2.16 Gene therapy

Gene therapy is a technique for correcting defective genes responsible for disease development. Gene therapy typically aims to supplement a defective mutant allele with a functional one. Scientist may use one of several approaches for correcting defective or abnormal genes:

- A normal gene may be inserted into a nonspecific location within the genome (*gene addition*). This is the most common approach.
- An abnormal gene can be replaced by a normal gene through homologous recombination (*gene replacement*).
- An abnormal gene can be repaired through selective reverse mutation, which returns the gene to its normal function.

Gene therapy may be *germ-line* or *somatic cell gene therapy*. Current gene therapy is exclusively somatic gene therapy which involves the introduction of genes into somatic cells of an affected individual. Germ-line gene therapy involves the permanent transmissible modification of the genome of a gamete, a zygote or an early embryo. The prospect of human germline gene therapy is currently not sanctioned.

Gene therapy may be *classical* and *nonclassical* gene therapy. In *classical* gene therapy genes are delivered to appropriate target cells with the aim of obtaining the optimal expression of the introduced genes. The idea of *nonclassical* gene therapy is to inhibit the expression of genes associated with the pathogenesis, or to correct a genetic defect for restoring the normal gene expression.

Potential use of somatic gene therapy

The potential use of this therapy is to cure genetic diseases. The first case of gene therapy occurred in 1990, at the NIH in Bethesda, Maryland. On that occasion, a four-year-old patient with a severe combined immuno- deficiency (due to *adenosine deaminase* enzyme deficiency) received an infusion of white blood cells that had been genetically modified to contain the gene that was non-functional in his genome. Since then, gene therapy has been studied and experimentally tested for several medical conditions.

Table 2.8 Human diseases that are target candidates for somatic cell gene therapy

Disease	*Target cells*	*Transfected gene(s)*
Hemophilia A	Liver, muscle, bone marrow cells	Factor VIII
Hemophilia B	Fibroblasts	Factor IX
Familial hypercholesterolemia	Liver	Low-density lipoprotein receptor
Severe combined immunodeficiency	Bone marrow cells, T-cells	Adenosine deaminase (ADA)
Hemoglobinopathies	Red blood precursor cells	α-globin, β-globin
Cystic fibrosis	Lung airway cells	Cystic fibrosis gene (CFTR)
Gaucher's disease	Bone marrow cells, macrophages	Glucocerebrosidase
Cancer	Tumor cells	p53, Rb, interleukins, growth-inhibitory genes, apoptosis genes

Methods for inserting and expressing a gene in a target cell

The genetic material may be transferred directly into cells within a patient (*in vivo* gene therapy), or cells may be removed from the patient and the genetic material inserted into them *in vitro*, prior to transplanting the modified cells back into the patient (*ex vivo* gene therapy).

Ex vivo gene transfer

This initially involves the transfer of cloned genes into cells grown in culture. Those cells which have been transfected successfully are selected, expanded by cell culture *in vitro* and then introduced into the patient. This approach is only applicable to tissues that can be removed from the body, altered genetically and returned to the patient where they will engraft and survive for a long period of time (e.g. cells of the hematopoietic system and skin cells).

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In vivo gene transfer

The cloned genes are transferred directly into the tissues of the patient. This may be the only possible option in tissues where individual cells cannot be cultured *in vitro* in sufficient numbers (e.g. brain cells) and/or where cultured cells cannot be re-implanted efficiently in patients.

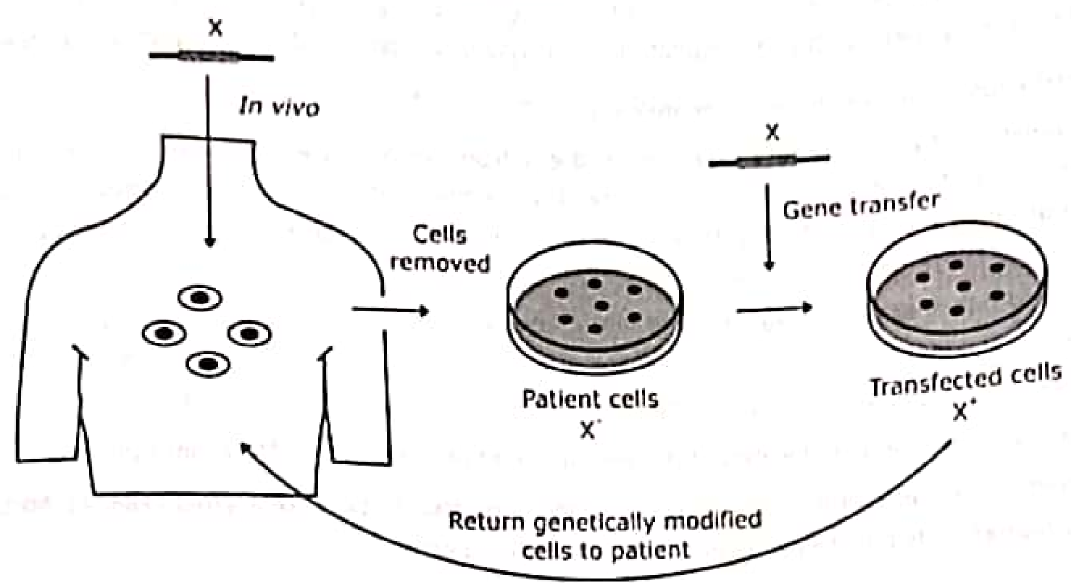

Figure 2.33 *In vivo* and *ex vivo* gene therapy.

Gene delivery system

One of the biggest problems in somatic gene therapy is to find an efficient gene delivery system. Appropriate methods to deliver DNA used in gene therapy are vital, as the targeted tissues must properly receive the appropriate genes. Gene therapy can be carried out using naked DNA delivered directly into the target cells. However, this procedure of introducing isolated DNA molecules has a very low efficiency rate. To increase the efficiency of DNA uptake by the target cells, special vectors have been engineered for gene transfer.

Two basic gene delivery methods are:

- *Virus mediated transduction methods* and
- *Non-viral transfection* methods

Virus mediated transduction methods

Viruses attack their hosts to insert their genetic material into the genetic material of the host. This genetic material contains instructions to produce these viruses. The host cell will carry out these instructions and produce the viruses. In addition to the instructions producing the components of the virus itself, viruses can carry additional genes containing instructions for creating other kinds of proteins.

Three types of viruses are currently used as vectors in gene therapy: *retroviruses*, *adenoviruses* and *adeno-associated viruses*. They differ in their mechanisms of action and results.

Retroviruses

The genetic material in retroviruses is in the form of RNA molecules, while the genetic material of their hosts is in the form of DNA. When a retrovirus infects a host cell, it will introduce its RNA together with some enzymes into the cell. This RNA molecule from the retrovirus must produce a DNA copy from its RNA molecule before it can be considered part of the genetic material of the host cell. The process of producing a DNA copy from an RNA molecule is termed reverse transcription. It is carried out by one of the enzymes carried in the virus, called *reverse transcriptase*. After this DNA copy is produced and is free in the nucleus of the host cell, it must be incorporated into the genome of the host cell. That is, it must be inserted into the large DNA molecules in the cell, or the chromosomes of the cell. This process is done by another enzyme in the virus called *integrase*.

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Now that the genetic material of the virus is incorporated and has become part of the genetic material of the host cell, we can say that the host cell is now modified to contain a new gene. When this host cell divides later, its descendants will all contain the new genes. One of the problems of gene therapy using retroviruses is that the integrase enzyme can insert the genetic material of the virus in any arbitrary position in the genome of the host. If genetic material happens to be inserted in the middle of one of the original genes of the host cell, the gene will be disrupted. If the gene happens to be one regulating cell division, uncontrolled cell division (i.e. cancer) can occur.

Strategy for retrovirus vector-mediated gene delivery

It involves the deletion of essential genes *gag*, *pol* and *env* from retrovirus which make it replication deficient. It creates a space for insertion of an expression cassette. The modified retrovirus acts as a vector for the expression cassette, although replication defective, it is still infectious. A number of *cis-acting* viral elements are required in the retroviral vector. These elements include:

1. A promoter and polyadenylation signal in the viral genome
2. A viral packaging signal
3. Reverse transcription signal
4. Short partially inverted repeats located at the terminal end of the viral 5' LTR for integration.

The most commonly used retroviral vector is based on Moloney Murine Leukemia Virus (MMLV). MMLV can infect mouse cells and human cells but they only infect rapidly dividing cells.

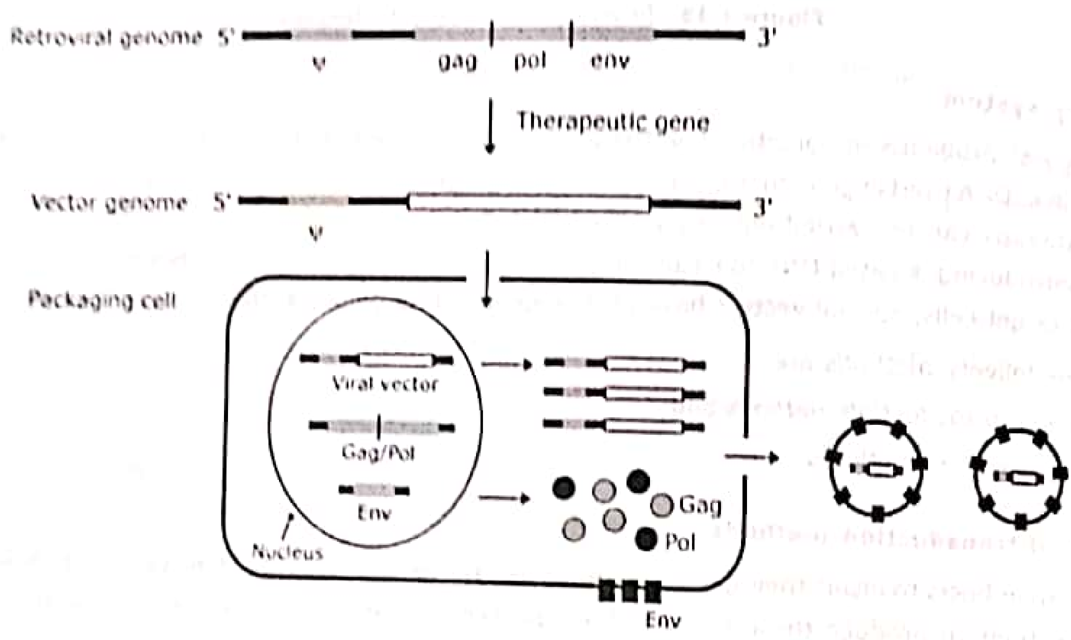

Figure 2.34 The gag, pol and env genes are deleted from the retroviral genome and replaced by the therapeutic gene. The ψ sequence is retained; this is recognized by viral proteins for assembly of the RNA into a virus particle. Gag, pol and env functions are supplied by the packaging cell, but the genes are on physically separate molecule and there is no ψ sequence. This is done in order to minimize the risk of producing replication competent viruses. Recombinant viral genomes are packaged into infective but replication deficient virus particles which bud off from the cell and are recovered from the supernatant. *Adapted and modified from Human Molecular Genetics, Tom Strachan and Andrew P. Read, Garland Science.*

Infection of a packaging cell line that carries intact *gag*, *pol* and *env* genes allows the modified retrovirus to reproduce and the packaged retroviral virus can be collected and used to infect a patient. In the cytosol of the patient cells, a DNA copy of the viral RNA is synthesized by viral reverse transcriptase, which accompanies the viral RNA into the cell. This DNA is then randomly integrated into the host cell genome, where its expression leads to production of the expression cassette product.

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Adenoviruses

Adenoviruses are DNA viruses with a linear, double-stranded genome of approximately 36 kbp. When these viruses infect a host cell, they introduce their DNA molecule into the host. The genetic material of the adenoviruses is not incorporated into the host cells genetic material. The linear dsDNA genome remains non integrated as an episome within the cell nucleus and the instructions in this extra DNA molecule are transcribed just like any other gene. These extra genes also do not replicate when the cell is about to undergo cell division. So, the descendants of that cell will not have the extra gene. This means that treatment with the adenovirus will require regular doses to add the missing gene every time.

Adeno-associated viruses

Adeno-associated viruses (AAV), from the parvovirus family, are non-pathogenic single-stranded DNA viruses. They rely on co-infection by an adeno or herpes helper virus to replicate. There are a few disadvantages to using adeno-associated viruses, mainly the small amount of DNA (up to 4.5 kb) it can carry and the difficulty in producing it. This type of virus is being used, however, because it is non-pathogenic (most people carry this harmless virus). In contrast to adenoviruses, most people treated with adeno-associated viruses will not build an immune response to remove the virus and the cells that have been successfully treated with it.

Potential advantages and disadvantages of various gene delivery systems

Retrovirus

Advantages

- Integrates into host cell's genome and provides stable gene expression.
- Contains no viral genes.
- Few immunity problems.

Disadvantages

- Infects only dividing cells.
- Random integration may cause insertional mutations.

Adenovirus

Advantages

- Can contain >30 kbp of nonviral DNA.
- Infects both non-dividing and dividing cells.

Disadvantages

- Does not provide long-term gene expression due to lack of integration.
- Very immunogenic.

Adeno-associated virus

Advantages

- Integrates into host cell's genome (chromosome 19) and provides stable expression.
- Contains no viral genes.
- Non-pathogenic.
- No immunity problems.
- Infects both non-dividing and dividing cells.

Disadvantages

- Low carrying capacity for foreign gene due to small genome size (about 4.7 kilobase).
- Difficult to obtain large amounts of viral stock.

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