

Medical Applications of Biotechnology • part 2

Gene therapy

LEARNING OBJECTIVES

- **Describe** the Human Genome Project and its impact on the medical field.
- **Explain** gene therapy and antisense therapy.
- **Describe** the medical implications of human gene therapy.
- **Define** stem cells, and describe their potential medical uses.
- **Describe** the cloning process and its potential medical uses.

This second article in a three-part series provides an overview of gene therapy. Topics discussed include the Human Genome Project, human gene therapy, stem cell therapy and cloning.

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The Human Genome Project

The Human Genome Project is a cooperative international project, begun in 1990, that has deciphered the genes contained in the 23 pairs of human chromosomes. In 2000, a series of technological breakthroughs and competition between the public and private sector led

to the completion of a working draft that identified approximately 99% of the human DNA sequence, known as the genome.

The findings from this project have implications for basic medical research, diagnosis of disease, development of new drugs and gene therapy.

Basic medical research. Efforts are currently underway to identify correlations between genetic mutations and susceptibility to disease. Scientists are also looking at Human Genome Project findings to better understand complex diseases such as cancer and HIV.

The National Cancer Institute has begun a major initiative in conjunction with the Human Genome Project. This program, called the Cancer Genome Anatomy Project (CGAP), is compiling an index currently containing more than 40,500 genes active in cancers. This tumor gene index lists all genes found, groups them according to their associated cancers, and compares gene expression in normal, precancerous and cancerous cells. This ability to report patterns of gene activity in cells has been developed using DNA microarrays.

Microarray technology is a new means of evaluating gene expression in tissue using a complementary DNA (cDNA) probe. Within minutes, it can show how an experimental drug might interact, for example, with the gene that causes breast cancer. This technology has applications not just in cancer, but also in many other disease and developmental processes.

Diagnosis of disease. Diagnosis using

genetic testing has the potential to radically improve medical practice. The identification of a genetic marker associated with a specific disease can be used to confirm a medical diagnosis, or to test for a disease prior to the development of symptoms. Testing patients who display disease symptoms can provide data that help in selecting the optimal therapy for a patient. Presymptomatic genetic testing can identify individuals for whom prophylactic therapy can prevent development of a serious disease.

Drug development. The explosion of genetic information from the Human Genome Project, combined with the use of microarray technology, has produced an astonishing number of compounds to be evaluated for potential use as drugs. The potential market for these drugs may be smaller than for more traditional drugs because they will target smaller groups of patients. However, this may be offset by reductions in development costs compared with traditional pharmaceuticals. It is expected that safety and efficacy can be demonstrated without many large clinical trials because of the ability to accurately select patient groups.

The Human Genome Project is devoting research, time and money to studying the ethical, legal and social implications of its own discoveries, addressing such issues as:

- Fairness in the use of genetic information (i.e., who should have access).
- Privacy and confidentiality.
- Stigmatization due to genetic differences.
- Reproductive issues (i.e., the use of genetic information in reproductive decision-making).

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Human gene therapy

Human gene therapy approaches disease treatment by replacing a malfunctioning gene or adding a new gene to human cells to direct the production of a missing or abnormal protein. Gene therapy may have applications in diseases such as cystic fibrosis, caused by a single mutant gene, and in diseases such as cancer or heart disease, caused by many altered genes and environmental factors.

Gene therapy can be administered *in vivo* (genes are directly inserted into cells inside the body) or *ex vivo* (genes are transferred into cells outside the body and then reinserted into the body). However, the real key to gene therapy involves inserting the desired genetic material into cells.

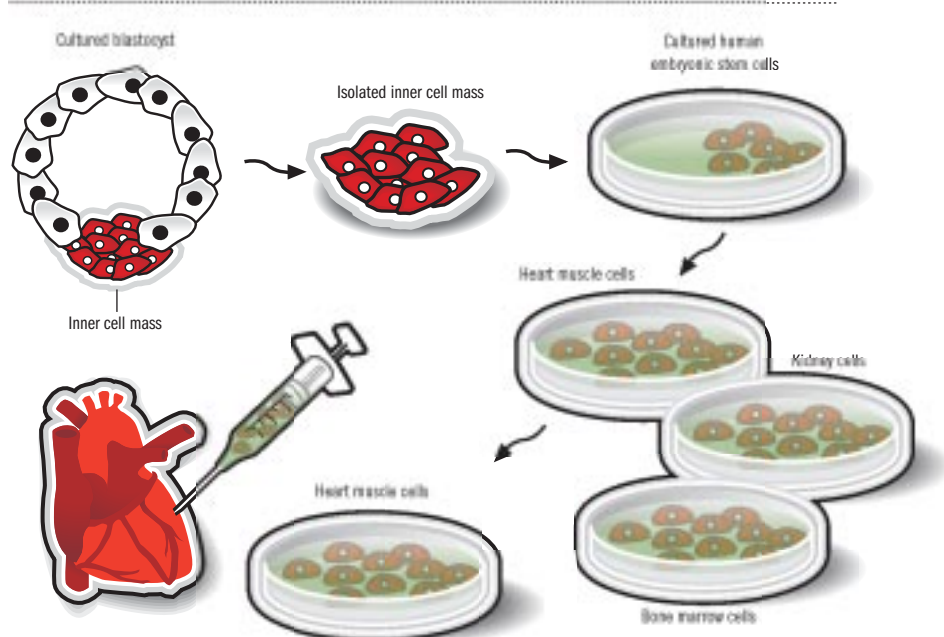
This is typically accomplished through gene transfer, using viral and nonviral vectors. Viral vectors are viruses that are modified so that they do not cause disease but retain the ability to penetrate cells; types include adenoviral vectors and retroviral vectors. Nonviral vectors are created using liposomes, naked DNA or molecular conjugates (protein-DNA complexes that can target specific cells).

Antisense therapy. This form of gene therapy uses small pieces of DNA to block the ability of specific genes to produce disease-causing proteins. In cells, DNA is stored in the nucleus in a double-stranded form, and it transmits information used to create proteins via single-stranded messenger RNA (mRNA), called the “sense” strand. In antisense therapy, a mirror image of the mRNA strand is created. This antisense strand then binds with the mRNA to prevent it from producing the encoded protein. Eventually, it is hoped that antisense therapy can be used to treat a wide variety of infections, inflammatory diseases and cancers with minimal adverse effects. At present, antisense drugs for rheumatoid arthritis, Crohn’s disease and various cancers are in clinical trials.

Medical applications of gene therapy

Gene therapy has been evaluated in clinical trials for the treatment of cancer, genetic diseases and infectious diseases in more

Culturing human embryonic stem cells



than 4,000 patients. One of the first successes of gene therapy involved the treatment of severe combined immune deficiency, a genetic disease that prevents the normal function of the immune system. In initial trials, gene therapy has also been successfully used to treat another genetic disease, familial hypercholesterolemia, by inserting low-density lipoprotein (LDL) receptor cells into patients’ livers and restoring normal cholesterol levels.

A number of gene therapy approaches are under evaluation for the treatment of a variety of cancers, and many show promising results. These include:

- **Suppression of oncogenes:** Antisense therapy blocks the expression of tumor-producing genes.
- **Insertion of tumor suppressor genes:** This approach restores the function of tumor-suppressor genes.
- **Suicide gene therapy:** Materials are introduced preferentially into cancer cells to make them susceptible to substances that will not harm normal cells.
- **Immunomodulatory gene therapy:** Genes that direct the production of tumor-killing cytokines are introduced into tumor cells.

Serious adverse events, including deaths, have been associated with the use of gene

therapy relying on adenoviral vectors. This has led the National Institutes of Health to revise its recombinant DNA (rDNA) guidelines. Significant improvements in targeted gene delivery and associated protein expression will be required before gene therapy becomes a practical clinical approach.

Two very new approaches within the realm of genetic therapies are stem cell therapy and cloning.

Stem cell research and therapy

When a sperm fertilizes an egg, the resulting cell has the potential to form an entire human. This cell divides rapidly, and within four days it forms a hollow sphere of cells called a blastocyst. The outer layer of the blastocyst contains the cells that will form the placenta and other tissues to support the developing fetus. The inner layer of the blastocyst contains stem cells, capable of forming all the cells required to form the human body.

Approaches for obtaining stem cells include:

- Isolating cells from the inner layer of blastocytes using embryos donated following *in vitro* fertilization procedures.
- Taking fetal tissue cells from regions destined to develop into the testes or ovaries

of embryos obtained from terminated pregnancies.

- Using somatic cell nuclear transfer, in which a normal human egg has its nucleus removed and then is fused with a somatic (non-egg or sperm related) cell.

Stem cells can produce virtually unlimited quantities of any cell in the body; therefore their potential medical impact is enormous. Scientists believe that these cells can be genetically engineered so that, if used in transplantation, they will not be rejected by their host.

The following are some of the types of cells that might be developed for medical use with stem cell technology:

- Cardiomyocytes – to replace heart muscle cells damaged by injury or ischemia in patients with congestive heart failure or myocardial infarction.
- Hematopoietic stem cells – to promote blood cell development in patients with HIV or sickle cell anemia, or those undergoing bone marrow transplantation.
- Endothelial cells – to repair damaged blood vessels in patients with atherosclerosis or ischemic areas of the brain or lower extremities, and to treat angina, stroke and arterial insufficiency.
- Islet cells – to produce insulin in the pancreas, or provide a cure for insulin-dependent diabetes.
- Neurons – to replace damaged nervous system cells in patients with Parkinson’s disease and Alzheimer’s disease.

It is expected that stem cells may provide a permanent, stable source of human cells to use to screen substances for therapeutic potential and to test for toxicity. They may also replace animal models for determining the potential of drugs to cause birth defects. In addition, stem cells may be useful in researching infertility disorders, premature pregnancy loss and birth defects, as well as producing tissues for transplantation. The future of stem cell therapy depends on the ability to create differentiated cells from stem cells and to develop large-scale production technology.

Cloning

Cloning involves transferring the complete genetic material from the nucleus of a cultured donor cell to a mature recipient egg

from which the nucleus has been removed. This process, called nuclear transfer, will result in offspring that are genetically identical to the organism from which the nucleus was taken.

The cloning of animals represents a potential source for pharmaceutical and nutritional proteins. Protein drugs that require complex modifications, or are needed in large supplies, can be produced by genetically modifying an animal to produce the desired protein in its milk.

The process begins when the gene for the desired protein is combined with a DNA signal that directs its production in the mammary gland. This DNA combination is then injected into the embryo of an appropriate animal, which is then implanted into a recipient mother. The resulting transgenic animal has the gene for the desired protein in all of its cells. However, the gene is instructed to function only in the mammary gland, so that the protein is made only in the animal’s milk. Thus, there is no danger of disease or harm to the animal in creating the desired protein.

This process, sometimes called “pharming,” is an rDNA technology that shows promise for creating a number of proteins with therapeutic value, including insulin, interferon, tissue plasminogen activator, hemoglobin and clotting factors used in treating hemophilia.

The following are a few benefits provided by the technology of cloning animals:

- All animals are born transgenic.
- Development of a production herd is significantly faster.
- Availability of products is accelerated, and clinical trials can begin sooner.
- Genes are removed selectively and replaced with the needed human equivalents.

Scottish scientists have successfully produced lambs from fetal cells that were genetically modified to carry genes for human proteins. Such animals, called founder organisms, could significantly lower the cost of producing proteins through rDNA technology by substantially increasing the ability to produce needed proteins.

It is thought that cloning may be used to develop animal organs for human transplantation. In fact, in March 2000, a major

step was taken toward creating genetically altered pigs whose organs and cells could be successfully transplanted into humans.

Some scientists assume that it is just a matter of time before a cloned human being is created. There has been speculation that this technology may be acceptable for the treatment of infertility. Human cloning has also been suggested to allow parents to seek donor bone marrow for an ill child. However, some ethicists have suggested that this uses a child as a means to an end and is not ethically appropriate. PR

The next article in the series will discuss monoclonal antibodies, and regulations and costs in biotechnology.



ARTICLE SUMMARY

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 - The findings from this research have implications for basic medical research, diagnosis of disease, development of new drugs and gene therapy.
- The Human Genome Project is studying the ethical, legal and social implications of its own discoveries.
- Gene therapy works by replacing a malfunctioning gene or adding a new gene to human cells to direct the production of a missing or abnormal protein.
 - Antisense therapy is a form of gene therapy that uses small pieces of DNA to block the ability of specific genes to produce disease-causing proteins.
 - Gene therapy has been evaluated in clinical trials for the treatment of cancer, genetic diseases and infectious diseases.
 - Significant improvements in procedures will be required before gene therapy becomes a practical clinical approach.
- Stem cells are able to form all the cells required to form the human body.
 - It is expected that stem cells may provide a permanent, stable source of human cells.
- Cloning involves transferring the complete genetic material from the nucleus of a cultured donor cell to a mature recipient egg from which the nucleus has been removed.
 - This process will result in offspring that are genetically identical to the organism from which the nucleus was taken.
 - The cloning of animals represents a potential source for pharmaceutical and nutritional proteins, as well as animal organs and cells for human transplantation.