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GENE THERAPY

Gene Therapy: A Comprehensive Review

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ABSTRACT

Background: **As gene therapy is one of the hottest topics of the new century, it carries the excitement of a cure to most of diseases, the controversy surrounding the altering of human imperfection, and the promise of a type of medical treatment most of us would never imagine possible.**

Material and methods: **A thorough search of literature was carried out through the National library of medicine (PubMed), SCOPUS, EMBASE databases using different Keywords. All the relevant articles were analyzed according to their importance and reviewed to evaluate the past present and future perspective of gene therapy.**

Results: **With a possibility to eliminate and prevent AIDS, malignancies, hereditary disorders and its conceivable cure for cardiac disorders, gene therapy is nothing short of a medical phenomenon.**

Conclusion: **Gene therapy has potential in treatment for most of the diseases.**

KEY WORDS

gene therapy, perspective, potential

INTRODUCTION

Numerous human geneticists have toyed with the idea of gene transfer for the treatment of inherited diseases. In the last few years that remarkable advances in recombinant DNA technology and cell biology have made it likely that this pipedream will become reality and furthermore, that gene therapy will not be restricted for the correction of single gene disorders, but will have applications for many branches of medicine[1)].

WHAT ARE GENES?

Genes are the fundamental physical and functional unit of heredity. A gene is an ordered sequence of nucleotides located in a particular position on a particular chromosome that encodes a specific functional product (i.e., a protein or RNA molecule). Gene is termed as a "biological units of heredity". Inherited from the parents, determines the unique traits - like the color of the eyes and color and texture of the hair. They also determine things like whether the child will be male or female, the amount of oxygen the blood can carry, and what the IQ will be[2)].

Genes are composed of long strands of a molecule called DNA and are located in single file within the chromosomes. The genetic message is encoded by subunits of the DNA called nucleotides. There are approximately three billion pairs of nucleotides in the chromosomes of a human cell. Each person's genetic makeup has a unique sequence of nucleotides, and this is what makes us different from one another. Scientists believe that every human has about 30,000 genes per cell. A mutation or imperfection in any one of these genes can result in a disease, physical disability or shortened life span. These mutations can be passed from one generation to another, inherited just like a mother's blond hair or a father's brown eyes. But with gene therapy, the treatment or elimination of inherited diseases or physical conditions due to these mutations could become a reality[3)].

GENE THERAPY

An experimental procedure aimed at replacing, manipulating, or supplementing nonfunctional or malfunctioning genes with healthy genes. Genes are specific sequences of bases that encode instructions on how to make proteins. Although genes get a lot of attention, it's the proteins that perform most life functions and even make up the majority of cellular structures. When genes are altered so that the encoded proteins are unable to carry out their normal functions, genetic disorders can result[4)].

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RESEARCHERS MAY USE ONE OF SEVERAL APPROACHES FOR CORRECTING FAULTY GENES[5)]

- A normal gene may be inserted into a nonspecific location within the genome to replace a nonfunctional gene. This approach is most common.
- An abnormal gene could be swapped for a normal gene through homologous recombination.
- The abnormal gene could be repaired through selective reverse mutation, which returns the gene to its normal function.
- The regulation (the degree to which a gene is turned on or off) of a particular gene could be altered.

TWO TYPES OF GENE THERAPY[6)]

1) **Somatic gene therapy**, involves introducing a "good" gene into targeted cells with the end result of treating the patient - but not the patient's future children because these genes do not get passed along to offspring. In other words, even though some of the patient's genes may be altered to treat a disease, the likelihood remains that the same disease will affect the patient's children. This is the form of gene therapy that is being done at most genetics laboratories throughout the world.

2) **Germline gene therapy** entail injecting foreign genes into fertilized eggs or in sperms producing cells, which will then pass any genetic changes to future generations as well. However, although it has potential for preventing inherited disease, this form of gene therapy is extremely controversial and currently very little research is being done in this area, both for technical and ethical reasons.

METHODS FOR GENE THERAPY[7)]

1) Physical
a) Direct injection of DNA
b) Liposome-mediated DNA transfer
c) Calcium phosphate transfection-
d) Electroporation
2) Retrovirus vectors
3) Other viral vectors
4) Targeted gene transfer via receptors
5) Artificial chromosomes
6) Site-directed recombination
7) Activation of genes of related function.

In most gene therapy studies, a "normal" gene is inserted into the genome to replace an "abnormal," disease-causing gene. A carrier molecule called a vector is used to deliver the therapeutic gene to the patient's target cells. Currently, the most common vector is a virus that has been genetically altered to carry normal human DNA. Viruses have evolved a way of encapsulating and delivering their genes to human cells in a pathogenic manner. Scientists have tried to take advantage of this capability and manipulate the virus genome to remove disease-causing genes and insert therapeutic genes[8)].

Some of the different types of viruses used as gene therapy vectors[9,10)]:

- **Retroviruses** - A class of viruses that can create double-stranded DNA copies of their RNA genomes. These copies of its genome can be integrated into the chromosomes of host cells. Human immunodeficiency virus (HIV) is a retrovirus.
- **Adenoviruses** - A class of viruses with double-stranded DNA genomes that cause respiratory, intestinal, and eye infections in humans. The virus that causes the common cold is an adenovirus.
- **Adeno-associated viruses** - A class of small, single-stranded DNA viruses that can insert their genetic material at a specific site on chromosome 19.
- **Herpes simplex viruses** - A class of double-stranded DNA viruses that infect a particular cell type, neurons. Herpes simplex virus type 1 is a common human pathogen that causes cold sores.

TARGET CELLS FOR GENE THERAPY ARE[11)]

1) Peripheral blood lymphocytes
2) Haemopoietic stem cells
3) Fibroblasts
4) Hepatocytes
5) Keratinocytes
6) Skeletal muscle myoblasts
7) Airway epithelial cells
8) Vascular endothelial cells
9) Tumor cells.

FACTORS INHIBITING THE GENE THERAPY[12,13)]

- Short-lived nature of gene therapy - Before gene therapy can become a permanent cure for any condition, the therapeutic DNA introduced into target cells must remain functional and the cells containing the therapeutic DNA must be long-lived and stable. Problems with integrating therapeutic DNA into the genome and the rapidly dividing nature of many cells prevent gene therapy from achieving any long-term benefits. Patients will have to undergo multiple rounds of gene therapy.
- **Immune response** - Anytime a foreign object is introduced into human tissues, the immune system is designed to attack the invader. The risk of stimulating the immune system in a way that reduces gene therapy effectiveness is always a potential risk. Furthermore, the immune system's enhanced response to invaders it has seen before makes it difficult for gene therapy to be repeated in patients.
- **Problems with viral vectors** - Viruses, while the carrier of choice in most gene therapy studies, present a variety of potential problems to the patient --toxicity, immune and inflammatory responses, and gene control and targeting issues. In addition, there is always the fear that the viral vector, once inside the patient, may recover its ability to cause disease.
- **Multigene disorders** - Conditions or disorders that arise from mutations in a single gene are the best candidates for gene therapy. Unfortunately, some the most commonly occurring disorders, such as heart disease, high blood pressure, Alzheimer's disease, arthritis, and diabetes, are caused by the combined effects of variations in many genes. Multigene or multifactorial disorders such as these would be especially difficult to treat effectively using gene therapy.

APPLICATIONS OF GENE THERAPY

Gene therapy is likely to have the greatest success with diseases that are cause by single gene defects. By the end of 1993, gene therapy had been approved for use on such diseases as severe combined immune deficiency, familial hypercholesterolemia, cystic fibrosis, and Gaucher's disease. Most protocols to date are aimed toward the treatment of cancer; a few are also targeted toward AIDS. Numerous disorders are discussed as candidates for gene therapy: Parkinson's and Alzheimer's diseases, arthritis, and heart disease. The Human Genome Project, an ongoing effort to identify the location of all the genes in the human genome, continues to identify genetic diseases[14,15)].

Eve Nichols describes the **criteria for selection of disease** for human gene therapy[16)]:

1) The disease is an incurable, life-threatening disease;
2) Organ, tissue and cell types affected by the disease have been identified
3) The normal counterpart of the defective gene has been isolated and cloned
4) The normal gene can be introduced into a substantial sub fraction of the cells from the affected tissue; or that introduction of the gene into the available target tissue, such as bone marrow, will somehow alter the disease process in the tissue affected by the disease.
5) The gene can be expressed adequately (it will direct the production of enough normal protein to make a difference)
6) Techniques are available to verify the safety of the procedure.

ADVANTAGES OF GENE THERAPY[17,18)]

1) Germ-line gene therapy offers a true cure, and not simply palliative or symptomatic treatment.
2) Germ-line gene therapy may be the only effective way of addressing some genetic diseases.
3) By preventing the transmission of disease genes, the expense and risk of somatic cell therapy for multiple generations is avoided.
4) Medicine should respond to the reproductive health needs of prospective parents at risk for transmitting serious genetic diseases
5) The scientific community has a right to free inquiry, within the bounds of acceptable human research.

While the development of germ-line gene therapy techniques will undoubtedly place some embryos at risk in the laboratory, once the successful techniques are developed, the therapy could help parents and researchers avoid the moral dilemma of disposing of "defective" embryos in the lab if the embryos could be repaired.

DISADVANTAGES OF GENE THERAPY[17,18)]

1) Germ-line gene therapy experiments would involve too much scientific uncertainty and clinical risks, and the long term effects of such therapy are unknown.
2) Such gene therapy would open the door to attempts at altering human traits not associated with disease, which could exacerbate problems of social discrimination.
3) As germ-line gene therapy involves research on early embryos and effects their offspring, such research essentially creates generations of unconsenting research subjects.
4) Gene therapy is very expensive, and will never be cost effective enough to merit high social priority.
5) Germ-line gene therapy would violate the rights of subsequent generations to inherit a genetic endowment that has not been intentionally modified.

The ethical issues posed by both somatic and germ-line gene therapies are international in scope. The documents listed below serve to demonstrate the variety of reactions to gene therapy, and to illuminate the complexity of this continuing public debate.

ETHICAL ISSUES SURROUNDING GENE THERAPY[19)]

Because gene therapy involves making changes to the body's set of basic instructions, it raises many unique ethical concerns. The ethical questions surrounding gene therapy include:

- How can "good" and "bad" uses of gene therapy be distinguished?
- Who decides which traits are normal and which constitute a disability or disorder?
- Will the high costs of gene therapy make it available only to the wealthy?
- Could the widespread use of gene therapy make society less accepting of people who are different?
- Should people be allowed to use gene therapy to enhance basic human traits such as height, intelligence, or athletic ability?

IS GENE THERAPY SAFE?[20)]

Gene therapy is under study to determine whether it could be used to treat disease. Current research is evaluating the safety of gene therapy; future studies will test whether it is an effective treatment option. Several studies have already shown that this approach can have very serious health risks, such as toxicity, inflammation, and cancer. Because the techniques are relatively new, some of the risks may be unpredictable; however, medical researchers, institutions, and regulatory agencies are working to ensure that gene therapy research is as safe as possible.

TREATMENT MODALITIES USING GENE THERAPY

1) Mucocutaneous gene therapy

it offers exciting new treatment modalities for skin and mucosal lesions. Transient expression of naked plasmid DNA could be used as a local treatment of various skin and mucosal lesions where the corresponding gene product (protein) has therapeutic or immunization potential. Ulrich R. Hengge etal analyzed the time course, magnitude, and histologic _expression of the indicator plasmid DNA (pCMV:β-Gal) in mucosal epithelium and papilloma lesions. Upon direct injection of naked plasmid DNA (20 μg) into oral mucosa, _expression occurred at high local concentrations, up to 35-fold higher than in comparable injections into the epidermis. Due to the accelerated turnover of mucosal epithelium β-galactosidase positive epithelial cells were detected in the basal and suprabasal layers as early as 3 h after injection, whereas only the most superficial mucosal layers demonstrated β-galactosidase staining at 24 h post-injection. These biologic characteristics need to be taken into consideration when clinical applications of expressing naked plasmid DNA in epithelial tissues are considered[21)].

2) Herpes viral infection.

Herpes viruses are a diverse family of large DNA viruses, all of which have the capacity to establish life long latent infection. Delivery of reporter genes invitro and in vivo has been demonstrated using a variety of replication competent and replication defective vectors and significant physiological modification in the CNS has been achieved by HSV-mediated gene delivery[21)].

3) Epstein-Barr virus diseases

Is a herpes virus that infects majority of the individuals and persists in an asymptomatic state by a combination of chronic replication in the mucosa and latency in peripheral blood B cells. These EBV infected B cells are highly immunogenic and normally susceptible cytotoxic T lympocytes. By gene therapy genetic modification of lympocytes could be a potential treatment for these diseases[21)].

4) Hemoglobinopathies-Thalassemia[22)]

Gene therapy for thalassemia requires replacement with a normal functional β-globin gene and in some cases with γ-globin gene.

5) Haemophilia A and B[23)]

Are relatively rare, X-linked inherited bleeding disorders which are life threatening to the patients unless treated by regular injections of factors VIII and IX respectively.

Gene therapy offers the prospect of a cure for the disease, thus potentially freeing patient from the existing regimens of regular intravenous injections of proteins and risk of infection by contaminating viruses. Although gene therapy is very attractive to the patients and clinicans, in practice, preclinical experiments in animal models suggests that it may be difficult to obtain adequate therapeautic levels of either factor VIII or IX for long periods in patients unless improved can be devised.

6) Cystic fibrosis[24)]

Is a common severe autosomal recessive genetic disease which is caused by disfunction of epithelial cell surface cAMP activated Cl-channel.The effects of this disfunction are pleoitropic but the human morbidity results from the effects in the respiratory epithelium.

Gene therapy is an attractive possible treatment, the gene required is well characterized and only low-level expression is required.

Of all the clinical trials, all the vector system proposed for CF gene therapy, adenovectors are the most advanced with the results of many clinical trials already published.

7) HIV Infection[25)]

The genetic approach to HIV infection is still very young and a number of different stages in the viral life cycle **are** being studied as targets for gene therapy, using a wide variety of modalities for gene deliv-

ery. Several gene therapy protocols for AIDS have been approved.

Elimination of virus from an infected person is an unlikely eventuality, therefore the aims must be to maintain the virus in its latent period as long as possible and to protect uninfected cells from viral infection and perhaps to enhance the immune response against virus. These methodologies are particularly amenable to a gene therapy approach and it seems likely that in future the combination therapy including both pharnmacological agents and gene therapy agents, will be used in concert to minimize this spread of the HIV with in an infected individual and thus prolong their disease free life.A gene therapy based vaccine is also a serious possibility.

8) Cancer[26]

Several approaches to cancer therapy are being explored

1) Immune responses to tumors are being enhanced
2) Genes are being inserted into tumor cells to evoke cell suicide
3) Finally methods are being developed to modify tumor suppressor or anti-oncogenes.

The primary target for stimulating an immune response to a tumor is major histocompatability complex(MHC) class1-restricted tumor specific cytotoxic (CD8) T cell.

Two criteria must be met

1) The CD8 T cell receptors must be occupied by an MHC class 1 peptide complex
2) A helper T cell must be activated to secrete cytokines, which acts on CD 8 T cell.

This second signal could be bypassed by inducing CD 8 cell to produce their own cytokines.

Cell suicide involves insertion of Herpes simples virus thymidine kinase (HSV-TK) gene. Tumar suppressor genes are being inserted into human tumors. One protocol involves inserting a normal p53 gene inot non-small cell lung carcinomas that are p53 defective.

In another, antisense DNA is injected to try to suppress the activity of activated oncogenes, in this case k-ras in lung carcinoma.

9) Gene Therapy and Viral Vaccination[27]

Live viral vaccines have had a major impact on the incedence of acute viral infections world-wide. Virus infections recognize as future vaccine targets will require a modified approach based on the detailed understanding of the immunobiology of specific infections combined with the application of the new technologies designed to generate specific and appropriate protective immunity. A similar vector technology directed at in vivo gene delivery is currently being exploited both gene therapy and vaccination. The induction of an immune response to an expressed transgene represents potential hazards for a gene therapy protocol but is the object of a vaccine strategy. In Vivo gene delivery using replication-competent or replication-deficient viral vector systems and by direct transfer of naked DNA can generate an effective humoral, secretary and cell mediated immune response to expressed transgenes.

10) Also Gene therapy is a serious consideration in many diseases caused by Virus, Bacteria and other microorganisms.

FUTURE OF GENE THERAPY

To cure genetic diseases, scientists must first determine which gene or set of genes causes each disease. The Human Genome Project and other international efforts have recently completed the initial work of sequencing and mapping virtually all of the 30,000 genes in the human cell. This research will provide new strategies to diagnose, treat, cure, and possibly prevent human diseases[17].

Although this information will help scientists determine the genetic basis of many diseases, it will be a long time before diseases actually can be treated through gene therapy. "The Human Genome Project is just a start," Nicholson says. "It's going to locate genes for us, but it's not going to tell us what these genes do. That will be the next step. Once we have that information, we'll be able to take advantage of that knowledge to provide treatment and/or cures[28]."

Gene therapy's potential to revolutionize medicine in the future is exciting, and its expectations for curing and preventing childhood diseases are encouraging. One day it may be possible to treat an unborn child in utero for a genetic disease even before it comes in to this world[29].

Scientists are hoping, the mapping of the human genome will lead the way toward cures for many diseases and that the successes of current clinical trials will create new opportunities and challenges. For now, however, it's a wait-and-see situation, calling for cautious optimism.

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