GENE THERAPY

By
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- Gene therapy was once considered a fantasy. However, thousands of individuals have already undergone human clinical trials.
- A great leap in medical science has taken place on the 14th September 1990, when a girl suffering from Adenosine deaminase deficiency (severe Immunodeficiency) was treated by transferring the normal gene for adenosine deaminase.

1. What is Gene Therapy?

- It is intracellular delivery of genes to generate a therapeutic effect by correcting an existing abnormality.
- Only *somatic gene therapy*, by inserting the new gene into somatic cell of the patient is under trial (This type of gene therapy cannot be passed to a person's children.) Germ cell gene therapy is considered as unethical (it might affect the development of a fetus in unexpected ways or have long-term side effects that are not yet known.)

2. Summary of the Procedure

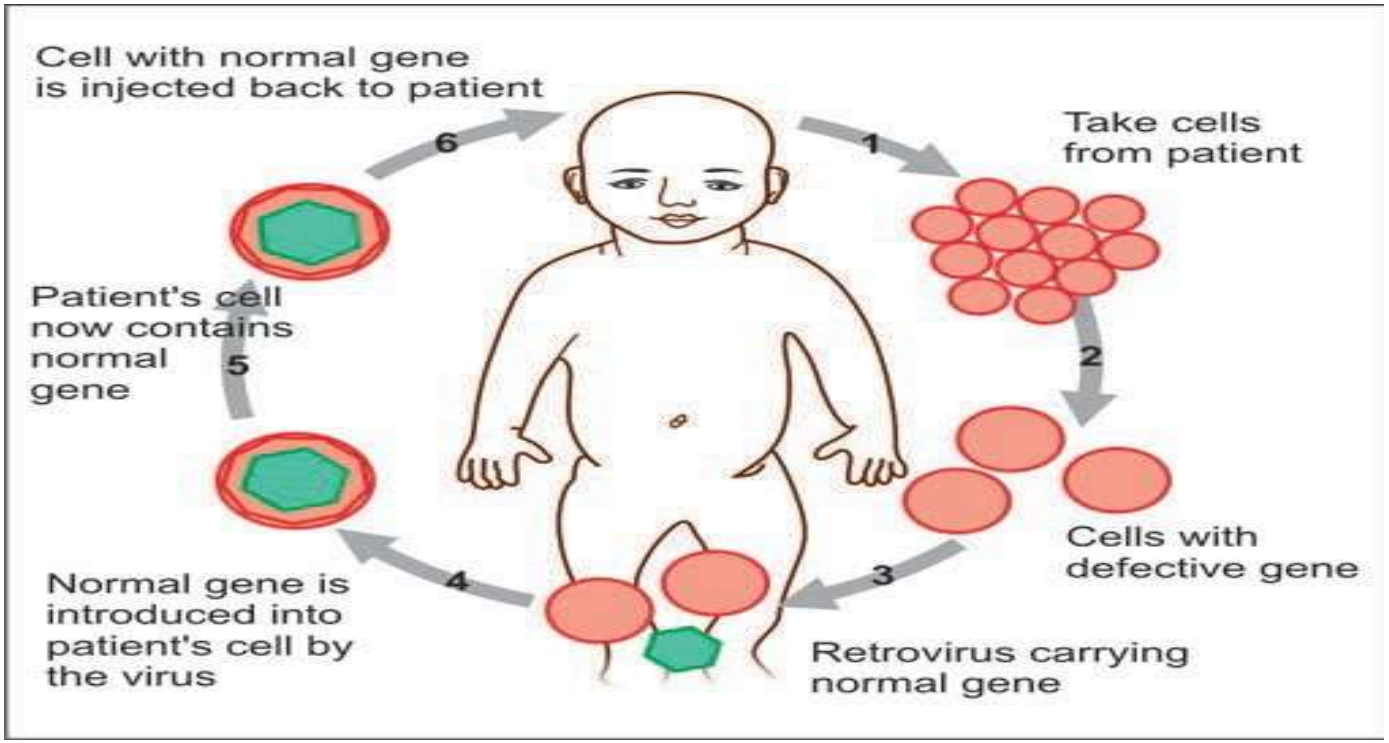
- **1.** Isolate the healthy gene **along with the sequence controlling its expression.**

- **2.** Incorporate this gene into a carrier or vector as ***an expression cassette***.

- **3.** Finally deliver the vector to the target cells.

3. How the Genes are Introduced?

- ***Ex vivo* strategy** where the patients' cells are cultured in the laboratory, the new genes are infused into the cells; and modified cells are administered back to the patient.



- ***In vivo* strategy**, where the vector is administered directly to the cell, e.g. CF (cystic fibrosis) gene to the respiratory tract cells.

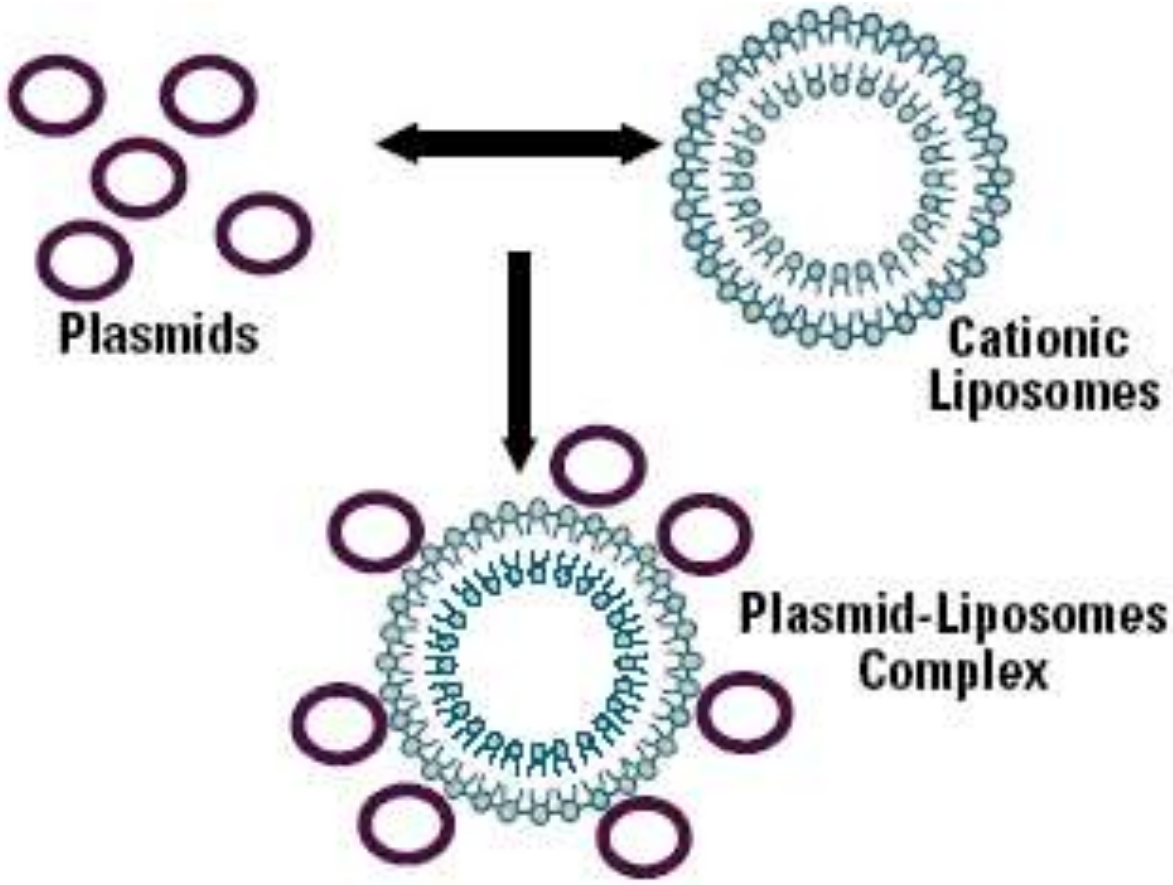
4. The Vectors

- Different vector (carrier) systems used for gene delivery are: Retroviruses, adenoviruses. Non-virus systems include liposomes, plasmids and physical methods.
- The viruses are modified so they can't cause disease when used in people.
- Some types of virus, such as retroviruses, integrate their genetic material (including the new gene) into a chromosome in the human cell.
- Other viruses, such as adenoviruses, introduce their DNA into the nucleus of the cell, but the DNA is not integrated into a chromosome. The DNA molecule is left free in the nucleus of the host cell, and the instructions in this extra DNA molecule are transcribed just like any other gene.

- **One of the problems of gene therapy using retroviruses** is insertional mutagenesis; 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 (i.e., cancer) can occur.

- **Disadvantages of adenovirus:** is that the expression is usually transient, the useful effect varying from a few weeks to months only, also they have the ability to trigger the immune system on subsequent exposure.

- **Plasmid Liposome Complex:**
- It is a non-viral vector system.
- **Liposomes** are artificial lipid bilayers, which could be incorporated with plasmids carrying the normal human DNA. The complexes can enter into the target cells by fusing with the plasma membrane. Cationic liposomes (positively charged) can form complexes spontaneously with DNA (negatively charged).
- **The advantages** with this strategy are that the vector can carry human gene of big size, do not replicate and evoke only very weak immune responses.
- **The disadvantage** is that most of the complexes are destroyed inside the host cell, and so the efficiency of gene transfer is less.


Plasmids
Cationic
Liposomes
Plasmid-Liposomes
Complex

- **Gene Gun Method:**
- Tungsten particles are coated with plasmid DNA, and accelerated by helium pressure discharge. This enables particles to penetrate the target tissues. It is quick, and could be used in almost all tissues.
- Cellular **damage** and **transient gene expression** are the draw backs.

5. Accomplishments

- Gene therapy is effective in inherited disorders caused by **single genes**. Several clinical trials have been conducted. Success stories are few. The most dazzling ones are shown in the followingTable:

Table 43.2. Success stories of gene therapy

Disease	Gene transferred by
1. Severe combined immunodeficiency (SCID)	Adenosine deaminase enzyme in chromosome 13 and 20 into lymphocytes; by retrovirus
2. Duchenne muscular dystrophy (DMD)	Dystrophin gene on short arm of X chromosome; by retrovirus
3. Cystic fibrosis (CF)	CFTR gene on chromosome 7 to bronchial epithelium;adenovirus
4. Familial hyper-cholesterolemia	LDL receptor gene on chrom 19 to hepatocytes; retrovirus
5. Hemophilia	A and B genes for factor VIII and IX into fibroblasts; retrovirus
6. Cancer	Activation of p53 (tumor suppressor gene) by liposome
7. Leber's Hereditary Optic Neuropathy	Introducing the gene for the enzyme (isomero hydrolase) using an adeno viral vector directly to the retina