Review Article

Use of viral vectors for Gene therapy

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ABSTRACT

Medicine is entering a new era for treating diseases and will enable physicians to treat the cause of a disease rather than the symptoms. Gene therapy is a promising treatment option for a number of diseases but the technique is still under investigation to make sure that it will be safe and effective. In the future, this technique may allow physicians to treat a disorder by inserting a gene to a patient’s cells instead of using drugs or surgery. One of the major hurdles to successful gene therapy of genetic and acquired diseases is the ability to introduce a foreign gene efficiently into the tissue of interest. Numerous viral and non-viral (synthetic) methods for gene delivery have been developed. This review highlights the use of viral vectors for gene therapy such as adenoviruses, adeno-associated viruses, retroviruses, herpes simplex viruses, poxviruses and baculoviruses. Keywords: Gene therapy, gene delivery systems, viral vectors.

Introduction

Gene therapy is an experimental technique that uses genes to treat or prevent disease¹. It is the treatment of a disease by the genetic modification of the patient's cell². Diseases that are suitable for treatment by gene therapy can be divided into genetic and acquired diseases³. Genetic diseases are typically caused by a single gene mutation or deletion (e.g. hemophilia, cystic fibrosis, etc.). The acquired diseases are those for which no single gene has been identified as the only cause of the disease state (e.g. cancer, Parkinson's disease, cardiovascular diseases, etc.).¹³ However, delivery of a single gene to the correct cell type(s) can potentially lead to the elimination of the disease state³. Basically it is the genetic diseases that cause the development of gene based therapeutics⁴. Prior to this endeavor, there were no alternatives to correcting a genetic disorder. Therapies were available to treat only the symptoms associated with the disease but not the actual cause of the disease. Through the efforts of gene therapy, the correction of the mutation and the subsequent return to normalcy is now feasible³. Researchers are considering several approaches to gene therapy including¹⁴:

1. Replacing a mutated gene (disease causing) with a healthy copy of the gene.
2. Inactivating or knocking out a mutated gene that is functioning improperly.
3. Introducing a new gene into the body to help fight a disease.

Types of gene therapy

Gene therapy can be classified into several ways. On the basis of cells, gene therapy may be divided into germ line and somatic gene therapy:

Germ Line Gene Therapy: In this type of therapy, genes are inserted into germ cells or embryonal cells²⁵⁷.

Somatic Gene Therapy: In this therapy, genes are inserted into somatic cells (undifferentiated body cells)²⁵⁷. There are many ethical concerns regarding gene therapy⁵. There are some controversies related to germ line gene therapy whereas somatic gene therapy is morally acceptable for treating diseases⁶⁸. Gene therapy can also be classified on the basis of therapy i.e. ex vivo and in vivo gene therapy:

Ex vivo Gene Therapy: In this, cells are removed...
from the patient's tissues (such as skin, liver, hematopoietic system) or tumors and cultured ex vivo in the laboratory. During the culture, the cells are provided with the therapeutic gene. This is followed by reinfusion or reimplantation of the transduced cells in to the patients. Ex vivo gene therapy is usually not preferred as it requires isolating and culturing services that can be provided by private or medical institutions^{2,3,6}.

In vivo Gene Therapy: Some organs (lungs, brain, heart etc.) are less suited for ex vivo gene therapy as culture of the target cells or retransplantation is not feasible. Therefore, the gene is directly administered in the somatic cells either locally or systematically. Clinically and pharmaceutically in vivo gene therapy is more acceptable as the therapeutic gene is directly administered to the patient via conventional routes and methods rather than isolating the patient’s cells, introducing the gene into those cells, and then reintroducing the modified cells back into the patient^{2,3,6}.

**Gene delivery systems**

The most concerned factor regarding gene therapy is how to deliver the gene safely at the target site. In most cases, a normal gene is inserted into the genome to replace an abnormal gene. The most common method of replacing/transferring gene is through a carrier (a molecule) known as vector, which is used to deliver the therapeutic gene(s) to the patient's target cells. A number of delivery systems have been developed and used for the effective transfer of genes including viral and non-viral vectors^{1-7}. But the most popular delivery system is the use of viruses as vectors. Viral Vectors for Gene Therapy.

There is a large and rapidly growing body of literature on methods for gene delivery involving the use of viral vectors. Genes are delivered more efficiently by viral vectors as compared to DNA transfection or non-viral approaches^{9}. Target cells of the human body such as liver or lung cells are infected with the viral vector, which then unloads its genetic material containing the therapeutic human gene into the cell.

This is the similar mechanism that viruses used in pathogenic manner^{1-7,9,10}. Different types of viruses that are used as vectors in gene therapy include:

1. **Adenoviruses**

They belong to the family Adenoviridae. They are non-enveloped, icosahedral viruses containing linear double stranded DNA that replicates in the nucleus of the infected cell. Mammalian adenoviruses belong to the genera Mastadenovirus^{9,11}. Adenovirus was first isolated in human adipose tissues in 1953 by Rowe et al^{12}. Since then this virus has been classified into six types i.e. from A–F^{9,11}. These species infect humans and are subdivided into over 50 infective serotypes^{13}. This virus class causes respiratory, intestinal and ocular infections in humans^{9,11,13}. So far researchers have concluded that serotypes 2 and 5 of species C are the most effective for creating viral vectors for use in gene therapy^{14,15}. Adenoviruses have many attractive features which have made them a popular vehicle for gene transfer including well-defined biology, capacity to accommodate large segments of DNA, and the ability to infect a wide variety of cell types, tissues, and species in a cell cycle-independent fashion. Adenovirus vectors are one of the most widely studied vector forms used in worldwide clinical trials^{9,11,13-15}. They are most commonly associated with treatment of different types of cancers^{16-22}, liver diseases^{23-25}, cardiovascular diseases^{26-29}, tuberculosis^{30-31}, etc.

2. **Adeno-associated viruses**

They belong to the genus Dependovirus of the family Parvoviridae. They are very small, non-enveloped, icosahedral viruses containing a linear single stranded DNA^{9,11,32}. They were first discovered in 1965 as a coinfecting agent of the adenoviruses and therefore are known as adeno-associated viruses (AAV)^{33}. This small virus is naturally replication-defective and requires the assistance of either a helper virus, such as the adenovirus or the herpes virus, or some form of genotoxic stress to replicate within a host cell nucleus^{9,34}. AAV are not considered to have any known role in disease^{35}. It has been suggested to have a role
in male infertility as its DNA is more commonly found in semen samples from men with abnormal semen. AAV have been used as vectors in tissue engineering of brain, muscle and retina cells, in the treatment of cystic fibrosis, hemophilia, Parkinson's disease, cancer, etc.

3. Retroviruses (γ-retroviruses, lentiviruses and spumaviruses)

These are enveloped, single stranded RNA viruses that belong to the family Retroviridae. Retroviruses are duplicated in host cells using the enzyme reverse transcriptase to produce DNA from its RNA genome. The virus thereafter replicates as part of the host cell's DNA. These viruses are most generally categorized as either simple (oncogenic retroviruses) or complex (lentiviruses and spumaviruses). Some simple/oncogenic retroviruses such as murine leukemia virus cause leukemia or lymphoma in humans whereas complex retroviruses such as lentivirus cause AIDS while spumavirus (e.g. human foamy virus / human spumaretrovirus) is non-pathogenic.

Retroviral vectors are used to infect dividing cells without producing any immunogenic viral proteins and hence become a permanent part of the host cell genome. They have proven extremely useful vectors in gene therapy research. These vectors are limited only by their relatively small carrying capacity and their inability to infect non-dividing cells; however, these disadvantages have not kept them from being the most widely used vectors in the research of gene and cell therapy. Retroviral vectors are widely used in studies of tissue repair and engineering, cancers, etc.

Lentiviruses, a subcategory of the retrovirus family, i.e. complex retroviruses, are similar to their simple retrovirus counterparts except that they are able to transduce non-dividing cells as well as they have a relatively large carrying capacity for foreign genomic material. This characteristic is advantageous in many gene therapeutic applications targeting post-mitotic, highly differentiated cells. Therefore, they can be used to produce other transgenic animal species. Lentiviruses have been used as a vector in gene therapy for β-thalassemia, severe combined immunodeficiency (SCID), CNS diseases, hematological diseases, cancer immunotherapy, etc.

Human foamy virus (HFV) was the first identified human retrovirus. It is non-pathogenic and considered as a promising vector for gene therapy due to its several unique features related to gene transfer. The potential advantages of HFV vectors include a broad host range, the largest packaging capacity of any retrovirus and the ability to persist in quiescent cells. Another advantage is that these vectors have a safer spectrum of insertional mutagenesis than murine leukemia virus (MLV) or human immunodeficiency virus (HIV). Because of these features, foamy vectors have the unique potential to safely and efficiently deliver several genes into a number of different cell types in vivo and are especially useful for transducing hematopoietic cells. HFV has been used in the therapy of neurological disorders such as Parkinson's disease, epilepsy, Huntington's disease, etc.

4. Herpes simplex viruses

It belongs to the family Herpesviridae. It has an icosahedral protein shell that is covered by a viral envelope composed of lipid and glycoproteins. It contains a double stranded DNA. Many different varieties of the herpes simplex virus (HSV) have been discovered. The most common of these, known as HSV-1, is well known by the average person as the viral cause for cold sores. One of the most intriguing aspects of this virus is its ability to infect a host and then remain latent for a period before reappearing again. Due to this property HSV has been utilized as vector in gene therapy. This virus also has a distinctive property of infecting neural cells. Therefore, HSV are used in treatment of Parkinson's disease, Alzheimer's disease, cancers, etc.

5. Poxviruses

The poxviruses belong to the family Poxviridae.
They are generally enveloped and contain a double stranded DNA. Poxviruses are the largest human and animal viruses. They are large enough to be seen by light microscopy by special staining techniques. They were one of the first animal viruses to be used as a gene-transfer vector. Poxviruses are known to cause infections in both humans and animals. Some important genera of this group and the diseases they cause includes: Orthopoxvirus (smallpox, vaccinia, cowpox, etc.), Parapoxvirus (orf, pseudo-cowpox, etc.), Yatapoxvirus (yabapox, tanapox) and Molluscipoxvirus (Molluscum contagiosum). Smallpox (variola) and Molluscum contagiosum infections are specific to humans only. Replication of the viral genome actually takes place in the host cell cytoplasm, as opposed to the host cell nucleus, this property is unique to poxviruses. A number of characteristics of their life cycle make poxviruses poor candidates for long-term expression. However, vaccinia and its relatives may be ideal in immunotheraphy applications, including their use as replicating agents that can be directed against solid tumors. These viruses have been used previously as vaccines against diseases like smallpox but they are now being studied as viral vectors against cancer and other viral, parasitic and bacterial diseases such as malaria, rabies, HIV, etc.

6. Baculoviruses

They belong to the family Baculoviridae. They are rod-shaped, enveloped and contain double stranded DNA. There are more than 500 types of baculoviruses. They are known to cause infection in invertebrates (e.g. mosquitoes, shrimps, etc.). They are capable of entering mammalian cells in culture but are non-pathogenic and also they are not known to be capable of replication in mammalian or other vertebrate animal cells. These are considerable safety advantages and may be a distinct advantage over other viral vectors. Baculoviral DNA has been known to automatically degrade inside host cells over time. Also, because the baculovirus only infects insects and invertebrates, humans do not appear to have pre-existing antibodies or T-cells specifically against baculoviruses. The main drawback associated with baculovirus is a rapid, complement mediated inactivation. To overcome this, researchers have successfully coated virus particles with polyethylenimine, protecting them against complement inactivation. They have been used in animal studies to deliver genes to a wide range of cell types including artheros artery, liver, brain, skeletal muscle, and also used in stem cell and bone tissue engineering.

Vectors derived from retroviruses and adenoviruses are used in the majority of gene therapy clinical trials to date. However, vectors derived from AAV, poxviruses, herpes simplex viruses, and baculoviruses are receiving increasingly more attention in the field of gene therapy. The viral vectors discussed above are those used in current clinical trials or under advanced preclinical development. Other viral vectors under development include those based on simian virus (SV)-40, γ-viruses, hepatitis viruses, negative strand RNA viruses (for example, influenza and ebola) and Epstein-Barr virus, etc. The choice of virus for gene therapy usually depends on the efficiency of transgene expression, packaging capacity, host range, cell- or tissue-specific targeting, replication competency, genome integration, ease of production, safety, toxicity and stability. No single vector system is likely to be optimal for all the potential gene therapy applications. Therefore, viral vectors are also being used in combination in various studies such as combination of adenovirus and AAV vectors. Combination of HSV and AAV vector improves the stability of transgene expression in human cells by site-specific integration. Similarly, retroviral genomes contained within an adenovirus have been claimed to integrate in the absence of the retroviral integrase activity.

Conclusion

The huge potential for gene therapy to cure a wide range of diseases has led to high expectations and a
great increase in research efforts in this area. Furthermore, with the sequencing of the human genome and the development of advanced technologies for the identification of genes and their function, the number of candidate diseases for gene therapy has continued to increase. However, the efficient transfer of a therapeutic gene into human cells depends upon the technology used for gene therapy. A number of viral delivery systems are in use. Further development and improvement of effective gene delivery vector systems will play a vital role to the promotion of human gene therapy.

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