

Delivery systems for gene therapy

Shrikant Mali

Department of Oral and Maxillofacial Surgery, CSMSS Dental College, Aurangabad, Maharashtra, India

The structure of DNA was unraveled by Watson and Crick in 1953, and two decades later Arber, Nathans and Smith discovered DNA restriction enzymes, which led to the rapid growth in the field of recombinant DNA technology. From expressing cloned genes in bacteria to expressing foreign DNA in transgenic animals, DNA is now slated to be used as a therapeutic agent to replace defective genes in patients suffering from genetic disorders or to kill tumor cells in cancer patients. Gene therapy provides modern medicine with new perspectives that were unthinkable two decades ago. Progress in molecular biology and especially, molecular medicine is now changing the basics of clinical medicine. A variety of viral and non-viral possibilities are available for basic and clinical research. This review summarizes the delivery routes and methods for gene transfer used in gene therapy.

Key words: Gene therapy, viral vectors, liposomes

Introduction

The goal of cancer gene therapy is to introduce new genetic material into target cells without toxicity to non-target tissues. The patient with recurrent or metastatic cancer is often considered incurable. A variety of chemotherapeutic agents has been used alone, and in combination, for the treatment of recurrent oral squamous cell carcinoma. However, chemotherapy is associated with well-known toxicities and has demonstrated no clear impact on survival in patients with

recurrent oral cancer. Local and regional disease control is paramount, underscoring an urgent need for more effective therapies. Gene therapy has the potential to target cancer cells while sparing normal tissues. Such a strategy may be useful for recurrent disease as well as in the adjuvant setting (i.e., at the resected tumor margins).

Although gene therapy as a treatment for disease holds great promise, progress in developing effective clinical protocols has been slow. The problem lies in the development of safe and efficient gene-delivery systems. This review will evaluate the problems and the potential solutions in this new field of medicine.

In order for target cells to manufacture the protein products of the introduced gene, the exogenous genetic material must be delivered to the cell's nucleus. This process of transfection exists in 2 classes of vectors: Viral and non-viral. The viral technique is associated with increased technical demands and an increased risk of virus-associated toxicity. However, viral vectors have been engineered for safety by making them replication incompetent. It is the viral ability to efficiently infect cells and in this process to transfer DNA to the host without invoking an immune response that makes viruses attractive as vectors. These altered viruses can be propagated in cell lines specialized to provide the necessary absent viral functions.^[1,2]

Genetic material can be transferred via a vector that is defined as the vehicle that is used to deliver the gene of interest. The ideal vector would transfer a precise amount of genetic material into each target cell, thereby allowing for expression of the gene product without causing toxicity.

An ideal vector should deliver gene to a specific cell type, accommodate foreign genes of sufficient size,

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Address for correspondence: Dr. Shrikant Mali, Flat No. 1, Varun Corner Osmanpura, Aurangabad, Maharashtra, India.
E-mail: shrikantmali@gmail.com

achieve the level and duration of trans-genic expression sufficient to correct the defect and be non-immunogenic and safe.^[1-3]

Gene transfer via the viral vectors is called transduction while transfer via the non-viral vectors is called transfection.^[4]

Chemical transfection introduces DNA by calcium phosphate, lipid, or protein complexes. Calcium phosphate, DEAE-dextran, liposomes, and lipoplexes (for oral delivery of gene) surfactants and perfluro chemical liquids for aerosol delivery of gene.

Lipid vectors are generated by a combination of plasmid DNA and a lipid solution that result in the formation of a liposome. This fuses with the cell membranes of a variety of cell types, introducing the plasmid DNA into the cytoplasm and nucleus, where it is transiently expressed. Many carcinoma cells, including oral squamous cancer cells, express high levels of folate receptor. Linkage of DNA or DNA-lipid complexes to folate can specifically target cancer cells. Pre-clinical studies have demonstrated the potential utility of linking targeting moieties to the gene therapy construct. The DNA can then be internalized via receptor-mediated endocytosis.

Physical transfection of genes can be accomplished by electroporation, microinjection, or use of ballistic particles. Parenteral injections, micro-injections, aerosol, electroporation (high voltage current is passed to the target cell to produce pores on the cell surface through which transgene enters the cell) and gene guns.^[5,6]

Electroporation therapy with intralesional bleomycin has been reported to be a technically simple outpatient technique where high-voltage electric impulses can be delivered into a neoplasm by transiently increasing cell membrane permeability to large molecules, including cytotoxic agents, thus causing localized progressive necrosis. Electroporation can treat bulky tumors (>2 cm) with complete penetration.^[5,7]

Viral Vectors

These are more promising system of gene delivery with various advantages over physical and chemical method:

1. Gene transferred is more efficient and specific than physical and chemical method
2. Multiple and repeated doses are required in case of physical and chemical method, whereas in case of viral vector even a single dose is sufficient.^[7,8]

Transfer of the Genetic Material to the Cells

Although, systemic intravenous route can be applied to deliver the genetic material to the cells, local delivery methods are more commonly used such as surgical, percutaneous, US and computed tomography guided and by means of catheters.

The viral vectors can be divided into two types: Integrating and non-integrating viral vectors. The former, such as, retroviral, lentiviral, and adeno-associated viral vectors, can integrate into the human genome; whereas the non-integrating vector (e.g., adenoviralvector) is maintained in the nucleus without integrating into the chromosomal DNA, so that the transgene is apt to lose during cell division and expression of the foreign gene is transient. In a packaging cell, the essential components for further propagating of viruses can be provided in trans, which enable the viral vectors to be packaged as the viral particles and to deliver genes to the targeted cells. Certainly, this is a dead-end infection, because the vectors lack the essential components for viruses' propagation. Recombinant viral vectors can lead to the generation of infectious parental viruses. This is a principle frequently used in viral vector design in gene therapy.^[4]

Retroviruses

Retroviruses are RNA viruses that carry a gene for a reverse transcriptase that transcribes the viral genetic material into a double stranded DNA intermediate. This DNA intermediate is then incorporated into the host DNA allowing the host cell machinery to produce all the necessary viral components. Additionally, because the viral genome is stably integrated into the host DNA, any modification that has been made will be passed to all daughter cells that are derived from the transfected cell currently; the most common retrovirus used is derived from the murine leukemia virus.

In general, retroviruses have been used for *ex vivo* gene therapy applications as they are unable to efficiently infect non-dividing cells.

Limitations of Retroviral Vectors

(a) low vector titer, (b) low transfection efficiency, demonstrated in *in vitro* experiments, (c) particle instability and difficulty to concentrate, and (d) inability to transduce non-dividing post-mitotic cells, particles infecting only proliferating cells.

The limitation of retroviral infection has been overcome, in part, by the use of lentiviral vectors, which have been shown to activate the immune system in pre-clinical animal models of oral cancer.

Using different viral envelope proteins that recognize different receptors can vary the range of cells that can be transduced, but still does not provide much specificity. The difficulty is that, because retroviral vectors cannot be generated at a high titer, it is not possible to get a large number of vector particles to the desired cell type *in vivo*. The viral particles would bind to many cells they encounter and therefore, would be diluted out before reaching their target.

The envelope protein has two functions: Binding to its receptor (by the surface [SU] moiety) and enabling the entry of the viral nucleoprotein core (carried out primarily by the trans membrane [TM] moiety). The SU protein binds to its receptor on the target cell surface and as a result, the SU/TM complex undergoes a conformational change that allows fusion of the viral and cellular membranes, followed by entry of the viral core (which carries the virus's genetic information) into the target cell's cytoplasm.

Two broad approaches to providing target cell specificity have been followed. First, the natural receptor-binding domain of the SU protein has been replaced with a ligand or single-chain antibody that recognizes a specific cell surface receptor.

Although, several creative systems have been designed, the most successful approach at present appears to be insertion of a ligand that recognizes an extracellular matrix (ECM) component into a part of the SU protein that does not disturb the natural

receptor-binding domain. This tethering concentrates the vector in the ECM in the vicinity of the target cells. Receptor binding and core entry can then occur through the natural envelope-receptor mechanism. Two ligands that appear particularly useful for tethering are those specific for fibronectin or collagen. Fibronectin is present in normal ECM and exposed collagen is present in areas of damage, for example after wound injury as in the cardiovascular system after angioplasty.^[2-4,7,8]

Lentiviruses (e.g., human immunodeficiency virus type 1: HIV-1) are a special group of retroviruses with the ability to infect both proliferating and quiescent cells. To expand the spectrum of target cells, it is possible to replace the genes for surface glycoproteins by genes from another viral genome in the packaging cell lines packaging cell lines (PCL) of the vector.

Lentiviral transfer systems ensure long-term expression and efficient transfer without inflammatory responses.^[4-6,9]

The adenovirus does not integrate its genome into the host genome. Instead, the adenoviral genome remains in the nucleus as an episome after the infection of the host cell. The advantages common to all adenoviral vectors include, the ease of purification and concentration and the high efficiency rate of host cell infection of various cell types, dividing or non-dividing. These advantages make adenoviral vectors a good candidate for direct *in vivo* gene transfer. The usefulness of these vectors is limited by 2 factors. In most tissues, the duration of transgene expression is limited to a few days to a week and viral genes are also transduced and expressed, eliciting an immune response to the transduced cells that ultimately results in their clearance.

Adenoviruses are DNA viruses that infect a cell, lose their protein coat, and transfer DNA into the nucleus, where it is transcribed. This DNA does not integrate into the host genome, and thus, its effects are transient (range: 7-42 days).

Therefore, multiple administrations of the vector are usually required. The advantage of adenoviral vectors is that most cells are susceptible to infection, regardless of their position in the cell cycle. In addition, adenoviruses can be produced at a relatively higher titer, thus increasing the efficiency of their administration. Since exposure to adenovirus is common, approximately, 90%

of humans have already formed antibodies against the virus. Pre-existing antibodies can limit the effectiveness of this strategy, particularly upon a second exposure to the vector. Transduction studies have demonstrated that direct injection, however, not topical application, of adenoviral constructs can transfect oral cancer cells *in vivo*.^[1,2,4,6-10]

Adeno-Associated Viruses

Adeno-associated viruses (AAV) demonstrate low immunogenicity, have no known pathogenicity, target non-proliferating cells, and may have discrete genome insertion sites. "Suicide" gene therapy has been shown to be feasible in oral cancer cell lines with the use of an AAV vector. AAV vectors have also been used successfully to transfer antisense or ribozyme genes in pre-clinical cancer models AAV vectors have been shown transduce brain, skeletal muscle, liver and possibly CD34 + blood cells efficiently. There are several drawbacks, however, some cells require a very high multiplicity of infection (the number of viral particles per cell required to achieve transduction); the AAV genome is small, only allowing room for about 4.8 kb of added DNA; and the production of viral particles is still very labor intensive because efficient packaging cells have not yet been developed.^[2-4,6,7,10]

The advantages of the herpes simplex virus (HSV) are its large size, the wide spectrum of action and the continuous expression of genes from long-lived infection. Little risk of insertional mutagenesis with the HSV, because it remains outside the nucleus (episomal). Unfortunately, the HSV also has its limitations, which include low infection efficiency, wild-type breakthrough, and a large genome size that makes it more difficult to manipulate than other viral vectors. Finally, HSV's tropism for neural cells limits its action; however, some researchers are trying to exploit this feature to target neurons.

Most herpes virus vectors are developed from strains of HSV type 1 (HSV-1). This double-stranded DNA virus has several interesting properties, including the ability to remain latent in tissues and to be re-activated at the original site of infection. After infecting a cell, HSV-1 replicates within the cell, causing cell lysis and infection

of surrounding cells. In addition, HSV-1 is a common pathogen in humans and rarely causes significant illnesses. HSV vectors can accommodate large pieces of foreign DNA and transfer genes rapidly and efficiently. A replication-conditional mutant of HSV has been shown to elicit anti-tumor responses in pre-clinical models of glioma and metastatic colon cancer.^[11-13]

Poxviruses

The large potential size (25 kb) of the gene insert, the absence of viral integration in to the host cellular genome, and the excellent immune stimulation induced by this virus all combine to make it an attractive candidate for immune based therapy in cancer. Vaccinia virus infects all cells; however, the host immune response to the vector does not abrogate the tumor immune response even following repeated injections. The availability of attenuated virus allows the use of vaccinia in immuno-delicate cancer patients and there is evidence that this vector enhances immunological rejection of the tumor.

Common toxicities included a local skin reaction at the site of the vaccine, usually of 4-5 days' duration, and mild flu-like symptoms of 1-2 days' duration. Cellular immune response did not correlate with the clinical response.^[2,11]

Alpha viruses are members of the Togaviridae family and are enveloped, single-stranded RNA viruses of icosahedral conformation. There are three different viruses with similar properties useful in gene therapy: Semliki forest virus, Sindbis virus, and Venezuelan equine encephalitis virus. Most alpha virus vectors used as gene vehicles are replication incompetent. They encode only the non-structural genes, while the structural genes necessary for replication are located in the helper plasmid genome.

To eliminate or at least reduce the possibility of generating infectious replication-competent particles, structural genes can be localized to two different helper plasmids. However, the replication-competency of alpha viral vectors can also be advantageous when used in intratumoral gene delivery. Replication-competent vectors contain all the genes of the wild-type genome, including the therapeutic gene, and are also able to transduce neighboring and surrounding tumor cells.

Because of their extensive host-cell toxicity and broad host range, alpha viruses are suitable for cancer gene therapy of various tumors. Many clinical trials have showed that intratumoral injection of recombinant alpha viruses induced apoptosis of tumor cells. The great potential of alpha viruses is also given by their immunogenicity and ability to induce cytotoxic T-cell responses. They are suitable for vaccine production and immunization against infectious agents, but also tumors.^[2,4,7,10,14]

Non-Viral Vectors

Non-viral vectors include naked-DNA and liposomes. They are based on plasmid, which is a closed, circular DNA strand. Therapeutic genes can be inserted directly into the plasmid, and then this recombinant plasmid can be introduced into cells in a variety of ways. For example, it can be injected directly into targeted tissues as naked-DNA.

Non-viral vectors are much cheaper and easier to produce in large amounts. These vectors have a limited immunogenicity, which allows for potential re-dosing, and they are considered safe, since there is no possibility of recombination that would result in a competent virus that could potentially cause disease. Less efficient gene transfer rate the “gene-gun,” does not require the presence of complicated and potentially toxic delivery systems. The gene material transfer is mediated by miniature particles of gold on which the DNA is bound. These particles are then shot into the cell under great pressure and speed (with the help of compressed helium) and so pass the membrane barrier.

In comparison to virus-derived vectors, non-viral vectors have several advantageous qualities, mainly the safety of administration without immunogenicity, but also almost unlimited transgene size and the possibility of repeated administration. Therapeutic gene can be introduced into the target cell either as an insert in plasmid with regulation sequences, what enables the regulation control of expression (inducible promoter) or as a PCR product. The simplest way of gene introduction is an injection of naked DNA in to target cells.

The use of non-viral vectors can be in the form of injections of naked DNA (usually plasmids), liposomes

or particle-mediated gene transfer (‘the gene gun’). The genetic material can be placed in liposomes in order to increase the DNA uptake in tissue culture. The last of these vectors uses a process by which the micro projectiles (e.g., gold or tungsten) are coated with DNA and then accelerated by either helium pressure or a high-voltage electrical discharge, thus, carrying enough energy to penetrate the cell membrane.

A novel strategy of non-viral gene transfer is to load cDNA onto a porous biomaterial scaffold and pack it directly into a wound with the subsequent transfer of the gene into endogenous cells migrating into the site. This technique is called gene-activated matrix and is an extension of research producing biodegradable polymers appropriate for tissue engineering.

Liposomes have no replication risk and are less immunogenic than viruses. Liposome-mediated gene transfer has been limited primarily by transfection efficiencies *in vivo*. Pegylated liposomes have been shown to localize to solid cancers and may deliver radio sensitizing agents preferentially to tumor tissue, potentially improving the therapeutic ratio of chemotherapy. The transferrin ligand has been used to target a cationic liposome delivery system, resulting in a significant increase in the transfection efficiency of the complex.

Delivery of wild-type (weight) p53 to a radiation-resistant squamous cell carcinoma cell line via this ligand targeted liposome complex was also able to modulate the radiation-resistant phenotype of these cells *in vitro*. These results indicate that this tumor-specific, ligand-liposome delivery system for p53 gene therapy, when used in concert with conventional radiotherapy, may provide a new and more effective means of cancer treatment.^[1,2,4-8,10-12]

Bacteria as Vectors for Blocking Angiogenesis Gene Therapy

Bactofection

The use of bacteria as a vector for the delivery of therapeutic genes to target cells is known as bactofection, and several studies have used this approach to deliver genes encoding anti-angiogenic molecules to tumor cells *in vivo*. However, if the product of the transgene

is secreted outside the target cell, it may still have a therapeutic effect on non-infected tumor cells.

DNA vaccination

It is known that bacteriofection of plasmids encoding a tumor-expressed antigens can lead to induction of humoral and cellular immune response in the host thereby providing protective defense against tumors (R. Xiang *et al.*, 2000). This approach, termed DNA vaccination, has been successfully implemented for anti-angiogenic therapy.

Alternative gene therapy

Another means of using bacteria for gene therapy is the so-called Alternative gene therapy (AGT) approach, which is also known as bacterial protein delivery. It is based on the transfer of bacterially expressed therapeutic proteins to the host organism using genetically modified (transformed) bacteria. As with bacteriofection, AGT is mostly used for treatment of tumors and employs primarily oncolytic and tumor-colonizing bacterial strains of Clostridia, Bifidobacteria or Salmonellae.

Bacterioference—bacteria-mediated RNA interference

Bacteria that have engineered to produce and deliver short interfering RNA represent a novel tool for the efficient induction of RNA interference in host cells.

Currently known and tested bacterial vectors can be divided into two groups. Strictly anaerobic bacteria (the species *Clostridium* and *Bifidobacterium*) are used in *in vivo* experiments. *Clostridium* is the most important bacterial species for use as a vector.

The second group consists of attenuated auxotrophic strains of *Salmonella typhimurium* that require the presence of tumor specific nutrition factors for selective replication. They use these factors for their own metabolism, thus, prohibiting the tumor cells from utilizing them and growing.^[4,12]

Conclusion

Gene therapy is good for single gene defect but more research should be carried out for multiple

gene defects. Viruses form good carrier of genes however, they also have their limitations. New and effective gene carriers need to be developed by further research to increase target specificity and decrease harm to adjacent healthy tissues. Gene therapy is very good method of treatment of genetic disorders and cancers.

References

1. Shillitoe EJ. Gene therapy: The end of the rainbow? *Head Neck Oncol* 2009;1:7.
2. Gardlik R, Pálffy R, Hodossy J, Lukács J, Turna J, Celec P. Vectors and delivery systems in gene therapy. *Med Sci Monit* 2005;11:RA110-21.
3. Kay MA, Liu D, Hoogerbrugge PM. Gene therapy. *Proc Natl Acad Sci U S A* 1997;94:12744-6.
4. Biçeroğlu S, Memiş A. Gene therapy: Applications in interventional radiology. *Diagn Interv Radiol* 2005;11:113-8.
5. Sharma R, Khajuria R, Sharma CL, Kapoor B, Goswami KC, Kohli K. Gene therapy: Current concepts. *JK Sci* 2004;6:62-6.
6. Mulherkar R. Gene therapy for cancer. *Curr Sci* 2001;81:555-60.
7. Xi S, Grandis JR. Gene therapy for the treatment of oral squamous cell carcinoma. *J Dent Res* 2003;82:11-6.
8. Gleich LL, Salamone FN. Molecular genetics of head and neck cancer. *Cancer Control* 2002;9:369-78.
9. Scholl SM, Michaelis S, McDermott R. Gene therapy applications to cancer treatment. *J Biomed Biotechnol* 2003;2003:35-47.
10. Zhou HS, Liu DP, Liang CC. Challenges and strategies: The immune responses in gene therapy. *Med Res Rev* 2004;24:748-61.
11. Ayllón Barbellido S, Campo Trapero J, Cano Sánchez J, Perea García MA, Escudero Castaño N, Bascones Martínez A. Gene therapy in the management of oral cancer: Review of the literature. *Med Oral Patol Oral Cir Bucal* 2008;13:E15-21.
12. Gardlik R, Celec P, Bernadic M. Targeting angiogenesis for cancer (gene) therapy. *Bratisl Lek Listy* 2011;112:428-34.
13. Gardlik R, Dovinova I, Chan JY. Approaches in gene therapy of cancer and cardiovascular diseases. In: Kang C, editor. *Gene Therapy Applications*. InTech; 2011. p. 59-84. Available from: http://www.intechopen.com/source/pdfs/17760/InTech-Approaches_in_gene_therapy_of_cancer_and_cardiovascular_diseases.pdf. [Last Accessed on 2013 Mar 7].
14. Anderson WF. Human gene therapy. *Nature* 1998;392 Suppl 6679:25-30.

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