GENE THERAPY

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Contents

Summary

Gene therapy has been used to correct genetic defects or to express therapeutically useful genetic material. This paper reviews gene transfer vectors, gene delivery methods and clinical applications. An efficient gene delivery is a crucial step in reaching a reasonable therapeutic effect. Where therapeutic strategies allow, genes can be delivered to a patient via *ex vivo* gene delivery methods. Another approach, and the ultimate goal of gene therapy, is *in vivo* delivery that involves the direct administration of gene into a patient. Genes are delivered to tissues with either non-viral or viral vectors. Many

different viruses, such as retroviruses, adenoviruses, and adeno-associated viruses have been used as gene transfer vectors. Over the last decade, more than thousand clinical trials have been started all of which involve gene transfer to somatic cells. Gene transfer to germ-line has not been tried in humans and is regarded unethical. Most of the trials are in cancer, vascular diseases, infectious diseases, AIDS, and inherited monogenic disorders such as cystic fibrosis. Also, some trials have focused on the treatment of neurologic illnesses, autoimmune diseases, regeneration of tissues, or tissue transplantation. Gene therapy is a novel form of molecular medicine that holds great promise to significantly improve conventional medicine. Rather than focusing on symptoms, gene therapy is aimed on the treatment of causes and pathogenesis of the diseases.

1. Introduction

The development of molecular techniques has made it possible to understand genetic background of several diseases. Gene therapy can be used for the treatment of inherited and acquired diseases. So far, more than thousand phase I and phase II gene-based clinical trials have been started (see Table 1). Gene Therapy can be defined as the introduction of nucleic acids into cells of an individual with a resulting therapeutic effect. Initially gene therapy was developed as a strategy to treat inherited, monogenic diseases, such as cystic fibrosis or adenosine deaminase deficiency. The original concept of gene therapy as "gene supplementation" has been broadened to cover any strategy that employs therapeutically beneficial genetic material to treat diseases, many of which do not involve germ-line mutations. Promising approaches utilizing genes that supply a therapeutic function have been applied for variable acquired conditions such as cardiovascular diseases, cancer, and AIDS. Compared to traditional medicine, a strong theoretical advantage of gene therapy is the possibility to achieve a long-lasting therapeutic effect in the target tissue by a single administration of the gene, without systemic side effects. Despite progress in gene transfer technology, further developments in efficiency, safety, and duration of the transgene expression are needed. However, gene transfer technology's potential for treating various human diseases is enormous: gene therapy can lead to significant enhancement of the quality of a patient's life, or even to a full recovery from the disease.

2. Vectors for Gene Transfer

The success of gene therapy depends on the efficacy and accuracy of gene delivery into the target tissue. The most efficient ways to deliver genetic material into a wide variety of cells or tissues rely on non-viral systems or utilize the ability of viruses to deliver genes into the host. Figure 1 shows the vectors used in the studies involving gene therapy from year 1985 to 2000. The delivery always requires an interaction between the plasma membrane of the target cell and the vector. The interaction can be non-specific and based on different charges; or specific and based on particular types of cell membrane proteins such as receptors. Each vector has characteristic advantages and disadvantages that determine their usefulness in therapy. Vectors used at present do not fulfill all the requirements for an ideal vector, and further development is needed. In addition to the supplementation of a therapeutic gene, an ideal vector would have the following properties:

- it should be immunologically inert,
- the expression should be regulated allowing short- or long -term expression,
- vectors should be cell- or tissue-targetable by surface-binding or by selected promoters at transcriptional level (see: Regulated and Targeted Vectors in bibliography),
- depending on the therapeutic purpose the vector should allow either episomal expression, or a site-specific integration into the genome in a safe well-known site and finally
- it should be easy to produce on a commercial scale. This eventually raises a need for a wide variety of vectors and problems like toxicity, immune responses and a possibility for insertional mutagenesis by integrative vectors may be observed.

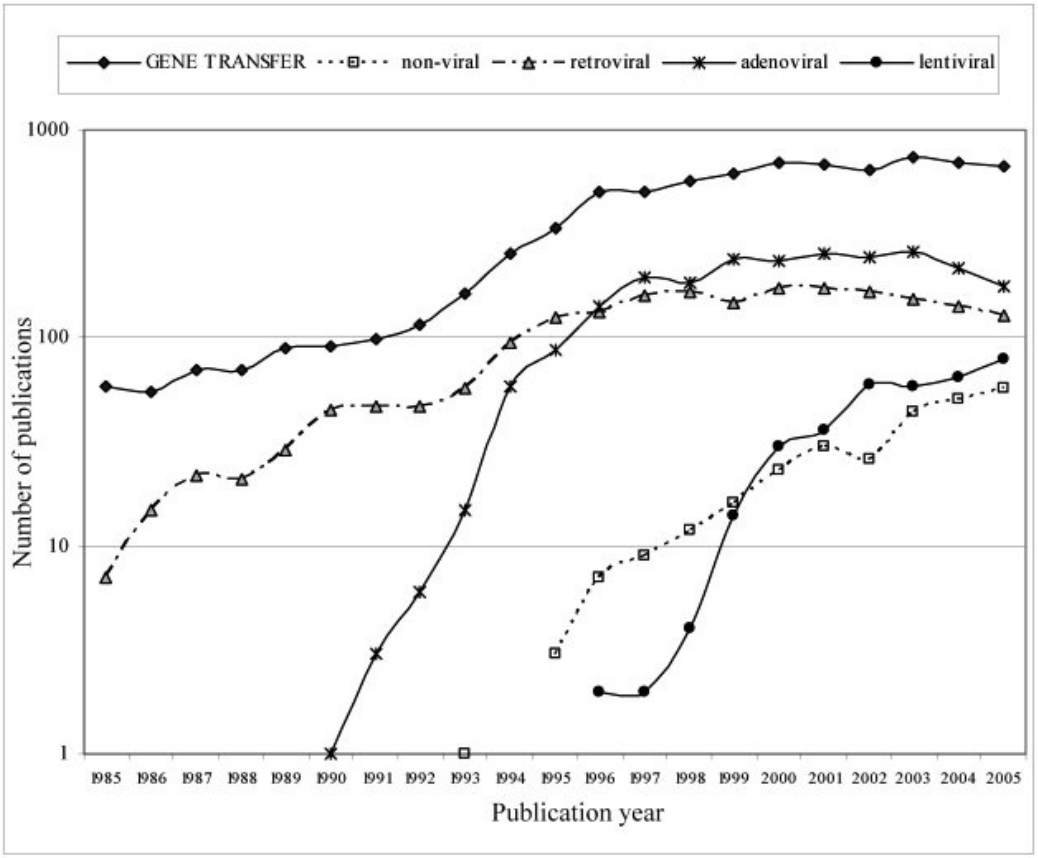

Figure 1: Figure shows the number of publications in the field of Gene Therapy and Gene Transfer.

Retroviral vectors have been important in the technical and conceptual development of viral vectors as gene delivery vehicles since the end of 1980s. The history of lentiviruses really starts in 1995. Their usage has increased similarly to non-viral vectors. There has been a remarkable increase in clinical gene therapy trials based on adenoviral vectors over the last few years. In 1996 only 15% of RAC approved clinical trials used adenoviral vectors whereas in the years 1999–2000 the figure was 36%. Other vectors in 1999–2000 RAC-approved clinical protocols were: retroviral vectors:

22%, nonviral vectors: 25%, Adeno-associated viral vectors:: 4%, and other vectors: 13%.

2.1. Non-viral Vectors

Non-viral vectors include oligonucleotides or naked plasmid DNA alone or in combination with liposomes or polymers. Although liposomes and polymer complexes improve the delivery of foreign DNA into the cell, they usually have low gene-transfer efficiency and they direct transient transgene expression. The delivery of transgene into the cell occurs after fusion with plasma membrane or by endocytosis. One of the limitations is the degradation of non-viral vectors in lysosomes. Therefore, usage of fusogenic peptides that destabilize and break endosomal membranes permitting the transgene release into the cytoplasm has greatly increased the efficacy of non-viral vectors. In the long run non-viral vectors are attractive vectors for development—they are usually not immunogenic, are easy to synthesize, and relatively safe. These advantages have made them useful for gene therapy. With a few exceptions, such as skeletal muscle and skin, naked DNA is not an efficient vector for gene delivery. The most widely used non-viral vectors are liposomes, which have been used in several *in vivo* gene transfer trials for objectives including the treatment of skin cancer, melanoma, and restenosis. Liposomes have also been used to enhance the gene transfer efficiency of retroviral and adenoviral vectors.

2.2. Viral Vectors

In virus-based gene delivery vehicles most of the viral genetic material has been removed and replaced with therapeutic genetic material. Essential components for the formation of a virus particle, such as structural and envelope proteins, are provided *in trans* by specific packaging cell lines. The modified virus carries the therapeutic gene into the host cell but is weakened and cannot cause a disease or propagate *in vitro*. As for all gene therapy vectors, the interaction with plasma membrane is crucial. By modulation of the virus envelope ("coat") protein, the host range, and specificity can be altered. Most of the viruses interact with specific receptors, are released into the cytoplasm and either remain episomal or integrate into the genome. At the moment, the main non-integrating viral vectors are derived from adenoviruses; and the three main integrating viral vectors are based on retroviruses, lentiviruses, or adeno-associated viruses. In contrast to non-viral vectors, viral vectors have caused manysome safety concerns but are also usually much more efficient in transducing genetic material in to the target cells. In 1999, gene therapy suffered a major setback with the death of a patient that was participating in a gene therapy trial for ornithine transcarboxylase deficiency. His death is believed to have been triggered by a severe immune response to the adenovirus carrier. Another setback, related to safety concern arousing a risk of carsinogenesis if retroviral integration occurs into the potential proto-oncogene, occurred in 2003 when EMEA and FDA placed a temporary halt on gene therapy trials using retroviral vectors in blood stem cells due to the development of a leukemia-like condition in 3 out of 11 treated SCID-XI patients. For lentiviruses (HIV) a risk of carsinogenesis seems to be rarer phenomenon.

2.2.1. Adenoviral Vectors

Adenoviral vectors belong to the family of *adenoviridae*, which mostly cause benign respiratory, gastrointestinal, and ocular infections in humans. Adenoviral vectors are DNA-viruses, can be generated at high titers, and efficiently deliver transgene into both dividing and non-dividing cells. Human cells are generally good candidates for adenoviral mediated gene delivery. Adenoviral vectors remain episomal in the nucleus and direct transient gene expression. Cell division leads to rapid loss of the transgene. The infectivity and expression of adenoviral vectors is also dependent on the expression of adenovirus-specific receptors on the target cells and immune reactions. The receptor for most adenovirus types has been identified as a cell-surface protein: coxsackievirus and adenovirus receptor (CAR). The adenovirus envelope fiber protein is responsible for the initial attachment to the host cell and penton protein is involved in virus internalization.

Current adenoviral vectors are highly immunogenic and therefore only a short-term expression of the transgene (usually between 5 and 20 days) is observed. In addition to eliciting inflammatory and toxic reactions in the host, immune responses may reduce gene transfer efficiency by eliminating transduced cells and limiting the number of readministrations to patients by generation of neutralizing antibodies. In some pathological conditions such as cancer, immune responses may also be beneficial for the host. Adenoviral vectors could successfully be employed in a variety of pathological conditions, such as in cancer and angiogenesis, where a high-level transient expression of the transgene is required.

2.2.2. Retroviral and Lentiviral Vectors

Retroviral and lentiviral vectors are RNA-viruses and belong to the family of *retroviridae*. These viruses possess a long history of efficient cross-species infections, including mammalian cells. Retroviral vectors are mainly based on Moloney murine leukemia virus (MoML) and they have been used in many clinical trials for the treatment of cancer, inherited and acquired monogenic disorders, and AIDS. Lentiviral vectors are based on human immunodeficiency virus-1 (HIV-1), non-human simian immunodeficiency virus (SIV), or feline immunodeficiency viruses (FIV). Lentiviral vectors hold a great promise as an efficient and stable gene delivery tool to various cells like stem cells. Minimizing the possibility of recombination among various viral genetic elements has increased the safety of lentiviral vectors. This has been gained by including less than 5% of the original viral genome into the vector and generating self-inactivating vectors.

Interaction between the envelope of retroviral and lentiviral vectors and a cell surface receptor is required for cell entry and infection. These viruses utilize a diverse set of proteins for cell entry that offer a possibility for vector modification and targeting. Amphotropic(i), xenotropic(ii), ecotropic(iii) and polytropic(iv) surface proteins of the virus determine the interaction of MuLV particles with subgroup-specific receptors. Some of these receptors are sodium-dependent phosphate transporters. The entry of HIV-1 requires the CD4 receptor that is found primarily on T-lymphocytes. For further

information about retrovirus receptors see reference Miller AD (1996) from bibliography. The viral tropism is altered by envelope pseudotyping. MoML or HIV-1 based vectors are often pseudotyped with vesicular stomatitis virus G-protein (VSV-G) to increase the infectivity and stability of viral particles. Also, expression of antibodies or ligands on the viral surface has conferred altered specificity to target cell receptors.

Retroviral and lentiviral vectors can lead to a stable integration of the transfected gene into the host genome and a long lasting expression of the transgene. A limitation of the retroviral vectors is their relatively low titer, which reduces *in vivo* gene transfer efficiency. Retroviral vectors need to cross the nuclear membrane for integration and can only infect dividing cells. This is a limitation and retroviral vectors are not suitable for the treatment of non-dividing cells like neurons. On the other hand, the selective transduction of dividing cells has made them potential vectors in the treatment of cancer. In contrast to retroviruses lentiviral vectors have the ability to transduce non-dividing cells as well as dividing cells. Stabile retrovirus- or lentivirus-mediated gene transfer is desirable for the treatment of diseases that require long-term transgene expression, such as genetic disorders.

2.2.3. Adeno-associated Viral Vectors

Adeno-associated viral vectors (AAV) are non-pathogenic DNA-viruses that belong to the family of *parvoviridae*. AAV require adenoviruses or herpes viruses as a helper for replication. AAV can infect non-dividing cells and a wide variety of cell types leading to sustained expression in muscle, liver, and the brain. Their wide tropism is based on the mechanism of virus entry, which involves binding to the plasma membrane heparan sulfate proteoglycan and V5 integrin. AAV viruses have two interesting features regarding chromosomal integration. Wild-type AAV has the capability to integrate site-specifically into chromosome 19. Unfortunately this feature is not conserved in modified AAV vectors followed by deletion of *rep*-gene (a gene coding for replication proteins). Also, AAV is susceptible to homologous recombination at low frequency. This feature has been shown to correct point mutations and deletions (see reference Inoue et al in bibliography of AAV).. Other limitations for the vector are a difficulty in large-scale production and a limited capacity to include transgene(s).

2.2.4. Other Viral Vectors

Some other vectors, such as the herpes simplex virus (HSV), do not integrate into the genome but allow a relatively long-term expression in some cell types, such as in neuronal cells. HSV can be a useful vector for cancer gene therapy, since it can induce cytotoxic effects and destroy malignant cells. Poxviruses have also been used for genetic vaccines. Baculoviral vectors are non-pathogenic insect viruses that have been used for *in vivo* gene transfer in central nervous system and in carotid arteries in rabbits. The gene transfer efficiency in these studies was relatively high, indicating their potential as a new gene-delivery tool.

Human cytomegalovirus, semliki forest virus, SV-40 virus, Epstein–Barr virus, influenza-virus, hybrid viruses (adenovirus/retroviruses, adenovirus/AAV), and some other viral vectors have been used in preliminary gene transfer studies, but their

usefulness as *in vivo* gene transfer vectors still needs proper evaluation. See further information about these viral vectors in Other Viral Vectors in bibliography.

2.2.5. Replicating Viral Vectors

The vectors listed above are designed to be replication defective—they can only infect target cells once and cannot spread the infection. However, gene transfer efficiency *in vivo* is usually low and target cells may be hidden beneath layers of other cells or physiological barriers. One possibility to increase the gene transfer efficiency is to overcome these delivery blocks and to prolong transgene expression by the use of replicating virus vectors (see: Alemany et al in bibliography of Other Viral Vectors).. With self-replicating vectors, only a modest amount of virus is needed to initiate spreading of the infection in a restricted area. For this purpose, the replicating vector must be regulated or targeted at the biological or physiological level. This approach would be beneficial in the treatment of cancer by improving on poor distribution of the vector in the tumor.

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1991-95 Senior Lecturer in Medical Biochemistry, University of Tampere
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1992-95 Senior Research Fellow, Research Council for Medicine, Academy of Finland
1995- Professor of Molecular Medicine, University of Kuopio
1998-1999 Established Investigator, Academy of Finland
2005- Research Professor of the Finnish Academy of Sciences
2005-2006 Visiting Professor, Salk Institute, Laboratory of Genetics (Prof. Inder Verma), USA