Review

Gene therapy – its potential in surgery

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Advances in techniques have resulted in practical applications for gene therapy, which is becoming applicable for the treatment of human disease. This review outlines the advantages and disadvantages of the techniques available. Examples of research efforts in the treatment of diseases of relevance to the surgeon (cardiovascular diseases, cancer, wound healing, fracture repair, and in organ transplantation) are presented.

Key words: Gene therapy – Surgery – Cancer – Cardiovascular disease – Transplantation

The basic principle of gene therapy involves the treatment of a disease by the introduction of a gene (transgene) which confers a therapeutic effect either directly by the transgene product or via an additional agent, *e.g.* by activation of an inactive pro-drug. Once deprived of their natural deleterious abilities, viruses can be powerful vehicles to introduce therapeutic genes into human cells.¹

Methods of gene delivery

The choice of gene delivery system (vector) is crucial. Constraints include the requirement for targeting specific tissues or organs, the necessity to maintain prolonged expression of the transgene, the level of expression required, and the possibility of engineering cells *in vitro* and then re-implanting them. In addition, potential side-effects on the host, such as toxicity and immunization, must be prevented. Trials in humans have to date largely proved

safe, although the death in 1999 of one patient emphasizes the need for continued vigilance in the planning and performance of such trials (see below).²

Methods of gene transfer may be virus-independent (transfection) or virus-dependent (transduction; Table 1). The method chosen is dictated largely by the condition for which gene therapy is being utilized. Transfection may be required, for example, when the introduction of large pieces of DNA is required, as current viral vectors are limited in the size of the DNA sequence they can incorporate successfully.

Non-viral methods (transfection)

Transfection of 'naked' DNA into mammalian cells can be achieved using various chemical or physical methods, but is generally inefficient. Recently, however, the introduction of naked DNA encoding vascular endothelial growth factor (VEGF) has resulted in significant angiogenesis in patients with atherosclerosis and ischaemic heart disease.³

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Abbreviations: AAV, adeno-associated virus; MHC, major histocompatibility complex; PTCA, percutaneous transluminal coronary angioplasty; VEGF, vascular endothelial growth factor

Cationic lipid complexed with DNA provides a highly efficient means of *in vitro* transfection.⁴ Cationic lipids include liposomes, micelles, emulsions and nanoparticles. Liposome vectors are non-antigenic, allow transfer of large pieces of DNA, and are being used in clinical gene therapy for cancer⁵ and cystic fibrosis.⁶ Its efficiency *in vivo*, however, is low, and gene expression is transient. The complexing of inactivated haemagglutinating virus of Japan (HVJ) with liposomes improves transfection efficiency.

Viral methods (transduction)

Viral vectors are generally more efficient. Once in the cell, they utilize the cellular metabolism of the host to complete their replicative cycle. Regions of the virus genome that are dispensable are deleted and replaced with the foreign gene(s) to be introduced into the cell. This manipulation renders the virus incapable of replication (replication-defective) in the host.

There are, however, some potential hazards. Firstly, the integration of the virus in the genome of the targeted host cell may affect cell function. Mutagenesis of a cell toward a more tumourigenic state is clearly of concern. Re-assuringly, cancers seem to require multiple genetic events before full mutagenesis is observed, and thus viral vectors are unlikely to represent significant mutagens. Other potential hazards include the rescue of an infectious virus from the replication-defective virus by recombination with host retroviral sequences, causing clinical infection. Encouragingly, none of these potential adverse effects has been observed in gene therapy clinical trials to date.⁷ However, the death of a young man in 1999 from an intractable acute respiratory deficiency syndrome (from uncertain cause) following gene therapy with a high dose of recombinant adenovirus has been a matter of concern.²

Table 1 Comparison of viral and non-viral methods of gene delivery

Method	Advantages	Disadvantages
NON-VIRAL		
Naked	Safe (no possibility of plasmid inducing a replication-competent virus). Administration can be repeated	Less efficient; transient expression
Liposome	Safe. Better gene transfer efficiency than naked plasmid. Administration can be repeated	Transient expression
HVJ*-liposome	High gene transfer efficiency. Can deliver oligonucleotides. Administration can be repeated	Difficulty in inactivating HVJ*. Transient expression
VIRAL		
Retrovirus	Long-term gene expression. Non-immunogenic. Broad spectrum of specificity	Inability to infect non-dividing cells. Potential activation of host genes. Potential mutagenesis. Limitation of transgene size
Adenovirus	Efficient transduction. Infects both dividing and non-dividing cells	Transient gene expression. Immunogenicity of viral particles
Adeno-associated virus	Infects both dividing and non-dividing cells. Long-term gene expression	Difficulty in creating recombinant virus. Need for helper virus (e.g. adenovirus)

*HVJ, haemagglutinating virus of Japan.

Figure 1 A packaging cell line, which already produces viral proteins, is transfected by the transgene construct (1). Replication-defective recombinant retroviruses are released into the culture medium (2). The viral particles infect cells via the membrane receptor (3). Under the control of the retroviral genome, the viral genetic information is converted from single-stranded RNA into double-stranded DNA (4), and integrated into the host genome at random (5). The transgene is transcribed into RNA, which in turn is translated into proteins by using the expression machinery of the host cell (6).

Retroviral vectors

The key feature is the stable integration of the therapeutic transgene into the host genome (Fig. 1). If successful integration of the viral genome into the host cell genome is to take place, many retroviral vectors require that the

Figure 2 A vector carrying both the transgene and some parts of the adenoviral genome is constructed (1). An adenoviral genome from which the regulatory elements have been deleted is also prepared (2). A cell is co-transfected with the vector and parental viral DNA (3). Within the transfected cell, homologous recombination occurs and replication-defective recombinant adenoviruses are produced (4). The cells are lysed by the viruses, and viral particles are released into the medium (5). These particles attach to target cell membranes via specific receptors, and the DNA is carried into the nucleus by viral protein (6). Within the nucleus, adenoviral DNA is transcribed and translated into proteins without integrating into the genome of the host (7).

target cell be dividing during the transduction period, which limits the efficiency and application of this method. Direct destruction of the virus may also occur *in vivo* by immune processes.

Lentiviruses, which include the human immunodeficiency viruses, are unique among retroviruses because of their ability to infect quiescent non-dividing cells. However, the clinical use of lentiviral vectors will require an assay to detect potential replication-competent viruses that would be pathogenic, which does not exist at the moment. Lentiviral or certain other retroviral vectors offer potential for treatment of a wide variety of diseases, including cystic fibrosis, sickle cell anaemia, Alzheimer's disease, and cardiovascular diseases. Potential target cells include airway epithelia, haematopoietic stem cells, neurons and glial cells, and cardiomyocytes.

Adenoviral vectors

The adenovirus (Fig. 2) attaches to a specific glycoprotein receptor (the Coxsackie adenovirus receptor) present on most mammalian cell membranes and enters the target cell through the internalization of membrane-bound vesicles (endocytosis). The virus persists in the nucleus without integrating into the genome. Certain key genes are deleted to ensure that the recombinant adenoviral vector is replication-incompetent and lysis-defective.

Adenoviruses do not require host cell replication for them to enter the nucleus, and can carry a large amount of DNA. However, expression of the transgene is frequently transient (< 4 weeks), and can be associated with inflammatory reactions and/or an immune response, which compromises the long-term expression of the introduced gene.

Adeno-associated viral (AAV) vectors

The adeno-associated virus (AAV) requires co-infection with a helper virus, such as the herpes simplex virus or an adenovirus, to produce cell infection. AAV virus vectors have a wide host range, can carry several different transgenes into the host genome, even when the host cell is quiescent. Although wild-type AAV is unique in its ability to target a defined region of human chromosome 19, current recombinant AAV vectors lack the capacity for site-specific integration. The instability of AAV production remains a concern.

Hybrid viral vectors

Hybrid viral vectors have been designed to exploit elements from different viruses. One virus may facilitate viral entry into the cell and the other may increase its ability to integrate transgenes into non-dividing cells. Examples include the vesicular stomatitis virus G/lentivirus vector, where the tropism and high transfer efficiency of the vesicular stomatitis virus G protein are combined with the ability of the lentivirus to integrate the transgene into non-dividing cells, and the adenoviral/retroviral vector, where the adenovirus infects the target cell which then functions as a transient retroviral producer cell.

Gene therapy clinical trials

Safety has been a primary concern. Government agencies in Europe and North America have responded by drawing up guidelines for certification of biological materials and clinical trials. The treatment of human diseases by gene transfer began in the US in 1989 and, by May 2000, almost 3500 patients had been entered into gene therapy trials; over 425 clinical protocols are currently under investigation. The first successful protocol demonstrated that an exogenous gene could be safely transferred and detected in targeted cells.⁸

The first approved functioning-gene protocol, for correction of severe combined immunodeficiency disorder (adenosine deaminase deficiency), was initiated in 1990.⁹ A retrovirus was transferred into autologous haematopoietic cells *ex vivo*, and the cells transfused into the patient. The majority of subsequent gene therapy trials have been directed towards patient populations with poor-prognosis, often incurable, diseases, such as single-gene inherited disorders (e.g. familial hypercholesterolaemia), cancer, and AIDS.

Potential roles for gene therapy in clinical surgery

Cardiovascular surgery

Clinical trials relate to the treatment of atherosclerosis, coronary artery restenosis after percutaneous transluminal coronary angioplasty (PTCA), myocardial infarction, angina pectoris, and familial hypercholesterolaemia. For example, in some patients with advanced peripheral vascular disease, the gene for vascular endothelial growth factor (VEGF) has been introduced to initiate angiogenesis and improve circulation in the ischaemic areas.¹⁰

Furthermore, preliminary data suggest that the direct intramuscular injection of naked DNA carrying VEGF is effective in stimulating angiogenesis in patients with ischaemic heart disease.³ The over-expression of VEGF may possibly prevent restenosis after PTCA by augmenting the regeneration of endothelial cells.¹¹ Restenosis after aorto-coronary artery bypass grafting is probably caused by over-proliferation of endothelial cells. Activation of several genes that encode regulatory proteins appears to be involved, and techniques to attenuate their function are being developed.¹²

Cancer

Of the first 244 protocols assessed by the National Institutes of Health Recombinant DNA Advisory Committee, 147 were for the treatment of various forms of cancer.¹³ The major approaches have involved: (i) the introduction of what is known as a 'suicide' gene whose activity is triggered by drug therapy; (ii) stimulation of the immune system to kill malignant cells; (iii) replacement of a non-active or defective gene by an active transgene; (iv) the inhibition of neo-angiogenesis, thus depriving the tumour of its blood supply; and (v) the introduction of a so-called 'tumour suppressor gene'.

In 'suicide' gene therapy, a virus introduces the gene for an enzyme which has no direct effect itself, but is activated by a pro-drug administered to the patient. For example, cells have been transduced with herpes simplex virus thymidine kinase, which metabolizes the drug ganciclovir into an active form which disrupts DNA replication and inhibits the proliferation of tumour cells. A trial has been carried out in patients with local recurrence of prostatic cancer.¹⁴ In 5 of 15 patients with

recurrent malignant brain tumours, this approach induced tumour regression after ganciclovir administration.¹⁵

Cytokine genes have been introduced into tumour cells (metastatic breast cancer, melanoma, glioblastoma) and can induce an inflammatory response, which is destructive to the tumour.¹⁶ Angiogenesis in gliomas has been reduced by the delivery of a VEGF gene to tumour cells.¹⁷ The p53 gene is one of the most frequently mutated genes found in neoplastic cells, the p53 protein being crucial to the regulation of the cell cycle. Retrovirus-mediated delivery of a wild-type p53 gene has resulted in a decrease of tumour size in patients with non-small cell lung cancer.¹⁸

Few of the gene therapy approaches that have been tested to date have achieved results compatible with a cure and have, therefore, been less than totally acceptable to the average clinician.

Wound healing and fracture repair

In the inflammatory stage of wound healing, growth factors play a major role in activating neutrophils, macrophages, fibroblasts, *etc.* Attempts to improve or accelerate wound healing have involved the transfer of growth factor genes. The human platelet-derived growth factor B gene, introduced by adenoviral¹⁹ or retroviral²⁰ vectors, has been associated with improved wound healing in rabbits. Transfection of fibroblast growth factor has been demonstrated to improve wound healing in diabetic mice.²¹

Bone fractures are also a potential target for gene therapy. Bone morphogenetic protein is a member of the transforming growth factor-beta family and plays an important role in bone formation. One study demonstrated that animals receiving the bone morphogenetic protein-2 gene healed their fractures earlier than untreated controls.²²

Organ transplantation

Approaches utilizing gene transfer have largely been directed towards: (i) the modulation of the grafted organ; or (ii) the induction of immune tolerance or specific unresponsiveness to the organ in the host.²³ None of these approaches, however, has yet progressed into clinical trials.

Modulation of the donor organ has been directed towards the reduction of graft immunogenicity, thus decreasing the host's immune response, or towards increasing the graft's protective mechanisms, e.g. by overexpression of 'protective' or immunosuppressive cytokines within the graft. Gene transfer is carried out *ex vivo* during preservation of the donor tissues or organ. Donor cells (e.g. pancreatic islets), which can be maintained in culture, clearly lend themselves to manipulation by such therapy.

One example relates to transduction with an inducible nitric oxide synthase gene. Nitric oxide is known most notably for its physiological regulation of vasomotor tone

and its ability to inhibit platelet aggregation, as well as other actions. Transduction of experimental rat heart grafts with an inducible nitric oxide synthase gene, using an adenoviral vector, temporarily suppressed the development of allograft arteriosclerosis.

The perfusion of rat kidneys with recombinant adenoviruses carrying the Fas ligand gene (encoding for cell death and, therefore, leading to death of invading host cells) prior to allotransplantation has resulted in significantly prolonged survival of experimental renal grafts after transplantation. The use of an adenovirus vector for the CTLA4-Ig gene, a potent blocker of T-cell activation, has improved mouse cardiac and islet allograft survival.

However, none of the methods and approaches described above has yet permitted complete graft 'protection'.

Gene delivery of sequences encoding donor-type major histocompatibility complex (MHC) class I antigens to recipient (autologous) bone marrow cells has been shown to facilitate the development of tolerance to a subsequent allograft from a donor matched for the MHC sequences introduced by gene therapy. In order to create the same class II molecules in the recipient as those in the donor kidney, *ex vivo* transfer of a single foreign class II gene into recipient bone marrow has been performed by using a retroviral delivery system in swine. Pigs receiving class II gene-transduced autologous bone marrow subsequently accepted a renal allograft, which expressed the same class II, without the need for any immunosuppressive therapy.

Conclusions

Although in its infancy, gene therapy is a promising approach that is likely to make a significant impact on the treatment of many conditions, including some diseases/disorders that are currently treated surgically.²⁴ Today, the major impediments to the successful clinical application of this approach are technical issues, such as low levels of gene expression, inadequate delivery systems, and insufficient targeting of the transgene to the appropriate cell. The death of one patient, however, emphasizes that there are potential risks with this form of therapy, and that development must be carefully controlled.

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