

7 Gene Therapy



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Gene therapy has become an increasingly important topic in science-related news. The basic concept of gene therapy is to introduce a gene with the capacity to cure or prevent the progression of a disease. Gene therapy introduces a normal, functional copy of a gene into a cell in which that gene is defective. Cells, tissue, or even whole individuals (when germ-line cell therapy becomes available) modified by gene therapy are considered to be transgenic or genetically modified. Gene therapy could eventually target the correction of genetic defects, eliminate cancerous cells, prevent cardiovascular diseases, block neurological disorders, and even eliminate infectious pathogens. However, gene therapy should be distinguished from the use of genomics to discover new drugs and diagnosis techniques, although the two are related in some respects. The two main types of gene therapy are somatic cell gene therapy and reproductive or germ-line gene therapy. This chapter also discusses therapeutic cloning, which involves stem cell manipulation for tissue and organ production.

Germ-line cell therapy involves the introduction of corrective genes into reproductive cells (sperm and eggs) or zygotes, with the objective of creating a beneficial genetic change that is transmitted to the offspring. When genes are introduced in a reproductive cell, descendant cells can inherit the genes.

Gene therapy of somatic cells, those not directly related to reproduction, results in changes that are not transmitted to offspring. An example of gene therapy in somatic cells is the introduction of genes in an organ or tissue to induce the production of an enzyme. This alteration does not affect the individual's genetic makeup as a whole and it is not transmitted to its descendants. With somatic cell gene therapy, a disabled organ is better able to function normally. This technology has many applications to human health. One variant of somatic cell gene therapy is DNA vaccines, which allow cells of the immune system to fight certain diseases in a method similar to conventional vaccines.

Stem cell therapy involves the use of *pluripotent cells*, or cells that can differentiate into any other cell type. Stem cells are found in developing embryos and in some tissues of adult individuals. This therapy is similar to a conventional transplant, with the objective of regenerating or repairing a damaged organ or tissue. The procedure

has a reduced probability of rejection because it uses the individual's own cells. For instance, stem cells differentiated into nerve cells could be used by patients suffering from paralysis, with the goal of helping them recovering movement; or in cases of heart stroke, muscle cells might be used to rejuvenate the cardiac muscles. Furthermore, the future may bring the growth of stem cells from an individual's body to produce certain tissues or organs in vitro. Stem cell research could eventually blend gene therapy with genetic engineering to create healthy stem cells that can be used to generate healthy organs and tissue.

A fundamental requirement for gene therapy is the correct identification of genes coding for diseases. This can be accomplished at a spectacular speed with the information from the Human Genome Project. Scientific magazines have been announcing, with great frequency, the discovery of genes responsible for several medical conditions, from Alzheimer's disease to baldness. The knowledge of the genes involved in these traits allows unequivocal diagnosis of the disease in the patient, an essential step before treatment can be initiated for the genetic disease. Biotechnology is contributing to the development of the needed genetic tests for detection of defective genes.

The most complex phase in gene therapy is the development of mechanisms to deliver the therapeutic genes to the target organ in an accurate, controlled, and effective way. That step has been developing more slowly and is currently the most limiting factor for gene therapy.

GENETIC DEFECTS

Each human being carries normal as well as some defective genes. Usually, the individual does not become aware of the presence of a defective gene until a disease associated with the gene is manifested in him or her or in a relative. More than 4,000 medical disorders caused by defective genes have been identified, each with varying degrees of seriousness. About 10 percent of the human population will evidence, sooner or later, some type of disorder. Although genes are responsible for predisposition to disease, the environment, diet, and lifestyle can affect the onset of the illness.

An example of a genetic disease is cystic fibrosis, which frequently becomes evident in the first years of life for the child carrying the defective gene. The mutant gene causes the development of cysts and fibrous tissue in the patient's pancreas and the production of thick and viscous lung mucous. The mucous makes breathing very difficult and, in many cases, is fatal. On average, in Western countries, about 1 child in 2,500 has the disease. If the child receives two defective recessive alleles of the gene named CF (one from each parent), he or she will develop the disease. Patients with cystic fibrosis can reduce the symptoms of the disease with drugs developed through genetic engineering. A cure for cystic fibrosis may come through gene therapy. One possibility is a genetically engineered virus, carrying the corrective gene, which after being introduced into the patient's lung cells would allow the lungs to function properly. The introduced gene would allow the lung cells to produce a protein that eliminates the mucus.

Most people do not manifest genetic diseases because, most of the time, they are carriers of just a single defective copy of the CF gene. As most of the defective genes are recessive, meaning two copies are needed for expression of the disease, most people do not have the disease. This is the reason for the larger incidence of genetic diseases in children from related parents.

If the defective gene, however, is dominant, the disease is expressed in any people that carry the defective gene. Huntington's Disease, a disorder of the nervous system that usually occurs after the age of 45, is an example of a genetic disease caused by a dominant gene.

Having a defective gene does not make disease development a certainty. Besides the large effect from genetics, the environment is also important to the onset of many illnesses. Diseases such as heart disease do have a genetic component, but are largely dependent on diet and lifestyle. Some genetic diseases also have benefits. A classic example of a genetic disease that has a beneficial effect on human survival is sickle cell anemia. There exists in the human population a defective β -hemoglobin gene and individuals carrying two copies of the defective gene develop sickle cell anemia, a blood problem caused by defective hemoglobin and consequently misshapen red blood cells. The genetic mutation in the defective allele of this disease is a single nucleotide change, from an A in normal genes to a T in the mutant. This single nucleotide mutation results in a mutant β -hemoglobin that possesses the amino acid valine instead of

Table 7-1
 Medical Conditions for Which Gene Therapy Is Being Studied

ADA deficiency	Hemophilia
AIDS	Liver cancer
Asthma	Lung cancer
Brain tumor	Melanoma
Breast cancer	Muscular dystrophy
Colon cancer	Neurodegenerative conditions
Diabetes	Ovarian cancer
Heart diseases	Prostate cancer

glutamine. The mutant β -hemoglobin has less affinity to oxygen, becoming a poor oxygen transporter in the blood. However, carriers of a single copy of the defective allele do not have the disease, and they are also resistant to malaria. There is an obvious advantage of carrying a single allele of the defective hemoglobin gene, especially in regions where malaria is endemic, as in tropical regions of Africa.

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 immune system deficiency (adenosine deaminase enzyme [ADA] deficiency or bubble-boy disease) received an infusion of white blood cells that had been genetically modified to contain the gene that was absent in his genome. Since then, gene therapy has been studied and experimentally tested for several medical conditions.

Diseases caused by the absence of an enzyme or the presence of an inactive enzyme are potential targets for gene therapy. Cystic fibrosis, ADA deficiency, and many other genetic diseases are among the candidates for gene therapy. Table 7-1 lists other diseases for which gene therapy is being considered.

VECTORS FOR GENE DELIVERY

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. *Vectors* are plasmids or viruses that are used to move recombinant DNA from one cell to another. A retrovirus is a special class of RNA viruses that can insert its nucleic acid into host cells. The viruses possess a gene for production of the reverse transcriptase, an enzyme that transcribes RNA in DNA in the host cell. Adenovirus, retrotransposons, and liposomes are other vectors used for gene transfer in gene therapy. They are all able to transfer and integrate genes into new cells. Retroviruses used in gene therapy are engineered so that any genes that are harmful to man are removed. Corrective genes are then added to replace the removed genes, and the new, modified retrovirus is then introduced into the patient.

One of the challenges for vectors is to survive the patient's immune system so they can transfer the corrective genes from their genome into the patient's cells. In general, the immune system of the human body contains molecules that immobilize viruses or other microorganisms that could infect the organism. Viruses that escape the immune system need to penetrate the cellular membrane, an additional barrier to infection. Finally, the infecting retrovirus must integrate its genome with that of the host, thereby moving the corrective genes into the genome of the infected cell. This integration happens in a random manner. It should occur in an area of DNA that is not essential to the host genome, or a risk of other complications might occur. Furthermore, the introduced gene must be transcribed and expressed for the production of the correct enzyme. With all these processes at the molecular level, gene therapy becomes a very complex procedure.

Another promising strategy, which has been used for the introduction of therapeutic genes in lung cancer treatment, is the direct injection of the corrective genes into the target area. Using this strategy, scientists have injected a drug containing the normal version of the gene p53, which suppresses cell tumor growth, directly into the patient's cancerous tumor. This technique bypasses the immune system reaction to the invading vector, a problem frequently associated with gene therapy. Many scientists believe that as gene therapy develops, it will be possible in the near future to easily introduce genes into pa-

tients through intramuscular injection, especially for cases of anemia, hemophilia, diabetes, and other diseases related to the circulatory system.

GENE THERAPY RISKS

The first death associated with gene therapy occurred on September 18, 1999, at the University of Pennsylvania. Jesse Gelsinger was participating in a clinical trial, a biomedical experiment for evaluation of safety and efficiency of a therapy for a disease. Gelsinger, who was 18 years old at the time of the treatment, had a deficiency of ornithine transcarboamylase, an important enzyme in the metabolism of ammonia. Patients with this rare metabolic disorder must maintain a low-protein diet and take a series of medicines to avoid ammonia poisoning in the blood stream. The gene therapy Gelsinger took triggered a chain reaction in his immune system, resulting in hepatic and respiratory failure, and consequently, his death four days after being treated.

Since Gelsinger's death, the University of Pennsylvania has been reevaluating all procedures involved in the vector engineering and in the administration of the therapy. No flaw has been found that would explain such an extreme reaction by his immune defense system. Ever since, the public and the FDA, the agency responsible for oversight of clinical trials in the United States, have been more skeptical and doubtful about whether current scientific knowledge is enough to justify further investigations with humans. The credibility of gene therapy was seriously damaged, resulting in a temporary moratorium on human clinical trials.

Another challenge to gene therapy has been its ephemeral benefits to patients. This has been observed in several clinical trials with cystic fibrosis and ADA deficiency patients, whose cure faded after a few months of therapy, and was followed by a return of the disease symptoms. A possible explanation for that is that the genetically modified somatic cells (see Figure 7-1) decreased in amount. Because they are already differentiated and possess only a limited capability to multiply, it is expected that after they are gone, the treated organ could become diseased again.

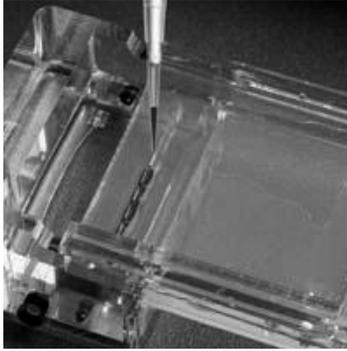


Figure 7-1
Isolation of corrective genes for use in gene therapy.

DNA VACCINES

A variation of gene therapy with somatic cells is the introduction of genes (naked DNA), with the objective of triggering the immune system to produce antibodies for certain infectious diseases, cancer, or some autoimmune diseases. Therefore, the objective is not repair of a defective gene in the individual's genome. Those genes can be introduced via intramuscular injections, inhalation, or oral ingestion. Cells that take up the gene in their genome can express the protein that stimulates the immune system to act against the disease.

The greatest challenge in this procedure is the transient effect of gene expression, because the modified cells can go through only a limited number of divisions before dying. Another challenge is the low efficiency of gene incorporation and expression in the target cells. Although in some cases the temporary gene expression is enough to trigger an effective immune response, most cases require a more lasting gene expression.

GERM-LINE CELL THERAPY

The main advantages of germ-line cell gene therapy are the following:

1. It offers the possibility for a true cure of several diseases and it is not only a temporary solution.

2. It might be the only way to treat some genetic diseases.
3. The benefits would be extended for several generations, because genetic defects are eliminated in the individual's genome and, consequently, the benefits would be passed to his or her offspring.

Some of the arguments presented against germ-line cell gene therapy are the following:

1. It involves many steps that are poorly understood, and the long-term results cannot be estimated.
2. It would open the door for genetic modifications in human traits with profound social and ethical implications.
3. It is very expensive and it would not benefit the common citizen.
4. The extension of the cure to a person's offspring would be possible only if the defective gene was directly modified, but probably not if a new gene was added to another part of the genome.

STEM CELL THERAPY

Stem cell therapy or therapeutic cloning does not involve gene therapy itself. However, in the future it might be used in conjunction with gene therapy for regeneration of tissue and organs after they have been treated with corrective genes. Visually, stem cells are not distinguishable from any other cells of the human body. Under a common microscope (magnification 20 to 40 times), those cells can only be observed using special dyes. Visually there is no significant difference in such cells. The real differences exist at the DNA level, where gene expression is amendable to signals influencing protein expression. The cells can differentiate into any of the 220 cell types of the human body (e.g., kidneys, heart, liver, skin, or retina), a phenomenon called *pluripotency*. At birth, stem cells can be harvested from an individual's bone marrow, fat tissue, and the umbilical cord. Embryonic stem cells are harvested from embryos up to a few days after fertilization.

Another characteristic of stem cells is their capability to grow indefinitely. Whereas the remaining body cells have a biological programming that limits the number of cell divisions they can go through before dying, stem cells can be maintained indefinitely in a petri dish with nutritive media.

Stem cell therapy provides hope for a cure for patients of incurable afflictions such as Parkinson's disease and Alzheimer's disease, and also for people suffering from paralysis resulting from spinal cord injuries.

At first, some opponents speculated that stem cells would be used in nurseries to produce organs such as livers, hearts, and virtually any other body part. However, most organs possess complex structures with ducts and valves, making it impossible to produce them outside of the organism. Stem cells have opened a new avenue for disease treatment. For example, the injection of stem cells into the liver of a patient with cirrhosis or hepatitis could result in new tissue capable of performing its role. Stem cell therapy also has great potential to cure rheumatoid arthritis and some heart diseases. Recent research has found that spine-injured mice suffering from paralysis were able to move their legs following an injection of stem cells.

Some people believe that if human stem cells are as versatile as those of mice, they might be the long sought after fountain of youth. The combination of stem cells with gene therapy might allow rebuilding of new body parts to substitute for old and defective ones. Right now, different procedures are being tested for curing ADA deficiency. Somatic cell gene therapies have the limitation of lasting for only a few months, which in turn requires repeated applications. With the use of stem cells to regenerate healthy bone marrow cells, a permanent cure is expected, as healthy cells have the capability to grow and divide continuously.

Embryonic stem cells, from embryos about four days old, have been at the center of a heated debate due to ethical issues. The main disagreement is whether or not a four-day-old embryo is already a human life. When would an embryo or a fetus reach the status of life? Those that support the use of embryonic stem cells would argue that human life would not begin until about the 14th day after the fertilization, whereas the opposition argues that

life begins at conception (i.e., at the moment of the fertilization of the egg by the sperm). For many, the destruction of embryos for the purpose of treating another human being is wrong. Recently, in the United States, the Bush administration broadened the definition of a child eligible for coverage under the Children’s Health Insurance Program by classifying a developing fetus as an “unborn child.” Many activists are arguing that the Bush administration’s proposal demonstrates its commitment to the strategy of undermining a woman’s right to choose abortion by ascribing legal rights to embryos. This subject is addressed further in Chapter 14, “Bioethics.”

FINAL CONSIDERATIONS

Although the idea of gene therapy has been around for only 20 years, the technique has been drawing a great deal of interest and curiosity through the world. The first trials generated great expectations within the scientific community. Although there have been several disappointments, many believe that it is just a matter of time before the technical and scientific details are mastered and the procedures become routine. This research is being advanced worldwide. In fact, Alain Fischer, a medical doctor in Paris, France, reported the complete cure of two children who had a rare immune deficiency condition.

Another promising result from stem cell research has been reported in type-B hemophilia patients at the Children’s Hospital in Philadelphia and at Stanford University, where patients treated with gene therapy presented a reduction in the period for blood coagulation. ADA deficiency, a disease caused by a defective gene for the ADA enzyme present on human chromosome 20 has been a focus for gene therapy in many institutions. In one of the cases, several patients treated with the corrective gene were able to reconstitute their immune systems and are living normal lives out of the isolated bubbles that are needed to maintain an environment free from microbes. The patients started to produce a correct ADA enzyme after receiving the gene therapy.

The potential use of this therapy to cure other more complicated diseases, such as cancer and coronary diseases, also seems promising. Gene therapy is still in its infancy, but it is believed that as it matures, it will become an effective treatment for the myriad of genetic diseases that affect humanity.