REVIEW ARTICLE

Gene Therapy: The Molecular Bandage for Treating Genetic Disorders

Md. Manjurul Karim

Professor of Microbiology, University of Dhaka

ABSTRACT

The concept of gene therapy involves the transfer of genetic material into a cell, tissue, or whole organ, with a view to curing a disease or at least improving the clinical status of a patient. Much of its success relies heavily on the development of an effective delivery system that is capable of efficient gene transfer in a variety of tissues, without causing any associated pathogenic effects. Viral vectors currently offer the best choice for efficient gene delivery, what has been discussed in this review article. Their performance and pathogenecity has been evaluated in animal models, and encouraging results form the basis for clinical trials to treat genetic disorders and acquired diseases. Despite some initial success in these trials, vector development remains a seminal concern for improved gene therapy technologies.

Key Words: Gene Therapy, Genetic Disorders, Molecular Bandage

Introduction

Gene therapy is an application of recombinant DNA technology in medicine. This is the name originally given to the methods that aim to cure an inherited metabolic disorder that lack an effective therapy, by providing the patient with a correct copy of the defective gene[1]. To date, we know more than four thousand diseases of this type where a defective gene causes an enzyme to be either absent or ineffective in catalyzing a particular metabolic reaction effectively[2]. The presence of a defective or faulty gene is replaced by a working gene, so that the body can make the correct enzyme or protein and consequently eliminate the root cause of the disease. It differs from traditional drug-based approaches, which may treat the problem, but which do not repair the underlying genetic flaw.

A survey in the United States showed that 1 infant in every 100 is born with a serious disorder caused by a defect in 1 or more of the estimated 31,000 genes found in the human body[2]. Thousands of children and adolescents die from these diseases each year, and tens of thousands suffer lifelong disability. Although gene therapy is not an approved medical therapy to treat disease, scientists expect that gene therapy will offer unprecedented opportunities to treat, cure, and ultimately prevent a vast range of diseases. Indeed, the gene therapists have expanded their horizon to treat other kinds of diseases which involve genetic susceptibility to illness, such as heart disease, diabetes, arthritis, and Alzheimer's disease. Today, nearly 75% of all clinical trials involving gene therapy are aimed at treatments for cancer and AIDS.

Review of Literature

1. Types of gene therapy: There are two distinctly different types of gene therapy: germ-line therapy and somatic-cell therapy. In germ-line therapy, genetic alterations are made to germ cells (such as sperm and eggs). A fertilized egg is provided with a copy of the correct version of the relevant gene and re-implanted into the mother. If successful, the gene is present and expressed in all cells of the resulting individual. Germ-line therapy is usually carried out by microinjection of a somatic cell followed by nuclear transfer into an oocyte, and theoretically could be used to treat any inherited disease[3]. This is highly controversial because such engineering would alter the genetic endowment of the unborn and could be passed on to future generations.

In somatic-cell therapy, on the other hand, gene surgeons attempt to fix genetic malfunctions in somatic (body) cells, such as blood cells and skin cells. Somatic cell therapy involves manipulation of cells, which either can be removed from the organism, transfected, and then placed back in the body, or transfected in situ without removal. The technique has most promise for inherited blood diseases (e.g., hemophilia and thalassaemia), with genes being introduced into stem cells from the bone marrow, which give rise to all the specialized cell types in the blood. The strategy is to prepare a bone extract containing several billion cells, transfect these with a retrovirus-based vector, and then re-implant the cells. Subsequent replication and differentiation of transfectants leads to the added gene being present in all the mature blood cells. In contrast to the germ-line therapy, somatic-cell therapy are restricted to the person being treated and cannot be passed on to his or her offspring.

2. Basic process: Basically, the therapy inserts a 'normal' gene into the genome to replace an 'abnormal,' or disease-causing gene that does not code for a functional protein: removal of the defective genes is unnecessary[3]. The situation is less easy with dominant genetic diseases, as with these it is the defective gene product itself that is responsible for the diseased state, and so the therapy must include not only addition of the correct gene but also removal of the defective version. This requires a gene delivery system that promotes recombination between the chromosomal and vector-borne versions of the gene, so that the defective chromosomal copy is replaced by the gene from the vector[3]. To achieve this goal, gene therapy requires technologies capable of gene transfer into a wide variety of cells, tissues, and organs. One of the biggest stumbling blocks to successful widespread application of such genetic treatments is the development of safe and effective vectors with which to ferry genetic material into a cell[1].

2.1 Vectors: The vectors that have been developed to overcome these obstacles fall into two broad categories: nonviral and viral vectors[4].

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 take advantage of this capability and manipulate the virus genome to remove disease-causing genes and insert therapeutic genes. Target cells such as the patient's liver or lung cells are infected with the vector. The vector then unloads its genetic material containing the therapeutic human gene into the target cell. The generation of a functional protein product from the therapeutic gene restores the target cell to a normal state (Figure 1).

2.1.1 Virus Vectors: Scientists use three types of viruses in gene therapy experiments- retroviruses, adenoviruses, and adeno-associated viruses.

2.1.1.1 Retroviruses: These viruses were the first to be used in gene therapy experiments. They are unique in a sense as they carry RNA as their primary carrier of genetic information instead of DNA. After invading a cell, they use an enzyme called reverse transcriptase to make a DNA copy of their genes. Later, a viral enzyme integrase incorporates this DNA copy into the infected cell's DNA.

In one application, scientists use a retrovirus that causes leukemia in mice but no known disease in humans. The researchers remove the genes that cause disease and, in their place, insert an RNA copy of a healthy gene into the virus. They also add a piece of genetic information called a promoter. The promoter is like an "on/off switch" for a gene. When it is turned on, usually with a drug, it tells the ribosomes to begin expressing the inserted gene.

Although widely used, some serious pitfalls question retroviruses' suitability as vectors. They can invade only cells that are actively dividing, limiting potential targets for therapy to blood cells, skin cells, and other fast-growing tissues. In addition, the viruses have no specific targets in the infected cells' chromosomes. Consequently, the genes they carry are inserted in a haphazard manner, which might result in

causing more damage than repair. Despite the presence of promoters, the added genes typically do not produce sufficient amounts of proteins to effectively treat disease. In addition, the patient's body generally recognizes retroviruses as foreign invaders, provoking adverse immune responses.

Researchers approached the use of retroviruses with caution because of concerns that they might attack inappropriate cells. To avoid this problem, researchers use the ex vivo strategy whereby the target cells from patient body is initially removed before treatment with the retrovirus (Figure 1). They then monitored the cells to ensure the therapy was working properly before returning the cells to the patient's body.

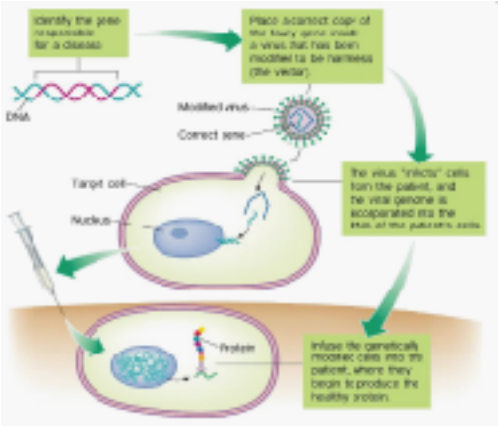

Figure 1: The ex vivo strategy of gene therapy. A new healthy gene is inserted into the RNA of the retroviruses (1). They are mixed with cells taken from a patient having a defective gene and cultured in a laboratory (2). The retroviruses insert the healthy gene into the DNA of the cells (3), which are later injected back into the patient (4).

2.1.1.2 Adenoviruses: To avoid the problem of inserting genes at the wrong sites, Adenoviruses are being used as recombinant vector, for, the viral genome is not integrated with the host cell genome, rather is left free in the nucleus of the host cell, while still allowing the messages in this extra DNA being transcribed just like any other gene. Researchers thus trade safety for impermanence, because the genes do not undergo replication, hence, the treatment requires readministration of the vector in a growing cell population. Adenoviruses can infect a broader variety of cells than retroviruses do, including cells that divide more slowly, such as lung cells. However, adenoviruses are also more likely to be attacked by the patient's immune system, sometimes provoking an undesirable inflammatory response. Despite these drawbacks, adenoviruses have been used in attempts to treat cancers of the liver and ovaries.

2.1.1.3 Adeno-Associated Virus (AAV): This virus has emerged as one of the most promising vectors, which infects a broad range of cells, including both dividing and nondividing cells. Importantly, these viruses can insert genetic material at a specific site on chromosome 19. Most people carry this virus, which is not pathogenic and do not provoke an immune response. Scientists have demonstrated that the AAV can be used to correct genetic defects in animals. It is now being used in preliminary studies to treat hemophilia in humans. The chief drawback of the AAV is that it is small, carrying only two genes in its natural state. Its payload is therefore relatively limited. Researchers have also had difficulties manufacturing large quantities of the altered virus.

2.1.2 Chimeraplasty: Chimeraplasty is a non-viral method of gene therapy which is intended to fix defective genes within a cell directly, making it unnecessary to insert new genes into cells. Researchers design short segments of DNA called chimeraplast, complementary to those sequences of a gene in which a defect occurs. When inserted into the cell's nucleus, The DNAs of the chimeraplast and the cell bind each other except in the middle of the strand, where the chimeraplast's sequence is different from that of the cell. The DNA repair enzymes see this "bump" in the DNA and interpret it as a signal to repair the defective gene. They replace the cells DNA with that of the chimeraplast. This leaves the chimeraplast's new sequence in the cell's DNA and the replaced DNA sequence then decays. Chimeraplasty's effectiveness has been questioned recently as it was found only 0.0002% effective in transforming yeast cells[5].

3. Debates about gene therapy: Critics and proponents all agree that the risks of gene therapy must not be substantially larger than the potential benefits. For this reason, most of the early gene therapy experiments have been on rare diseases that can cause intense suffering and for which no treatment exists. Initial experiments using gene therapy in the treatment of cancer and AIDS have been conducted primarily in patients for whom all other treatments have failed and who are near death, so the risks are small. This is the same procedure used to test new drugs against these diseases. But many people feel that because gene therapies use altered genes and potentially dangerous viruses, these treatments should be more extensively tested than drugs before being approved.

The potential risks are also at the center of the more contentious debate about germ-line therapy. Although the potential benefits of success are great-lifelong freedom from genetic disease and the elimination of dangerous genetic legacies in families-the risks are also much greater. For example, partial correction of a genetic defect might lead to the birth of a severely deformed child. Without the intervention of gene therapy, such a pregnancy might otherwise result in a miscarriage. The procedure might also bring unforeseen harm to the mother, through either unpredicted spread of the virus vector or other problems associated with the procedure.

Beyond scientific and medical risks, germ-line therapy opens thorny moral dilemmas. Critics argue that, for example, people do not have the right to sculpt the genetic blueprint of children-a decision in which the child has no voice. Others portray the experiments as the first step down the slippery slope to 'designer babies'.

4. Challenges for gene therapy: Gene therapy is a powerful technology, but many advances are necessary before it will make a noticeable impact on the treatment of disease. Researchers must make vectors that are more effective at invading large numbers of cells, must find new ways to manufacture the vectors in large quantities, and must produce better promoters so that larger quantities of proteins are produced.

Conclusion

The field of gene therapy is still very young and the rules governing gene therapy clinical trials are evolving as the field evolves. Despite some technical setbacks in the methods used in gene therapy, most experts believe that use of gene therapy in medicine is inevitable. They believe that it is the ultimate bandage that will be a matter of routine to correct simple genetic disorders, eliminating a number of diseases that now plague humankind.

References

1. Friedman T. 1999. The Development of Human Gene Therapy. Cold Spring Harbor, NY: Cold Spring Harbor Lab. Press
2. Microsoft Encarta Encyclopedia standard 2004.
3. Brown TA. 2010. Gene Cloning and DNA Analysis in Medicine. In: Gene cloning and DNA analysis, Wiley-Blackwell publishing
4. Verma IM and Weitzman MD. 2005. GENE THERAPY: Twenty-First Century Medicine. Annu. Rev. Biochem. 74:711-38.
5. http://en.wikipedia.org/wiki/Chimeraplasty; visited in January 5, 2012.