GENE THERAPY: PRINCIPLES AND POTENTIALS

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Many disease states are caused either by infection or genetic alteration. The occurrence of genetic diseases has been well documented in medical literature and to date there are more than 5,000 reported cases in the world. The incidence of an infant being born with a serious genetic defect is one in a hundred. With the progress of the infant's growth and development, the physical or mental abnormalities caused by the genetic defect become more evident causing suffering and death. Although drug therapy has been used to alleviate the pain and sufferings, cures for the genetic diseases have remained elusive. In addition to inherited disorders, many acquired disorders, such as cancer, have been shown to occur on the basis of acquired genetic defects. For the only way a genetic disease can be cured is by replacing the defective gene with a healthy gene. It may also be possible to reverse genetic lesion in acquired genetic diseases. This clinical approach is called gene therapy.

Like other fields of biological sciences, the medical sciences have also entered the age of genetic engineering which allows the introduction of exogenous genes to cells within or without an organism for the synthesis of desirable proteins. In the following we will discuss the principles and procedures involved in genetic engineering with specific reference to their potential for gene therapy.

Principles of Gene Therapy

A normal human cell (somatic cell) carries 2 sets of 23 chromosomes, one set inherited from the mother and the other from the father. The DNA in each chromosome carries thousands of genes, and each gene contains codes for a single protein. Genetic diseases arise when one or more related gene become altered such that the cell produces a different protein than that needed for normal cell metabolism. Such an altered gene is called a mutated gene. Thus, genetic diseases can result from mutation in a single gene or more than one gene. Because it is easier to correct a single mutation, the most promising candidates for gene therapy are the diseases caused by single mutation as opposed to diseases resulting from multiple mutations (*Table I*). Genetic alterations are not only inherited but also can be acquired during the life of a person. Cancers, for example, are caused by such acquired genetic alterations. Thus, gene therapy as a technique has the potential to be used in the treat-

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ment of both inherited and acquired genetic diseases.

TABLE I– *Diseases Caused by Single Gene Defects*

Disease	Defective gene
Severe combined immunodeficiency	Adenosine deaminase
Hemophilia A	Factor VIII
Hemophilia B	Factor IX
Gaucher's disease	Glucocerebrosidase
Emphysema	alpha-1-antitrypsin
Cystic fibrosis	Cysticfibrosis transmembrane regulator
Phenylketonuria	Phenylalanine hydroxylase
Muscular dystrophy	Dystrophin
Thalassaenua	B-globin
Sickel cell anemia	B-globin

The therapeutic gene of interest could be introduced to the patient's germ cells (sperms, eggs, early embryos) or somatic cells. Germ-line gene therapy would not cure the disease in the carrier patient, but would offer a permanent cure for offspring and subsequent generations. Somatic cell therapy, on the other hand, benefits only the patients without any promise for the descendants. Although germ-line gene therapy has been successfully carried out in mice, its application in human gene therapy would raise serious ethical, practical and social questions because of the possible impact on human heredity. Until legal and social answers are available for different real and imaginary controversies, somatic cell therapy, appears to be the only available alternative.

The subcellular mechanism involved in gene therapy is designated as gene replacement or gene augmentation. In gene replacement, the defective gene is removed and the healthy gene is introduced to the same site. So far, yeast is the only eucaryotic organisms where gene replacement is done regularly. In growing yeast cells, DNA fragments are efficiently integrated into the homologous chromosomal sites by general recombination events. In contrast, it is difficult, though not impossible, to achieve homologous recombination in mammalian cells. When a gene is introduced in to mammalian cells, presently there is no way to control the site and method of its integration to the host chromosome. Thus the success rate for mammalian gene replacement has been less than 1%. Neverthless, site specific recombination of foreign DNA (homologous recombination) has been demonstrated in several mammalian system(1,2) including the hypoxanthineguanine phosphoribosyl transferase (HPRT) and the int-2 loci in mouse embryonal cells. When optimized, gene replacement will be the ideal method of gene insertion because of two important advantages. Site-specific insertion increases the probability that the healthy gene will function correctly, and reduces the chances of activation of a dormant cancer cuasing oncogene.

Unlike gene replacement, gene augmentation involves correcting defective gene's function by introducing the healthy gene to a nonspecific site on the host genome (integrative) or to an extragenomic site (non-integrative) with the help of a vector (carrier of the healthy gene) without removing the defective gene. In the latter method, the functional gene introduced need not integrate to the host chromosome for expression. As long the

vector carrying the gene stays intact in the host cell, the therapeutic protein will be produced. Because the DNA delivered in this manner possesses no mechanism allowing its persistence, the therapy is transient, lasting only for days or weeks. In contrast, a more lasting effect is obtained when gene augmentation is achieved by integrating the healthy gene to a non-specific site of the host genome. Gene augmentation therapy can be directed to correct specific group of cells called target cells as in the protocol used to correct adenosine deaminase (ADA) deficiency(3) or to non-targeted somatic cells as in the case of alpha-1-antitrypsin(3). Using non-targeted gene augmentation, any cell that does not normally express a particular gene can be engineered to express that gene. For example the blood clotting factor (Factor IX) can be synthesized, in somatic cells outside the liver, its normal site of synthesis. Gene augmentation also could be used to cure cancers and viral diseases such as the human immunodeficiency virus (HIV) through the introduction of a gene that encodes the ribozyme capable of degrading HIV RNA(5). There are major limitations associated with gene augmentation. This form of therapy may not be helpful where overproduction of the defective protein by the mutated gene (as in the case of sickle cell anemia) can not be compensated by the smaller amount of normal protein produced by the therapeutic gene. Also, it may be deleterious because of the non specific integration, the therapeutic gene may activate an inactive oncogene (a cancer inducer) causing cancer. Despite these possible risks, gene augmentation is the most widely used gene therapy procedure. The development of a successful gene therapy protocol using the described strategies depends on three important criteria: (*a*) efficient delivery; (*b*) optimum expression and (*c*) safety.

Procedures in Gene Therapy

Delivery strategy

Irrespective of the method of gene therapy (*i.e.*, gene replacement or augmentation) an appropriate method must be used to introduce the exogenous healthy gene to the patient. This can be accomplished through *ex vivo* or *in vivo* delivery. In *ex vivo* delivery, cells from the patient are removed for introduction of the healthy gene into these cells which are then returned to the patient. All current human protocols involve *ex vivo* delivery. The advantage of *ex vivo* delivery is that the correction is achieved only in the desired cells and at high frequency. So far *ex vivo* delivery is limited to circulating and dividing cells like bone marrow and fibroblasts because of the relative accessibility and the availability of these cells. The first federally approved gene therapy procedure to correct adenosine deaminase (ADA) deficiency in human was preformed in 1990 by W. French Anderson's group using bone marrow cells.

In *in vivo* delivery, the normal gene would be delivered directly to the target cells of the affected organ in an intact individual, thus avoiding cell transplant. This method, though extremely difficult and limited by inaccessibility of the target organ and target cell, is now being attempted with laboratory animals for development of a gene therapy method for diseases like Cystic Fibrosis and others.

Gene Transfer Vectors

Whether the selected gene delivery method is designated as *ex vivo* or *in vivo,* it essentially involves delivery or introduction

of the healthy exogenous gene into target cells of the patient. This is where the basic genetic engineering methods become an integral part of gene therapy. A healthy gene or parts thereof can be introduced to a recipient cell through nonbiological (physical and chemical) or biological methods (*Table II*). The physical methods involve DNA introduction through electroporation, *i.e.,* by increasing the cell membrane permeability by exposing the cells to rapid pulses of high-voltage, or, microinjection through fine glass pipets. Because these methods require handling of one cell at a time, they are not very effective when a masses of cells need correction, as would be the case in gene therapy. Quick and large-scale DNA introduction is accomplished through chemical methods where the exogenous DNA is coprecipitated with calcium phosphate, conjugated to polycations or lipids, or encapsidated into liposomes. When in contact with the recipient cells, the above chemicals alter the permeability of the membrane, thereby facilitating DNA entry. However, the chemical methods are limited by low efficiency and toxicity.

Alternatively, biological introduction of the exogenous gene is mediated through vectors such as viruses. When a virus is used as vector, a part of the viral genetic material (*i.e.,* usually the virulent portion) is replaced by the therapeutic gene. The many advantages of virus vectors are (*a*) their omnipresence and natural ability to enter the cells, (*b*) availability of techniques to remove their virulence through genetic engineering, and (*c*) their ability to integrate with the host chromosome or remain viable inside the cell without becoming a part of the genome. Depending on the initial genetic make up, a given virus can be a DNA or a RNA virus.

TABLE II—*Gene Transfer System (Vectors) Used in Gene Therapy Protocols*

Vector		Efficiency	Integration	Limitation on DNA size	Need for dividing cells	Cell specific delivery	Repeated dosing needed	Toxiciy
Nonbiological								
Chemical	Calcium phosphate	High	±	–	–	–	?	+
Fusion	Liposomes	High	±	–	–	–	?	+
Physical	Electroporation	Low	+?	–	–	–	?	+
	Microinjection	Low	+	–	–	–	?	+
Biological								
Viral	RNA virus (Retrovirus)	High	+	+	+	–	–	–
	DNA virus (Adenovirus)	High	–	+	–	–	+	–
Conjugated system	DNA-protein	High	+	–	–	+	–	–

Code explanation: ± Uncertain; + Yes; – No; ? Unknown.

To date retroviruses (*i.e.,* RNA virus) are considered as the most promising gene vectors because of the high efficiency of gene transfer, the integration of their DNA with the host cell's chromosome, and their infection of a broad spectrum of cells. The mechanisms of the entrance, insertion, and expression of the viral genome carrying the foreign DNA have been reviewed in many articles(6,7). The disadvantages of retroviral vectors are the requirement of dividing host cells for gene transfer, activation of oncogenes due to insertional mutagenesis and potential helper virus production. Because retroviruses are not very effective gene delivery vectors for low proliferating host cells such as airway epithelia, the possibility of using other viruses as vectors is under development. Recently, adenoviruses (DNA viruses) have been successfully used as gene delivery vectors. The major advantages of adenovirus vectors are their ability to infect nonproliferating cells and suitability for *in vivo* delivery especially in lung(8). The disadvantage of the adenovirus vector is its inability to integrate to the host chromosome causing short-term expression of the therapeutic gene. So the therapy has to be administered in short intervals. In absence of a better alternative a short-term therapy is desirable for some diseases.

The problem with the viral gene delivery systems is the limitations on the size of the therapeutic DNA which can be carried with the viral genome where as the problem with nonbiological system is non-specific delivery and toxicity. To minimize these disadvantages, the biological and non-biological vector producing techniques have been combined to develop a new vector where the therapeutic DNA is hooked to the outside of the viral coat instead of integrating it with the viral genome(9). The advantages of this conjugated system are relatively unrestricted therapeutic DNA size, cell specific delivery and less toxicity. When fully optimized, this gene delivery system may be a major tool in gene therapy.

Conclusions

This brief overview shows that gene therapy is no more a scientist's dream and is moving rapidly toward clinical applications. At present 17 clinical protocols are under development in different laboratories. Questions pertaining to safety, long term expression, and optimization of the protocol have to be answered before these protocols can be used as accepted medical procedures. Gene therapy, when perfected, will undoubtedly have a major impact in the health care in industrialized countries. At the current rate of progress, gene therapy is likely to be practised in many major hospitals around the world before the onset of the 21st century. But its applicability to and impact in developing countries like India is uncertain. The uncertainty is based on the fact that gene therapy in the present form requires specialized laboratories and the clinical procedures depend on highly skilled professionals, which are both very scarce and expensive. Also, the social and religious issues are unique depending on the country. In a society where pride runs deep in a person's heritage and family history and belief runs strong in predestination, acceptance of gene therapy as a cure for genetic diseases will depend on the national priority rather than social choice. It is hoped that proper education and understanding will shorten the time otherwise needed for the acceptance of gene therapy for social welfare at large and more especially for child welfare.

Acknowledgements

The authors thank Drs. Margaret Leigh and Subhash C. Mohapatra for their critical review and helpful suggestions.

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