GENE THERAPY

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Contents

Summary

Gene therapy can be broadly defined as the transfer of defined genetic material to specific target cells of a patient for the ultimate purpose of preventing or altering a particular disease state. Two main approaches to gene therapy include *in vivo* and *ex vivo*. *Ex vivo* gene transfer techniques usually involve the genetic alterations of cells (cell lines or human cells), mostly by use of viral vectors, prior to implanting these into the tissues of the living body. *In vivo* gene therapy means direct introduction of genetic material into the human body. It can be accomplished by use of non-viral vectors. In addition to gene transfer and *in vivo* production of therapeutic proteins, some techniques are aimed at repairing defective genes or suppressing the function of genes.

This article describes the use of various biotechnologies in development of gene therapy. The most frequent method of gene transfer is by viral vectors but several non-viral vectors are also being developed. Several routes of administration have been used although injection is still the most commonly used method. Efforts are also being made to develop orally administered gene therapy. Physical methods of gene transfer include ultrasound, electroporation, laser irradiation, and gene gun. Related methods include use of genetically modified cells, antisense therapy and RNA interference. Gene therapy is an excellent method of delivery of therapeutics and various methods of targeted and controlled gene therapy are described. Gene therapy can be considered for almost any disease. Major applications that are currently under investigation include cancer, cardiovascular diseases, neurological disorders and infections. Some complications of gene therapy have been encountered and these are being addressed. It is anticipated that gene therapy will become established as a part of human medicine during the next decade and will fit in with the concepts of personalized medicine.

1. Introduction

Gene therapy can be broadly defined as the transfer of defined genetic material to specific target cells of a patient for the ultimate purpose of preventing or altering a particular disease state. Applications of gene therapy would be narrow if one uses the term as a synonym for transfer of defined genetic material to specific target cells using carriers or delivery vehicles called vectors. Vectors are usually viral but several non-viral techniques are being used as well. Genes and DNA are now being introduced without the use of vectors and various techniques are being used to modify the function of genes *in vivo* without gene transfer. If one adds to this the cell therapy particularly with use of genetically modified cells, the scope of gene therapy becomes much broader. Gene therapy can now combined with antisense techniques such as RNA interference (RNAi), further increasing the therapeutic applications.

A study of the historical evolution of gene therapy gives an idea of the time lapsing between fundamental discoveries, animal experiments and human applications of a new

technology. Although the capability of viruses to transmit genes was recognized as far back as 1952, suggestion for their use as vectors for gene transfer was not made until 20 years later. Another decade passed before retrovirus vectors were used for gene transfer and the first authorized gene therapy experiment in humans did not take place until a further decade later in 1992. In 1999, the death in the US of Jesse Gelsinger following gene therapy led to a setback and an intense scrutiny of the supervision process in gene therapy trials. In 2001, the US FDA introduced stricter guidelines and regulations for gene therapy. There are several late stage clinical trials in cancer gene therapy. The first product for gene therapy of cancer was approved in China in 2003.

2. Relation of Gene Therapy to Other Biotechnologies

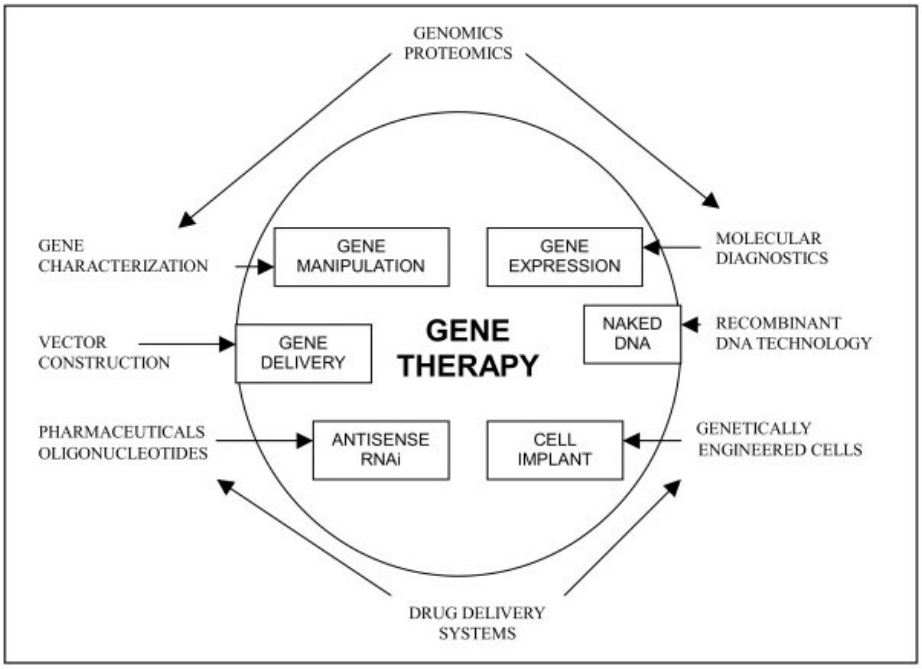

Figure 1. Relation of gene therapy to other biotechnologies

Gene therapy is also related to other biotechnologies, particularly molecular diagnostics and genomics/proteomics. Molecular diagnosis can be defined as the clinical application of molecular technologies to elucidate, diagnose, and monitor human diseases. Molecular technologies include the use of DNA (deoxyribonucleic acid) and RNA (ribonucleic acid) as well as recombinant antibodies. Polymerase chain reaction (PCR) is the best known of molecular diagnostics and has applications in gene therapy. Genomics is the study of all of the genes in an organism - their sequences, structure, regulation, interaction, and products. Pharmacogenomics, an offshoot of genomics, means the application of genomic technologies to drug discovery and development. The completion of the Human Genome Project and the advent of the postgenomic era have its impact on gene therapy as well. Commercial applications of the knowledge of the human genome include gene therapy - transfer of DNA sequences into cells to correct genetic defects, destroy abnormal cells or regulate cellular function. Relation of gene therapy to other biotechnologies is shown in Figure 1.

3. Gene Therapy Technologies

3.1. Classification of Gene Therapy Techniques

Essentially, gene therapy involves three steps: administration, delivery, and expression.

- Administration means introduction of the gene or a vector containing the gene into the body.
- Delivery means the transfer of the gene from the site of the administration to the nucleus of the target cell.
- Expression means the production of a therapeutic gene product in the cell.

A simplified classification of various methods of gene therapy is shown in Table 1.

Gene transfer
Chemical: calcium phosphate transfection
Physical: ultrasound, electroporation, laser irradiation, gene gun
Transduction with recombinant virus vectors
Adeno-associated virus
Adenovirus
Herpes simplex virus
Lentivirus
Moloney murine leukemia virus
Retroviral vectors
Vaccinia virus
Other viruses
Nonviral vectors for gene therapy
Liposomes
Ligand-polylysine-DNA complexes
Dendrimers and other polycationic polymers
Synthetic peptide complexes
Artificial viral vectors
Artificial chromosomes
Use of genetically modified microorganisms as oncolytic agents.
Genetically modified viruses
Genetically modified bacteria
Cell therapy
Administration of cells modified ex vivo
Implantation of genetically engineered cells to produce therapeutic substances
Gene/DNA administration
Direct injection of naked DNA or genes: systemic or at target site
Receptor-mediated endocytosis
Use of refined methods of drug delivery: e.g., microspheres
Gene regulation
Regulation of expression of delivered genes in target cells by locus control region technology
Light Activated Gene Therapy
Molecular switch to control expression of genes in vivo
Promoter element-triggered gene therapy

Repair of defective genes
Involves correction of the gene in situ, e.g., chimeraplasty
Gene repair mediated by single-stranded oligonucleotides
Gene replacement
Excision or replacement of the defective gene by a normal gene
Spliceosome-mediated RNA trans-splicing
Inhibition of gene expression
Antisense oligodeoxynucleotides
Antisense RNA
Ribozymes
RNA interference

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Table 1. Classification of methods of gene therapy

3.2. Physical Methods of Gene Transfer

3.2.1. Electroporation

This is a method of physical transfection for temporarily creating small openings or pores in cell membranes to introduce macromolecules such as DNA. The openings are created by applying an electrical field to the cell for a few microseconds to a few milliseconds; their size varies according to the amplitude and duration of the pulses. Once the DNA has diffused into the cell, the power is discontinued and the pores reseal themselves.

Although DNA can be transported into cells by electroporating their membranes, the molecular basis of this process is controversial. Currently there are two major hypotheses. In the first, DNA is transported through large stable pores that form without significant interaction with DNA and then reseal. The second, more widely supported, hypothesis suggests that DNA is transported into cells by direct interaction with lipids and intermediates that involve a complex of lipid components and DNA. In this case, permeabilization by an electric field might cause a number of smaller pores that by themselves are not large enough to allow DNA transport but make the membrane amenable to structural modifications that allow translocation of DNA. The simulation of the large bilayer shows holes that have a diameter of up to 10 nm, which would be sufficiently large to allow the transport of double-stranded DNA but and it remains to be shown that pores of this size are reversible and will reseal when the electric field is removed. Computer simulations of lipid bilayers in the presence of an external electric field show that pore formation is promoted by an increased likelihood of transmembrane water defects caused by the interaction of water dipoles with the electric field gradient at the water/lipid or water/octane interface.

3.2.2. Particle Bombardment

This method is also known as the "gene gun" technology or biolistic or ballistic microprojectile method. Gene gun is a convenient, hand-held device that provides rapid

and direct gene transfer into a range of targets under *in vivo* or in situ conditions. Particle-mediated gene transfer advanced with the introduction of the helium chamber in which helium replaced gunpowder as the source of acceleration. Modern units employ an adjustable, low pressure helium pulse to sweep DNA- or RNA-coated gold microcarriers from the inner wall of a small plastic cartridge directly into the target. Targeted gene delivery is now a reality for *in vivo* research applications. The microcarriers accelerate for maximum penetration as they move through the barrel, while the helium pulse diffuses outward. The spacer maintains the optimal target distance for *in vivo* applications and vents the helium gas away from the target to minimize cell surface impact.

3.2.3. Ultrasound-mediated Transfection

Several studies during the past decade have suggested that transfection may be stimulated by ultrasound but the transfection efficiency was low. Ultrasound increases the permeability of the cell membrane and facilitates the passive diffusion of plasmids into the cells. Cavitation (microbubbles) caused by ultrasound energy produces breaches in cell membrane and is a key component in ultrasound-mediated transfection. Ultrasound is a promising method for introducing foreign DNA or other macromolecules into cell. Advantages of ultrasound-mediated transfection are:

- Ultrasound energy can be transmitted to the walls of containers normally used to grow cells *in vitro*.
- Ultrasound may be used for transfection *in vivo* because it can be focused into restricted areas or cavities of the body.

Tremendous enhancement in gene expression can be achieved with ultrasound alone following either intratumoral, intramuscular or intravenous injection of a gene product. Ultrasound-targeted microbubble destruction can be used to deliver adenoviral or plasmid DNA to the myocardium. This technique holds great promise in applying the rapidly expanding repertoire of gene therapies being developed for cardiac disease. Cationic acoustic liposomes, whose composition and structure enables them to reflect ultrasound, have been used to improve the efficacy of liposomal gene transfer. The ultrasound increases DNA release from liposomes at much lower power than is required to produce the same effect with standard liposomes. Acoustic liposomes conjugated to antibodies have been shown to target sites of vascular disease. This technology will enable simultaneous identification of the disease site and activation of the therapeutic agent in situ.

3.2.4. Molecular Vibration

Electric field-induced molecular vibration enables foreign DNA molecules to penetrate the plasma membrane and enter the cytoplasm of both primary mesenchymal progenitor cells and established cell lines of various species, at high efficiency and with low cell mortality. This procedure requires no special reagents, allows stable expression of transduced DNA, and does not interfere with the normal cellular differentiation activities of human and chick mesenchymal progenitors.

3.2.5. Gene Transfection using Laser Irradiation

Laser beams have been used for gene transfection. The principle of this procedure is that a small hole is made in a cell membrane by pulse-wave Nd:YAG (neodymium:yttrium aluminum-garnet) laser irradiation, and a gene contained in a medium is transferred into the cytoplasm through the hole. This method enables transfection of targeted cells, i.e. cells in suspension as well as attached cells. This method is useful for gene transfection in cellular biotechnology. Holmium YAG laser (Ho:YAG) is also a promising new gene transfer strategy.

3.2.6. Photochemical Transfection

Most synthetic gene delivery vectors are taken up in the cell by endocytosis, and inefficient escape of the transgene from endocytic vesicles often is a major barrier for gene transfer by such vectors. To improve endosomal release, photochemical internalization (PCI) is used. PCI is based on photochemical reactions initiated by photosensitizing compounds localized in endocytic vesicles, inducing rupture of these vesicles upon light exposure. PCI constitutes an efficient light-inducible gene transfer method *in vitro*, which potentially can be developed into a site-specific method for gene delivery for *in vivo* gene therapy. PCI treatment by the photosensitizer aluminum phthalocyanine strongly improves transfection mediated by cationic polymers (e.g. poly-L-lysine and polyethylenimine), while the effect on transfection with cationic lipids is more variable. The timing of the light treatment relative to the transfection period is also important, indicating that release of the DNA from early endosomes is important for the outcome of PCI-induced transfection.

3.2.7. Chemical Methods of Gene Transfer

The technique relies on precipitates of plasmid DNA formed by its interaction with calcium ions. It is an inexpensive and simple technique. Plasmid DNA is mixed in a solution of calcium chloride, and then is added to a phosphate- buffered solution. Transfer efficiency is usually quite low (less than 1%) and reaches levels greater than 10% in only a few specific cell lines. The DNA precipitates enter the cell by endocytosis. Although this technique has minimal cellular toxicity and is both simple as well as inexpensive, the low level of transgene expression prompted development of other techniques. Little or no transgene expression has been observed *in vivo* following calcium phosphate transfection.

Specific molecules and molecular complexes are used to improved gene permeation of cellular membranes. Chemical techniques enable water soluble DNA to pass through the fatty membranes of the cells, either by opening "holes" in the membrane for the genes to pass through, or by complexing the DNA with liposomes that allow its transmembrane passage via normal transport mechanisms. This technique is occasionally used in research but there is no commercial development for gene therapy.

3.3. *Ex vivo* and *In vivo* Gene Therapy

Gene transfer to human patients may be *ex vivo* or *in vivo* as shown in Figure 2.

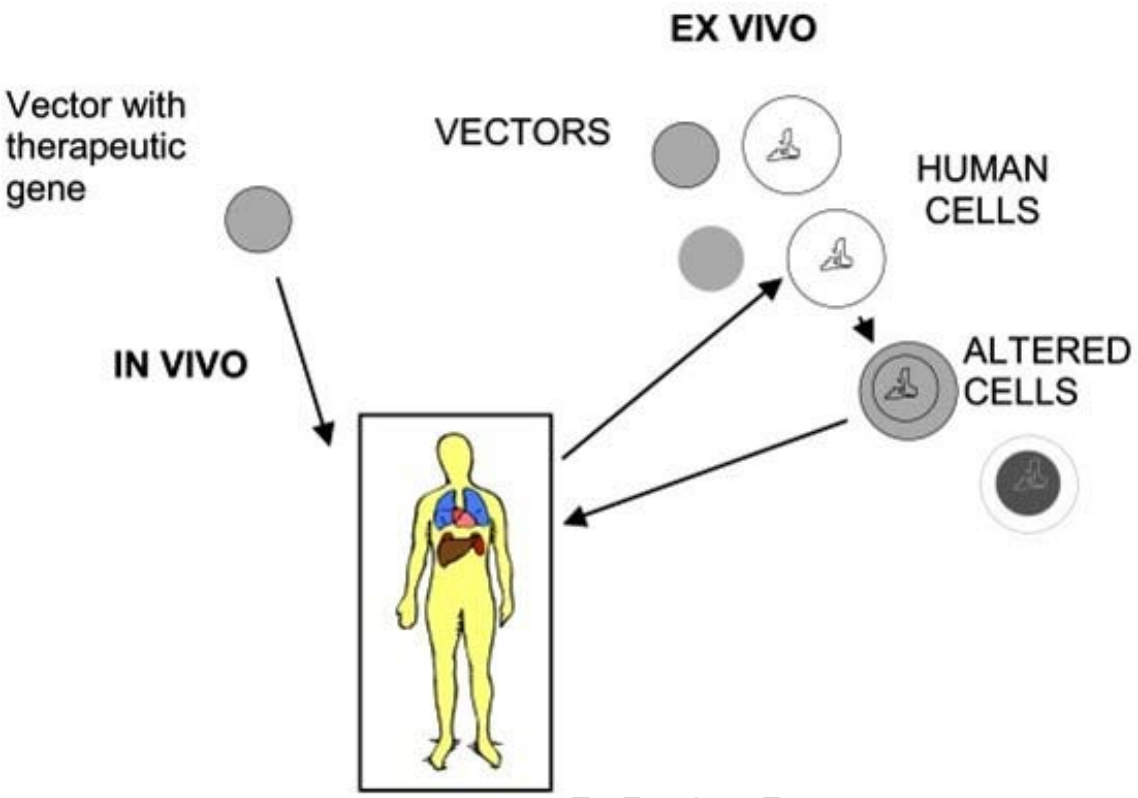

Figure 2. *Ex vivo* and *in vivo* techniques of gene therapy

3.3.1. *Ex vivo* Gene Therapy

Ex vivo gene transfer techniques usually involve the genetic alterations of cells (cell lines or human cells), mostly by use of viral vectors, prior to implanting these into the tissues of the living body. These methods have been frequently used in clinical trials because they are usually more efficient than *in vivo* methods. One disadvantage is that reimplantation of genetically engineered cells grown in culture may not result in long-term survival of a large portion of the cells unless they are protected by encapsulation before injection.

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Biographical Sketch

Professor K. K. Jain is a neurologist/neurosurgeon by training and has been working in the biotechnology/biopharmaceuticals industry for several years. He received graduate training in both Europe and USA, has held academic positions in several countries, and is a Fellow of the Faculty of Pharmaceutical Medicine of the Royal Colleges of UK. Currently, he is a consultant at Jain PharmaBiotech. Prof. Jain is the author of over 370 publications including 12 books and 46 special reports, which have covered important areas in biotechnology, gene therapy and biopharmaceuticals.