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1 author:

Manoj Mishra
Shambhunath Institute of Pharmacy, Prayagraj, India
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A REVIEW ON GENE THERAPY: HISTORY, VECTORS, TECHNOLOGIES AND APPLICATION

Garima Verma* and Manoj Kumar Mishra

Shambhunath Institute of Pharmacy, Jhalwa, Allahabad, Uttar Pradesh-211012, India.

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***Corresponding Author**
Garima Verma
Shambhunath Institute of Pharmacy, Jhalwa, Allahabad, Uttar Pradesh-211012, India.

ABSTRACT

Gene therapy can be broadly defined as the transfer of genetic material to cure a disease or at least to improve the clinical status of a patient. One of the basic concepts of gene therapy is to transform viruses into genetic shuttles, which will deliver the gene of interest into the target cells. Safe methods have been devised to do this, using several viral and non-viral vectors. Two main approaches emerged: *in vivo* modification and ex vivo modification. Retrovirus, adenovirus, adeno-associated virus are suitable for gene therapeutic approaches which are based on permanent expression of the therapeutic gene. Non-viral vectors are far less efficient than viral vectors, but they have advantages due to their low immunogenicity and their large capacity for therapeutic DNA. To improve the function of non-viral vectors, the addition of viral functions such as receptor mediated uptake and nuclear translocation of DNA may finally lead to the development of an artificial virus. Gene transfer protocols have been approved for human use in inherited diseases, cancers and acquired disorders. Although the available vector systems are able to deliver genes *in vivo* into cells, the ideal delivery vehicle has not been found. Thus, the present viral vectors should be used only with great caution in human beings and further progress in vector development is necessary.

KEYWORDS: Gene Therapy, Viral vectors, Immunogenicity, Adeno- associated virus.

INTRODUCTION

Gene therapy typically involves the insertion of a functioning gene into cells to correct a cellular dysfunction or to provide a new cellular function.[1] For example, diseases such as cystic fibrosis, combined immunodeficiency syndromes, muscular dystrophy, hemophilia, and many cancers result from the presence of defective genes. Gene therapy can be used to

correct or replace the defective genes responsible. Gene therapy has been especially successful in the treatment of combined immunodeficiency syndromes, showing lasting and remarkable therapeutic benefit.[2-4]

For gene transfer, either a messenger ribonucleic acid (mRNA) or genetic material that codes for mRNA needs to be transferred into the appropriate cell and expressed at sufficient levels. In most cases, a relatively large piece of genetic material (>1 kb) is required that includes the promoter sequences that activate expression of the gene, the coding sequences that direct production of a protein and signaling sequences that direct RNA processing such as polyadenylation. A second class of gene therapy involves altering the expression of an endogenous gene in a cell. This can be achieved by transferring a relatively short piece of genetic material (20 to 50bp) that is complementary to the mRNA. This transfer would affect gene expression by any of a variety of mechanisms through blocking translational initiation, mRNA processing, or leading to destruction of the mRNA. Alternatively, a gene that encodes antisense RNA that is complementary to a cellular RNA can function in a similar fashion.

Gene Therapy History

The first clinical study using gene transfer was reported.[5] Rosenberg and his colleagues used a retroviral vector to transfer the neomycin resistance marker gene into tumor-infiltrating lymphocytes obtained from five patients with metastatic melanoma. These lymphocytes then were expanded *in vitro* and later re-infused into the respective patients. Since this first study showed that retroviral gene transfer was safe and practical, it led to many other studies. Indeed, since 1989, more than 900 clinical trials have been approved worldwide.[6] What made gene therapy possible between 1963 and 1990 was the development of recombinant DNA technology.

In 2003 as well as in November 2005 China approved the first gene therapy drugs for the treatment of certain malignant tumors. A first European application for the approval of a gene therapy drug for the treatment of an aggressive brain tumor was submitted to the European Agency for the Evaluation of Medicinal Products (EMEA) in 2005. Despite continued great difficulties in the technical implementation, the successes of gene therapy can doubtlessly be confirmed today. For example, successful therapies have been developed during the past five years for patients with severe hereditary immunodeficiency diseases. These treatments are visibly beneficial to these patients with life-threatening conditions. The death of a patient in the USA in 1999 as a result of a very high systemically administered dose of adenoviral

vectors were tragic events that were viewed by the public as a setback for gene therapy. Nevertheless, the same principles apply to gene therapy as to other medical interventions: Effective procedures are associated with potential side effects which can be reduced by improving the procedures when the underlying mechanisms are understood. German scientists have made important contributions in this field, from basic research of the vector-host interaction to clinical studies. Among other things, in 2006 they reported on the correction of a severe immunodeficiency in adult patients through gene therapy.

Vectors

Facilitating the transfer of genetic information into a cell are vehicles simply called as vectors. Vectors can be divided into viral and nonviral delivery systems. The most commonly used viral vectors are derived from retrovirus, adenovirus and adeno-associated virus (AAV). Other viral vectors that have been less extensively used are derived from herpes simplex virus 1 (HSV-1), vaccinia virus, or baculovirus. Nonviral vectors can be either plasmid deoxyribonucleic acid (DNA), which is a circle of double-stranded DNA that replicates in bacteria or chemically synthesized compounds that are or resemble oligodeoxynucleotides. Major considerations in determining the optimal vector and delivery system are (a) the target cells and its characteristics, that is, the ability to be virally transduced ex vivo and reinfused to the patient, (b) the longevity of expression required and (c) the size of the genetic material to be transferred. Characteristics of viruses that have been used to generate viral vectors is shown in Table-1.

TABLE 1: Characteristics of Viruses that have been used to Generate Viral Vectors

Virus	Size and Type genome	Viral Proteins	Physical Properties	Disease in Animals
Retrovirus	7–10kb of single stranded RNA	Gag[a], Pol[b], Env[c]	100nm diameter; enveloped	Rapid or slow induction of tumors; acquired immunodeficiency syndrome (AIDS
Adenovirus	36kb double stranded linear DNA	Over 25 proteins	70–100nm in diameter; nonenveloped	Cold; conjunctivitis; gastroenteritis
Adenovirus associated virus	4-7 kb single stranded linear DNA	Rep[d] and Cap[e]	18–26nm in diameter; nonenveloped	No known disease
Herpes simplex virus 1 (HSV1)	152kb of double stranded linear DNA	Over 81proteins	110nm in diameter	Mouth ulcers; genital warts; encephalitis
Vaccinia virus	190kb of double stranded linear DNA	Over 198 open reading frames virus	350 by 270nm rectangles; enveloped	Attenuated virus that was used to vaccinate against smallpox

Baculovirus	130kb of double stranded circular DNA	Over 60 proteins	270 by 45 nm rectangles; enveloped	None in mammals; insect pathogen
Gag is a polyprotein and is an acronym for group antigen (ag), b- Pol is reverse transcriptase, c- Env is envelop protein, d- Rep is replication viral gene, e- Cap is capsid viral gene.				

Viral Vectors

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.

eg:- One of the problems of gene therapy using retroviruses is that the integrase enzyme can insert the genetic material of the virus into any arbitrary position in the genome of the host; it randomly inserts the genetic material into a chromosome. If genetic material happens to be inserted in the middle of one of the original genes of the host cell, this gene will be disrupted (insertional mutagenesis). If the gene happens to be one regulating cell division, uncontrolled cell division (i.e., cancer) can occur. This problem has recently begun to be addressed by utilizing zinc finger nucleases[7] or by including certain sequences such as the beta-globin locus control region to direct the site of integration to specific chromosomal sites.

Adenovirus[8]

To avoid problem of inserting genes at wrong sites, some 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 variety of cells than retrovirus, including cells that divide more slowly, such as lungs cells. However, adenovirus also are 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. Despite these drawbacks, this 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, adenoviral product.[9]

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 chromosome 19 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.18 It is now being used in preliminary studies to treat hereditary blood disease hemophilia, muscle and eye disease. The chief drawback of AAV is that it is small, carrying only two genes in its natural state. Its payload therefore is relatively limited. It can produce unintended genetic damage because the virus inserts its genes directly into host cell's DNA. Researchers have also had difficulties in manufacturing large quantities of the altered virus. The production problem has recently being solved by Amsterdam Molecular Therapeutics.[10]

Herpes simplex viruses (HSV)

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.

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, nerve and lung cells. The wild type of HSV-1 virus is able to infect neurons which are not rejected by immune system.[11] Antibodies to HSV-1 are common in humans, however complications due to herpes infections are somewhat rare.

Non-viral methods

In comparison with virus-derived vectors, non-viral vectors have several advantages, such as the safety of administration without immunogenicity, almost unlimited transgene size and the possibility of repeated administration.[12] Non-viral gene delivery systems generally consist of three categories: (a) naked DNA delivery, (b) lipid-based and (c) polymer-based delivery.[13]

Naked plasmid DNA

The simplest technique of non-viral gene transfer is the use of so called naked DNA. A series of approaches for naked plasmid DNA based gene delivery strategies have been reported in

recent years like, naked plasmid DNA transfer method wherein a cytotoxic T-lymphocyte antigen 4- immunoglobulin (CTLA4-Ig) gene was delivered using a naked plasmid DNA.[14] Naked DNA was used for antiangiogenic therapy where the fetal liver kinase-1 gene was delivered.[15] One more interesting area that is the use of naked plasmid DNA gene delivery as electro gene therapy which is done after injection of naked plasmid DNA and delivery of electric pulses directly to the tissue the expression of gene of interest can be obtained[16], because of its inherent simplicity naked DNA is an attractive non-viral vector and moreover its ease of production in bacteria and manipulation using standard recombinant DNA techniques substantiates its use as non-viral gene delivery system. The other important advantage in using naked DNA gene delivery system is its ability to show very little dissemination and transfection at distant sites following delivery and also can be administered several times as it does not show any antibody response against itself.[17]

Cationic lipids

Cationic liposomes are an important class of compounds suitable for carrying negatively charged DNA. There are at present several commercial transfection reagents that are based on cationic lipids like DOTMA(Lipofectin), DOTAP, DOSPA, DOSPER, DDAB, DODAC, Neophectin (PCL-2), DMRIE, DC-Chol, DOGS (Transfectam). However use of these reagents *in vivo* is plagued by their inherent toxicities. Cationic lipids consist of a positively charged head group, a hydrophobic tail and a linker connecting the head to the tail group. The charged head groups are usually quaternary amines, tails are saturated or unsaturated alkyl chains or cholesteryl groups. Cationic liposomes in contrast to neutral and anionic liposomes, which need DNA implementation into the vehicle, cationic liposomes naturally, create complexes with negatively charged DNA. Their positive charge, moreover, allows interactions with the negatively charged cell membrane and thus penetration into the cell is permitted.[18]

There are numerous reports on the use of various cationic lipids as non-viral gene delivery vectors. The use of cationic liposomes has made great strides between the initial report by Felgner et al. in 1987 and their use in the world's first human gene therapy clinical trial by Nabeleta.[19] cationic liposomes are used in recent times is siRNA delivery. In a recent study by sato et al. siRNA complexed with galactosylated cationic liposomes for liver parenchymal cell selective delivery of siRNA has shown that siRNA did not undergo nuclease digestion and urinary excretion and moreover was delivered efficiently to the liver and was detected in

parenchymal cells rather than liver non parenchymal cells. The endogenous gene (ubc13 gene) expression in the liver was inhibited up to 80% when complexes of ubc13-siRNA and galactosylated liposomes were administered to mice.[20] Though cationic liposomes have been extensively used as transfection agents *in vitro*. There, *in vivo* success is plagued by toxicity. In a recent study it was found that the mechanism behind the toxicity of cationic liposomes is largely induction of apoptosis. A cDNA micro array study showed that up regulation of 45 genes related to apoptosis, transcription regulation and immune response was due to lipofection.[21]

Polymeric gene carriers

Synthetic polycationic polymers have gained wide attention as non-viral vectors for gene delivery. A number of reviews and book issues have already been published which illustrates various mechanisms through which they act and also various biochemical and therapeutic aspects of these systems. Polyplexes form these polymers spontaneously as a result of electrostatic interaction between phosphate groups of DNA and oppositely charged groups of polycationic polymer.[22-24]

PEI (polyethyleneimine) is more appropriate as it has set a gold standard for nonviral gene delivery. Their ability, to condense large DNA molecules and eventually leading to homogenous spherical molecules of 100nm size or less as, they are capable of transfecting into cells efficiently for both *in vitro* and *in vivo.*[23] The other synthetic polymers showing promising results in gene delivery are poly-L-lysine. It is one of the first polymers to be studied for nonviral gene delivery because of its peptidic nature i.e.it is biodegradable and hence it is more suitable for in vivo use.[25] Imidazole containing polymers have been reported to have efficient transfection properties. -amino groups of poly-Lysine were modified. In various approaches with histidine or other imidazole-containing structures proved to be better transfecting agents than the unmodified poly-L-lysine.[26-28] Transfection and cytotoxicity studies were carried on amino methacrylate polymer where quaternary amine groups are connected to uncharged hydrophilic polymer of similar structure which is poly (N-Hydroxypropyl methacrylamide)-b-poly(trimethylamino methylmethacrylate) (PHPMA-b-PTMAEM). It was found that while toxicity has not been changed much but the transfection efficiency has been increased with the addition of PHPMA block.[29]

Gene Therapy Technologies

The transfer of genetic material can be accomplished *in vivo* through local or systemic inoculation or *ex vivo* where the target of interest is collected and modified outside of the organism before return to the host. Transfer of synthetic DNA can be accomplished by transduction or transfection. Such methods of transfer include either direct injection of DNA into the recipient cells, or utilising methods to induce membranes permeation, receptor-mediated uptake or endocytosis. Transduction utilises recombinant virus as a vector for gene transfer. Entry of these vectors is mediated by cell-surface receptors. Concerns regarding the immunogenicity of viral vector systems due to activation of memory responses against constituent viral proteins or a primary response to neoantigens has spawned the evolution of synthetic gene delivery systems which exploit transfection, the transfer of DNA via physical, chemical or electrical methods.[30,31] Benefits of non-viral methods for DNA transfer include a reduction of risks associated with viruses (immune response, insertional mutagenesis) and limitations to gene delivery (such as length of the transgene cassette).[32]

Physical Methods to Enhance Delivery

1. Electroporation

Electroporation is a method that uses short pulses of high voltage to carry DNA across the cell membrane. This shock is thought to cause temporary formation of pores in the cell membrane, allowing DNA molecules to pass through. Electroporation is generally efficient and works across a broad range of cell types. However, a high rate of cell death following electroporation has limited its use, including clinical applications.

2. Gene Gun

The use of particle bombardment, or the gene gun, is another physical method of DNA transfection. In this technique, DNA is coated with gold particles and loaded into a device which generates a force to achieve penetration of DNA/gold into the cells.eg:- If the DNA is integrated in the wrong place in the genome, for example in a tumor suppressor gene, it could induce a tumor. This has occurred in clinical trials for X-linked severe combined immunodeficiency (X-SCID) patients, in which hematopoietic stem cells were transduced with a corrective transgene using a retrovirus, and this led to the development of T cell leukemia in 3 of 20 patients.[33]

3. Sonoporation

Sonoporation uses ultrasonic frequencies to deliver DNA into cells. The process of acoustic cavitation is thought to disrupt the cell membrane and allow DNA to move into cells.

4. Magnetofection

In a method termed magnetofection, DNA is complexed to a magnetic particles and a magnet is placed underneath the tissue culture dish to bring DNA complexes into contact with a cell monolayer.

Chemical Methods to Enhance Delivery

1. Oligonucleotides

The use of synthetic oligonucleotides in gene therapy is to inactivate the genes involved in the disease process. There are several methods by which this is achieved. One strategy uses antisense specific to the target gene to disrupt the transcription of the faulty gene. Another uses small molecules of RNA called siRNA to signal the cell to cleave specific unique sequences in the mRNA transcript of the faulty gene, disrupting translation of the faulty mRNA and therefore expression of the gene.

2. Lipoplexes and polyplexes

To improve the delivery of the new DNA into the cell, the DNA must be protected from damage and (positively charged). Initially, anionic and neutral lipids were used for the construction of lipoplexes for synthetic vectors.

3. Dendrimers

A dendrimer is a highly branched macromolecule with a spherical shape. The surface of the particle may be functionalized in many ways and many of the properties of the resulting construct are determined by its surface. In particular it is possible to construct a cationic dendrimer, i.e. one with a positive surface charge. When in the presence of genetic material such as DNA or RNA, charge complimentarily leads to a temporary association of the nucleic acid with the cationic dendrimer. On reaching its destination the dendrimer-nucleic acid complex is then taken into the cell via endocytosis.

4. Hybrid methods

Due to every method of gene transfer having shortcomings, there have been some hybrid methods developed that combine two or more techniques. Virosomes[34] are one example;

they combine liposomes with an inactivated HIV or influenza virus. This has been shown to have more efficient gene transfer in respiratory epithelial cells than either viral or liposomal methods[35] alone. Other methods involve mixing other viral vectors with cationic lipids or hybridising viruses.

Electrical methods

Electrotransfer are more well-established. Applying an electrical field to cells alters the resting transmembrane potential, which can induce permeability though the formation of reversible structural membrane changes (electropores).[36] A large number of animal studies have been performed across on a range of tissues, with the main application being immunotherapy.[36] Therapeutic levels of gene expression have been achieved, as well the cotransfer of multiple plasmids.[37] Although more efficient than chemical or physical methods, the efficiency of electrotransfer is still less than that seen with viral vectors.

The choice between transfection strategies compared to transduction with a virus will largely depend on the therapeutic goal. For transient gene expression or repeat dosing scenarios, synthetic delivery systems herald obvious advantages. Conversely, correction of missing protein disorders which require long-term, stable gene expression may be better served by viral vectors which can lead to integration of the transgene with host DNA and more stable constitutive protein expression. Synthetic delivery holds potential benefits in term terms of safety, low frequency of gene integration, ability to introduce larger portion of genes and ease of production.[38,39] Another consideration is the efficacy of expression: in general, viral vectors achieve higher efficiency of expression than synthetic systems.[40,41] The development of artificial viral systems (synthetic viruses) remains a future strategy to harness the advantages of viral and synthetic systems.

Gene Therapy in Diseases

Gene Therapy for Oral Squamous Cell Carcinoma

The current treatment strategies for oral squamous cell carcinoma (OSCC) include a combination of surgery, radiation therapy and chemotherapy. However, surgical resection of tumors frequently causes profound defects in oral functions such as speech and swallowing as well as in cosmetic aspects.[42] Chemotherapy is associated with well-known toxicity and has demonstrated no clear impact on the survival of patients with recurrent oral cancer. Recurrence develops in approximately one third of the patients despite definitive treatment.[43] Two thirds of the patients dying of this disease have no evidence of

symptomatic distant metastasis. Therefore, local and regional disease control is paramount, underscoring an urgent need for more effective therapy.

Several reports have indicated that the combination of radiation and gene therapies has synergistic suppressive effects on various cancer cells, including colorectal, ovarian, nasopharyngeal and head / neck cancer cells.[44] Gene therapy can also be used as an adjuvant to surgery (at the resected tumor margins). This review highlights various gene therapy methods that are available for combating OSCC.

Gene Therapy in Periodontics

Periodontal diseases have a broad spectrum of inflammatory and destructive responses, and are thought to be multifactorial in origin. Genetic variance has been considered as a major risk factor for periodontitis. With the advent of gene therapy in dentistry, significant progress has been made to control periodontal disease and reconstruct the dentoalveolar apparatus.[45] Gene therapy is a field of Biomedicine. A broad definition of gene therapy is the genetic modification of cells for therapeutic purposes.[46] Genes are specific sequences of bases present in the chromosome that form the basic unit of heredity. Each person's genetic constitution is different and the changes in the genes determine the differences between individuals. Some changes usually in a single gene, may cause serious diseases. More often, gene variants interact with the environment to predispose some individuals to various ailments.

The goal of gene therapy is to transfer the DNA of interest, for example, growth factor and thrombolytic genes into cells, thereby allowing the DNA to be synthesized in these cells and its proteins (termed recombinant protein) expressed. Gene therapy may involve (1) supplying or increasing the expression of a mutant gene that is insufficiently expressed (e.g., to treat enzymatic deficiencies); (2) blocking a gene that is detrimental (e.g., using antisense constructs to inhibit tumor proliferation); or (3) adding a foreign gene to treat a situation beyond the capability of the normal genome (e.g., introduce an enzyme into a cell or tissue that allows the tissue to become more sensitive to the effects of a pharmacologic agent).

Gene Therapy for Cystic Fibrosis Lung Disease

Gene therapy for the treatment of cystic fibrosis should be a "natural": Cystic fibrosis (CF) is a recessive disease associated with loss of function mutations in the CF transmembrane conductance regulator (CFTR) gene, which has a well-characterized gene product;

heterozygotes, as predicted, appear to be phenotypically perfectly normal; the level of expression of CFTR in affected cells generally appears to be low; and the dysfunctional epithelial lining cells in the organ most affected by CF (the lung) are available for direct vector delivery via topical administration.[47] However, despite an impressive amount of research in this area, there is little evidence to suggest that an effective gene-transfer approach for the treatment of CF lung disease is imminent. The inability to produce such a therapy reflects in part the learning curve with respect to vector technology and the failure to appreciate the capacity of the airway epithelial cells to defend themselves against the penetration by moieties, including gene-therapy vectors, from the outside world. This Perspective will focus on the issues that impact on moving this field forward.

Gene Therapy for Parkinson's Disease

Parkinson's disease (PD) is a chronic, progressive neurodegenerative disease most widely recognized for the profound degeneration of mid-brain dopamine nigrostriatal neurons linked to serious motor symptoms. However, PD is far more complex than commonly appreciated, with multiple etiologic variables and pathogenic pathways, complex pathologies and a wide range of central nervous system (CNS) and non-CNS symptoms. The drugs' effectiveness decline with progressive pathology, leading to gradual incapacitation of patients by increased "off" time (*i.e.*, periods of no symptomatic relief) and increasing side effects such as peak-dose dyskinesias. Thus, adequate treatment of the nigrostriatal-mediated motor impairments continues to represent a significant unmet medical need, affecting over 4 million people worldwide.[48] Though a number of solutions have been conceived to improve the function of the degenerating dopaminergic system, translating these biopharmaceutical concepts to the clinic has been challenging due to obstacles associated with delivering macromolecules to the central nervous system in a persistent and targeted fashion.

Progress achieved in the realm of gene therapy (GT) over the past decade has offered solutions to many of the delivery constraints[49] and several aspects of PD present it overtly as an ideal clinical indication to target using GT: (i) the well-defined, localizable and targetable neuronal systems involved with major motor symptoms, (ii) the need for relatively small titer and volume of vector targeted to those sites, which avoids the systemic circulation of immunogenic materials and (iii) the large and increasing demand for improved therapeutics with an aging population, which in whole bolsters impact and financial support for research and development.

Gene Therapy for Infectious Diseases

Gene therapy is being investigated as an alternative treatment for a wide range of infectious diseases that are not amenable to standard clinical management.[50-57] Gene therapy for infectious diseases requires the introduction of genes designed to specifically block or inhibit the gene expression or function of gene products, such that the replication of the infectious agent is blocked or limited. In addition to this intracellular intervention, gene therapy may be used to intervene in the spread of the infectious agent at the extracellular level. This could be achieved by sustained expression in vivo of a secreted inhibitory protein or by stimulation of a specific immune response.

Approaches to gene therapy for infectious diseases can be divided into three broad categories: (i) gene therapies based on nucleic acid moieties, including antisense DNA and RNA, RNA decoys and catalytic RNA moieties (ribozymes); (ii) protein approaches such as transdominant negative proteins (TNPs) and single-chain antibodies; and (iii) immunotherapeutic approaches involving genetic vaccines or pathogen-specific lymphocytes. It is further possible that combinations of the aforementioned approaches will be used simultaneously to inhibit multiple stages of the viral life cycle. The extent to which gene therapy will be effective against infectious agents is the direct result of several key factors: (i) selection of the appropriate target cell or tissue for gene therapy; (ii) the efficiency of the gene delivery system; (iii) appropriate expression, regulation and stability of the gene therapy product(s); and (iv) the efficiency of the inhibition of replication by the gene inhibition product.

Gene Therapy for Arthritis

Rheumatoid arthritis is an autoimmune disease with intra-articular inflammation and synovial hyperplasia that results in progressive degradation of cartilage and bone, in severe cases it causes systemic complications. Recently, biological agents that suppress the activities of proinflammatory cytokines have shown efficacy as antiarthritic drugs, but require frequent administration. Thus, gene transfer approaches are being developed as an alternative approach for targeted, more efficient and sustained delivery of inhibitors of inflammatory cytokines as well as other therapeutic agents.

Recently, biological agents that modulate the proinflammatory activities of TNF-α and IL-1β have shown efficacy as novel antiarthritic drugs.[58-61] However, arthritis therapies that employ biological agents are currently limited by possible systemic side effects such as the

occurrence and re-emergence of viral and bacterial infections as well as their exorbitant expense. There are several different approaches that can be utilized for the treatment of arthritis.[62-64] Genes can be delivered locally at the site of disease pathology such as the joint by intra-articular injection. Alternatively, therapeutic genes can be delivered using specific circulating cell types such as T cells.[65-67] or antigen-presenting cells (APCs) such as dendritic cells (DC).[68-70] Although these types of cells result in more systemic delivery of therapeutic proteins, the ability of certain immune regulatory cells to home sites of inflammation can also allow for local treatments following systemic injection. It is also possible to increase the levels of circulating therapeutic proteins by delivery of the gene to tissues such as muscle or liver.[71,72]

Gene Therapy in Diabetic Neuropathy

Gene therapy shows promise in treating diabetic polyneuropathy, a disorder that commonly affects diabetics who've had the disease for many years, a new study finds. Researchers in Boston found that intramuscular injections of vascular endothelial growth factor (VEGF) gene may help patients with diabetic polyneuropathy.

The study included 39 patients who received three sets of injections of VEGF gene in one leg and 11 patients who received a placebo. Loss of sensation and pain in the legs and feet, weakness, and balance problems are among the symptoms associated with diabetic neuropathy. The loss of sensation means that ulcerations on the feet may go undetected, which can lead to amputation.

Targeting Gene Delivery

An important considering in gene therapy is ensuring that the pharmacophore is delivered to an area that maximise its therapeutic benefit. This can be especially complex in the living organism due to shared receptors between tissues, circulatory anomalies (such as the blood-brain barrier) and ability of serum proteins to destabilise synthetic vector complexes. In some cases, direct application of the vector to the dysfunctional tissue may be required to maximise effect. In the case of a cationic liposome complexed to plasmid DNA encoding chloramphenicol acetyltransferase, direct injection into murine hepatic tumors resulted in higher levels of gene expression than were achieved with systemic or portal vein inoculation.[73] Lipoplexes complexed to the bcl-2 gene have demonstrated reduced neural apoptosis after transient cerebral ischaemia in an animal model, circumventing the blood-brain barrier by utilising direct intra-thecal injection.[74] Furthermore, the cystic fibrosis

transmembrane regulator gene has been successfully packaged with both cationic liposomes and polymers and safely delivered intranasally to cystic fibrosis directly targeting airway mucosa.[74-76]

Other targeting techniques include altering the charge of the synthetic vector-DNA plasmid particle: cationic liposomes have been shown to preferentially distribute to the lung after systemic administration, an effect which is lost which decreasing positivity.[77] Size also plays a role as large molecules may be unable to extravasate from the circulation to reach target cells within organ parenchyma. Additionally, constitutive expression of specific ligands on targets cells can be manipulated to design advantage, for example the use of dextran-spermine polycation complexing with DNA to target the liver by preferential binding to galactose receptors on hepatic parenchyma.[78] Here is some target cells for gene therapy shown in Table: 2.

Table 2: Target sites for Gene Therapy

Disease	Target
Cancer	Tumor cells antigen presenting cells (APCs), blood progenitor cells, T cells, fibroblasts, muscle cells
Inherited monogenic disease	Lung epithelial cells, macrophages, T cells, blood progenitor cells, hepatocytes, muscle cells
Cardiovascular disease	Endothelial cells, muscle cells
Rheumatoid arthiritis	Sinovial lining cells
Cubital tunnel Syndrome	Nerve cells
Infectious disease	T cells, blood progenitor cells, antigen presenting cells (APCs), muscle cells

CONCLUSION

Most scientists believe the potential for gene therapy is the most exciting application of DNA science, yet undertaken. How widely this therapy will be applied, depends on the simplification of procedure. As gene therapy is uprising in the field of medicine, scientists believe that after 20 years, this will be the last cure of every genetic disease. Genes may ultimately be used as medicine and given as simple intravenous injection of gene transfer vehicle that will seek our target cells for stable, site-specific chromosomal integration and subsequent gene expression. And now that a draft of the human genome map is complete, research is focusing on the function of each gene and the role of the faulty gene play in disease. Gene therapy will ultimately change our lives forever.

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