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Genetic Therapy

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Abstract
The rise of new genetic technologies and their implied power has engendered concerns among religious, scientific, and civic leaders that these new technologies may be growing more rapidly than our ability to prudently control and productively use them. The ability to insert human genes into human patients to treat specific genetic diseases is known as human gene therapy–has been one of the concerns noted by those observing the evolution of genetic technologies. Advances in biotechnology have brought gene therapy to the forefront of medical research. The prelude to successful gene therapy i.e. the efficient transfer and expression of a variety of human gene into target cells has already been accomplished in several systems.

Keywords: Vector, Gene therapy, Genetics, Gene, Functional gene, Gene delivery

Introduction
Gene therapy is a technique for correcting defective genes responsible for disease development. The most common approach for correcting faulty genes is to insert a “normal” gene into the genome to replace an “abnormal” disease causing gene. A normal gene can be inserted to a specific location within a genome to replace a non functional gene. This is the most common approach. An abnormal gene can be swapped for a normal gene for homologous recombination. An abnormal gene could be repaired through selective reverse mutation, which returns the gene through its normal function. Gene therapy is the insertion, alteration or removal of genes from an individual cells and biological tissues to treat diseases. It is a technique for correcting defective genes that are responsible for disease development.

Fig 1

Principles of Gene Therapy
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 particular gene can be turn on or off] of gene can be altered. A normal gene could be inserted into a nonspecific location within the genome to replace the nonfunctional gene.

Types of gene therapy
1. Germ line gene therapy
Where germ cells (sperm or egg) are modified by the introduction of functional genes, which are integrated into their genome. Therefore changes due to therapy would be heritable and would be passed on to later generation. Theoretically, this approach should be highly effective in counteracting genetic disease and hereditary disorders. But at present many jurisdictions, a variety oftechnical difficulties and ethical reasons make it unlikely that germ line therapy would be tried in human beings in near future.

2. Somatic gene therapy
Where therapeutic genes are transferred into the somatic cells of a patient. Any modifications and effects will be restricted to the individual patient only and will not be inherited by the patients offspring or any later generation..

Different mechanisms of gene therapy
Gene therapy refers to the insertion of genetic material to correct a defect. Gene therapy can take several forms:
- Gene insertion, in which a new version of a gene is introduced into a cell;
- Gene modification, in which a gene already in place is altered; and
- Gene surgery, in which a particular gene is excised and may also, be replaced by its normal counterpart.

Such genetic alterations would involve insertion of new material that directly codes for proteins or that affects how existing genes are expressed by suppressing or enhancing production of particular proteins. Current prospects for human gene therapy do not include either gene modification or gene surgery because these are more complex than merely adding new genes to cells, Such complicated manipulations can now be performed, however, in some viruses, yeast, and bacteria, and the necessary technologies may later be discovered that would permit gene surgery or controlled genetic modification in animals and humans. Through the remainder of this background paper, gene therapy will refer to gene insertion, because this is the form likely to be applied

first. The distinction is technically relevant, regulate the cell. Certain sequences of DNA contain information for specific proteins such as enzymes, hemoglobin (the oxygen-containing protein in red blood cells), or the variety of receptors on the cell's surface. Stretches of DNA that contain the information for a specific product are called genes. The DNA of the gene would not be different for somatic versus germ line therapy, although there might be different sequences added adjacent to the gene depending on how the gene would be regulated in a particular experiment or treatment. The difference between somatic and germ line therapy is which type of cell is treated with DNA.

Techniques of gene therapy

Gene therapy involves isolating a gene, putting it into cells where it will be used, and ensuring that the inserted gene functions in the new cells in a way that does not harm the patient.

Genes are copied and passed on by DNA replication

Genetic information is transmitted from one cell to its progeny by duplication, or replication, of its DNA. When a cell divides, it copies its DNA and distributes a copy to each of two offspring cells. A new therapeutic gene introduced into a cell in the laboratory can thus be reproduced through the process of cell division when the cell is placed into a patient and proliferates. Many break throughs in molecular genetics have come from discoveries about how DNA replicates, how it can be specifically cut and reassembled, and how to re-introduce the altered DNA back into cells in such a way that its expression, or translation into protein, can be controlled (Judson, 1980). Many of the techniques for splicing and controlling the expression of genes were first discovered between 1970 and 1974, using some of the same techniques that led to the development of recombinant DNA (Watson, 1984).

Isolation and cloning of the normal gene

The usual first step in approaching gene therapy is identification of the abnormal gene. This step can be skipped when the corresponding normal genes are already available, as was the case for sickle cell disease. Once the abnormal gene has been found, then copies of the corresponding normal gene must be isolated and copied. There are several ways to identify abnormal genes. These involve analysis of patterns of inheritance of a disease, study of the metabolism of patients who have the disease, and analysis of the genes of those who have the disease, identification of the gene that causes a particular disease requires hundreds of experiments, luck, and extensive resort to recombinant DNA technology. Once the gene that causes a disease has been identified, the corresponding normal gene must be isolated, unless it is already available because it has been studied for some other purpose. Using an abnormal gene to find its normal counterpart is usually done by exploiting the extensive similarity between the sequences of the normal and defective genes; they rarely differ greatly in overall sequence. After the normal gene has been identified and isolated, then it must be copied. The process of making multiple copies of a single gene is called cloning. Cloning involves combining the gene of interest with DNA sequences that allow it to be copied in lower organisms—usually bacteria or yeasts. The DNA containing the gene of interest is then inserted into bacteria or yeast or into some types of mammalian cells growing in culture. The DNA is copied as the cells proliferate. The numerous copies of DNA are then purified from other cell components, and the gene of interest can be cut away from unwanted DNA sequences, now has millions or billions of copies of a single gene. These copies are then combined with DNA that is suitable for insertion into human cells.

Insertion into human cells

The DNA that contains the normal gene can be administered to human cells in several ways:

Using viruses physically injecting it, treating the DNA chemically so that cells take it up they are induced to take in the DNA or by fusing the cells with membranes that contain the DNA. In the distant future, designed viruses or genetic elements may be used to transfer genes to specifically targeted human cells. At present more primitive methods are used.

Chemical and physical methods

Some early experiments in gene transfer employed mixing DNA with chemicals and subsequently applying the DNA to a large number of cells. Most cells would pick up the DNA, and some would insert it into their own DNA, and, in some cases, express it. The usual chemical treatment employed calcium phosphate with relatively large amounts of the desired DNA. The most common physical method involved in which electrical treatment of the cells induced uptake of DNA and other constituents from the fluids bathing the cells.

Disadvantages

- The DNA is only stably incorporated into a small proportion of cells, usually only one in ten thousand to one in a million.
- This feature requires that cells that take up and incorporate the desired DNA must somehow be separated from cells that do not, and there must be a very large number of cells to treat in the first place.
- The DNA usually inserts at random into the cell's genome, and often in multiple copies. DATA insertion following physical insertion methods is thus relatively uncontrolled and unpredictable.

Membrane Fusion

The final way to get DNA into cells involves putting it inside of membranes that can then be fused with the outer membrane of target cells, allowing the contents to spill into the cells. The membrane sacs, called liposome's, can be made of artificially constructed lipid mixtures or derived from specially treated cells such as red blood cells or bacteria. The advantage of cell fusion is that it is relatively simple, and large numbers of cells can be treated. It is, like chemical treatment, unreliable and nonspecific at delivery. The technique might prove useful in the future, however, if membranes are constructed that target specific cells with highly reliable delivery. Safe methods are devised to do this, using several viral and non viral vectors. Two main approaches emerged are vivo modification and vitro modification.

Viral Vector

One of the most promising vectors currently being used is harmless viruses. 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 viral genome and replace them with working human gene. This altered virus can then be used to smuggle genes into cells with great efficiency. Some of the viruses insert their genes into the host genome, but do not actually enter the cell. Others penetrate the cell membrane disguised as protein molecule and enter the cell. Once the transplanted gene is 'switched on' the right location within the cell of an infected person, it can then issue instructions necessary for the cell to make the protein, that was previously missed or altered. Some of the different types of viruses used as gene therapy vectors.

Retrovirus

First viruses to be used as vectors in gene therapy experiments were retroviruses. They belong to a class of viruses which can create double stranded DNA copies with the enzyme reverse transcriptase. These copies of its genome can be integrated into the chromosome of host cell by another enzyme carried the virus called as integrase. Now thehost cell has been modified to contain a new gene. If such modified host cells divide later, their descendants will contain the new genes. Although retroviruses have been used in most gene therapy experiments so far, they present problems. One such problem is that integrase enzyme can insert genetic material of the virus into any arbitrary position in the genome of the host, which can lead to insertion mutagenesis (if insertion is in the middle of the gene) or uncontrolled cell division (if gene happens to be one regulating cell division) leading to cancer. This problem has recently begun to be addressed by utilizing zinc finger nuclease or by including certain sequences such as beta globin locus control region to direct the site of integration to specific chromosome.Gene therapy trial using retroviral vector to treat X-linked severe combined immune deficiency represent the most successful application till date. Also this has been tried to treat SCID due to ADA deficiency with relative success. As researchers havegrown more confident, they have begun injecting altered retroviruses directly into tissues where the corrected genes are needed.

Herpes simplex virus [HSV]

It is a human neurotropic virus, which is mostly used for gene transfer in nervous system. It has a large genome compared to other viruses, which enable scientist to insert more than one therapeutic gene into a single virus, paving the way for treatment of disorders caused by more than one gene defect. HSV makes an ideal vector as it can infect a wide range of tissues including muscle, liver, pancreas, and nerve and lung cells. The wild type of HSV-1 virus is able to infect neurons which are not rejected by immune system. Antibodies to HSV-1 are common in humans; however complications due to herpes infections are somewhat rare.

Adeno-associated viruses [AAVs]

One of the most promising potential vectors is a recently discovered virus called the AAV, which infects a broad range of cells including both dividing and non dividing cells. AAVs are small viruses from the Parvovirus family with a genome of single stranded DNA. It can insert genetic material at a specific site on chromosome19 with near 100% certainty. Researchers believe that most people carry AAV which do not cause disease and do not provoke an immune response. Scientists have demonstrated the animal experiments using AAV to correct genetic defects. It is now being used in preliminary studies to treat hereditary blood disease hemophilia, muscle and eye disease. Also clinical trials have been initiated to use AAV vectors to deliver genes to brain as the virus can infect non dividing cells like neurons in which their genome are expressed for a long time. The chief drawback of AAV limited is that it is small, carrying only 2 genes in its natural state. Its payload therefore is relatively. It can produce unintended genetic damage because the virus inserts its genes directly into host cell's DNA

Adenovirus

To avoid problem of inserting genes at wrong sites, genome researchers have turned to other types of viruses. A class of virus with double stranded DNA genome that can cause respiratory, intestinal and eye infection (especially the common cold).When these viruses infect a host cell; they introduce their DNA molecule into the host. The genetic material of the adenovirus is not incorporated into the host cell's genetic material. The DNA molecule is left free in the nucleus of the host cell, and the instructions in this extra DNA molecule are transcribed just like any other gene. Adenovirus also can infect a broader a broader variety of cells than retrovirus, including cells that divide more slowly, such as lungs cells. However, adenovirus also is more likely to be attacked by the patient's immune system, and the high levels of virus required for treatment often provoke an undesirable inflammatory response.

Drawbacks

- Vector system has been promoted for treating cancer of liver and ovaries and indeed the first gene therapy product to be licensed to treat head and neck cancer is Gendicine, based adenoviral product.
- Work using adenovirus vector has focused on genetically crippled version of the virus.

Non-Viral Methods

Simplest method of non-viral transfect ion is direct DNA injection. Clinical trials to inject naked DNA plasmids have been performed successfully. There have been trials with naked PCR products, which have had greater success. Research efforts have yielded several non-viral methods gene transfer such as electroporation (creation of electric field induced pores in plasma membrane), sonoporation (ultrasonic frequencies to disrupt cell membrane), magnetofection (use of magnetic particle complexes with DNA), gene guns (shoots DNA coated gold particles into cells by using high pressure) and receptor mediated gene transfer are being explored. Each method has its own advantages and disadvantages. Among the several no viral approaches, receptor mediated gene transfer currently holds the most promise. This application involves the use of DNA conjugated withspecific proteins (viral structural protein), or with liposome, or both. Under experimental ex-vivo conditions, liposome's containing DNA has been shown to

undergo cellular uptake through endocytosis, with subsequent transient exogenous gene expression. Nevertheless, the application of this approach will likely be limited until methods for the stable integration of the endocytosed DNA are devised and improvement in target ability; transfection efficiency and DNA carrying capacity are developed. Recently several chemical methods like use of synthetic oligonucleotides (to inactivate defective genes by using antisense specific to target gene), lipoplexes (made up of anionic and neutral lipids) and polyp lexes (complex of polymers with DNA) have been used to facilitate delivery of the DNA into cell. Recently there have been some hybrid methods developed that combine two or more techniques. For example-vibrosomes that combine liposome's with aninactivated HIV or influenza virus.

Target tissues for gene therapy

Hematopoietic cells derived from bone marrow (BM) may be readily obtained and manipulated ex-vivo in a variety of tissue culture system. Furthermore, therapies based on transfer of genetically modified hematopoietic cells are potentially applicable to a wide range disorders, including the hemoglobinopathies, AIDS and cancer. For these reasons, BM cells are attractive targets in gene therapy research. Gene transfer into human hematopoietic stem cells capable of proliferating in-vivo for long period of time, giving rise to large number of progeny expressing the desired product, remains an elusive goal. The main limitation is related to the fact that hematopoietic cells greatest potential for proliferation is generally quiescent and resistant to retrovirus mediated gene transfer. Meanwhile, the utility of genetically modified hematopoietic cells, with limited but predictable proliferative potential continues to be elevated. Research into the usefulness of other cell types as gene transfer vehicle remains very active. The known regenerative properties of the liver make it an attractive gene transfer target. Disorders theoretically amenable to this form of therapy include familial hypercholesterolemia, hemophilia, urea cycle defect, -1-antitrypsin deficiency and phenylketonuria. Other potential targets for therapy include skeletal muscle cells for amelioration of muscular dystrophies, respiratory epithelial cells for the treatment of respiratory insufficiency in cystic fibrosis, and central nervous system tissue for degenerative neurologic disorders.

Potential social implications of gene therapy

In case of genetic enhancement, such facilities could be a luxury available for the rich and powerful in the world. Wide spread use of it may lead to the development of new definition for 'normal' which would have a huge implication on persons with disabilities. This technology may permanently remove such disabilities. Nowadays gene therapy is mostly used to irradiate life threatening diseases. But in future when the technologies become more accessible. it can be used to treat more complex treatments like change in behavioral traits. germline gene therapy can forever change the genetic makeup of an individual's descendants. Hence human gene pool can be permanently affected, if these technologies are mostly used for human welfare an error in technology or judgments can lead to far reaching consequences.

Conclusion

The policy on genetic patient data is centered on determining the rights of privacy and access, pitting individual autonomy against relatives or third parties needs for information. The legitimacy of others 'needs are determined by the potential benefits to relatives, health providers, insurers, employers, or the general public compared to potential harm to the patient from disclosure. Several factors are included in such assessments, including the seriousness of the genetic condition, the genetic relationship between interested parties, and the probability of preventing harm or promoting good by disclosure. When no genetic relationship exists, as in the case of insurers and employers, issues of fairness arise. Continuing public scrutiny may be instrumental in the evolution of deciding on a hierarchy of conditions and people for whom disclosure of genetic patient data is important (Rosenfeld, 1984). General education is an issue of Federal, State and local interest, necessary so that all people can have an understanding of genetics sufficient to understand the complex issues of genetic patient data (President's Commission, 1983; Rowley, 1984). Some schools have responded to this need by making genetics a major focus of their biology courses. As the technology for identifying genetic disease improves, it is important that physicians become aware of that technology and how to use it with patients. Public policy on genetic patient data attempts to control access so that individual privacy is protected.

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