple gene are not treated effectively by gene therapy.
2. Gene therapy has short luda nature so the patient required multiple round gene therapy.
3. Gene therapy has few side effects includes drowsiness, nausea,etc.
4. Multigame disorders such as arthritis,blood pressure,alzheimer,etc are difficult to treat completely by using gene therapy.
5. The cost of gene therapy is very high.
6. Immune response of body to the transferred gene stimulate the potential risk to gene therapy.

Applications of gene therapy :

1. Parkinson's disease :

Parkinson's disease is the neurodegenerative disease and it is estimated up to 5.8 million people in world wide.PD is characterized by muscle rigidity,hypokinesia,impaired gaie,tremor are major symptoms and disturbed emotions sleep pattern,autonomous dysfunction are non motor symptoms.According to reports the effectiveness of gene therapy is proven for example the chemical level of GABA,which absence cause PD on which gene therapy shows 23% improvement.The randomised controlled trials estimated that by using gene therapy the improvement of advanced PD symptoms occur.The gene that producing the chemical agent glutamic acid decarboxylase (GAD) is transfer into the ganglia cell which controls the movement of cerebral area and this transfered gene called GAD which increases the level of GABA.

2. Alzheimer's disease :

Alzheimer's disease is the major cause of dementia which leads to the memory loss and cognitive decline.AD is a neurodegenerative disease & it affects approximately 29 million people world wide.Aging is the major risk factor of Alzheimer's disease.It is characterized by the memoryloss,progressive cognitive decline,physiological symptoms of dementia,mood swings & personality change.pathologically it is characterized by AD brain includes the amyloid plaque and neurofibrillary tangles (NFT's).The concentration dependent A beta aggregation is the major cause of AD and other factors including apolipoprotein E (APOE) associated with inflammation &lipid.Acetylcholinesterase(ACHE) inhibitor such as galantamine and NMDA antagonist donepezile & rivastigmaine memutine are antidementia drugs are available.immunotherapy using beta vaccine disease modifying effects have been achieved.Gene therapy directly increases the enzymetic activity and decrease the level of bioactive substances.This achieved through delivery of the

transgene by vector.The virus that infect the host cells in which gene is expressed and by this way gene therapy is successfuly applied to the alzheimer disease.

3. Cystic fibrosis :

Cystic fibrosis is the autosomal recessive time limiting disease resulting from the mutation.The mutation occurs in the cystic fibrosis transmembrane conductance regulator (CFTR) gene.The patient with cystic fibrosis (CF), CFTR protein function is abnormal due to the lack of production (class 1 mutation) failure to reach its site of action due to the miss folding (class 2, commonest caucasian defect is phe 508 Del), defect in getting (class 3),conductance (class 4), abnormally low channel numbers(class 5) and decrease half life (class 5).The major effect of this type of mutation is on respiratory, gastrointestinal and reproductive tracts.The gene therapy is developed as a treatment for cystic fibrosis.The gene therapy includes the correct copies of cystic fibrosis are transmembrane conductance regulator (CFTR) DNA transfer to the epithelial cells in airways.Gene therapy for CF is safe & results in a small improvement in the lung function.

4. Diabetic neuropathy :

Diabetic neuropathy is a common untreatable of both type 1 & type 2 diabetes.Diabetic polyneuropathy is the major complications of both type of diabetes that is insulin dependent & non insulin dependent diabetes mallitus.The tight control of glycemia is the one of the treatment to improve sign & symptoms of neuropathy.There are two different gene therapy strategies are used.In first, gene transfer to the muscle by injection used to effect the continuous systematic delivery of the trophic factor.In second, gene is transfer to the dorsal root ganglion to effect the local release of trophic factor in nervous system.The phase 1/2 human trials of VEGF administered by injection of the plasmid containing VEGF gene in muscle for the treatment of diabetic neuropathy is now in progress.The NT-3 may be beneficial in the treatment of inherited neuropathy.

5. Cancer :

Cancer is a disease in which some of body cells are divide uncontrollably and destroy body tissue.The cancer is leading to cause death world wide, nearly 10 million deaths are occur in year.The most common cancers are lung,breast, rectum & colon cancers.The broad field of gene therapy is important in preventing deaths from cancer.In gene therapy of cancer there are three different gene treatment approaches: immunotherapy, oncolytic virotherapy & gene immunotherapy uses genetically modified cells & viral particles uses that replicate within the cancer cells to cause death of cells & this is an emerging treatment modality that show great result.The new treatment in that gene is introduce into a cancerous cell or to surrounding tissue to cause cell death or slow the growth of cancer cells.This is very flexible technique of treatment and a wide range of genes & vectors are used in clinical trials with sucessful outcomes.

Future work :

Gene therapy is one of the most important area in biotechnology, with respect to the current progress and future possibilities. The new development in gene therapy have paved a path for next generation technologies.Scientist currently work on the development of effective methodology and minimise the risk and side effects of treatment.scientist have determine the risk related to the gene therapy and working to alleviate them.researches have developed the various vectors to carry therapeutic genes into human cells such as viral vector, bacterial vector and plasmid DNA.

The new technologies includes the human gene editing technology and patient derived cellular gene therapy i.e removing cell from the patient, genetically modify them and replace in the body.The researches believe that developmentsin the engineering & profiling of non viral nanoparticles for gene transfer & recent approval of siRNA based drug would have large impact on future gene therapy.In future,new genetic therapies will be develop for both common and rare diseases.Researches stated that gene therapy show singnificant advances in coming years.

Conclusion :

The gene therapy can offer the opportunity to improve the symptomology or slow progression of non cancerous blood & neurologic disorders.The rapid changes in gene therapy have created number of innovative methods for treating cancer patients.Rhe gene therapy treatment now available for the diseases such as Alzheimer's disease, cystic fibrosis, Parkinson's disease and various cancers etc.Gene therapy includes the many methods for gene transfer.Gene therapy based on the initials clinical trial data, potential

impact on health care costs & patients life.on going researches will leads to the development of other treatments in future.

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