

# Gene therapy in the treatment of disease

## Stacey Efstathiou

Gene therapy offers great potential in treating diseases such as cancer. Stacey Efstathiou explores the current state of knowledge, focusing on the use of viral vectors.

The deduction of the complete nucleotide sequence of the human genome has provided a blueprint of life. Although we are probably many years away from a full characterization of the number, function and regulation of individual genes, it is clear that single gene defects account for over 4000 inherited disorders. Such disorders can lead to severe and often fatal disease early in life or

late onset debilitating disease in adults. Linking single gene defects to particular disease syndromes and the rapid development of recombinant DNA and 'gene knockout' mouse technologies have been the central platform on which the emerging science of gene therapy is based. However, although the basic principle of gene therapy is simple, its successful application to the treatment of a variety of disease states is proving a major challenge.

### ● The basic principle of gene therapy

Following the identification and linkage of a specific gene to a particular disorder it should be possible to correct a gene defect by supplying a fully functional copy of the gene in question either directly *in vivo* or *ex vivo*. This strategy is relatively straightforward in the case of recessive single gene defects since insertion of a functional allele of a defective gene is sufficient to reverse a given phenotype. In the case of dominant gene defects or acquired disorders, such as cancer or infectious disease,

the situation is more complex and it is often necessary to interfere with the expression of an 'abnormal' gene. Whether one is considering gene therapy of an inherited or acquired disorder the critical first step is efficient gene delivery to a particular cell or tissue type. Thereafter the particular attributes of a gene delivery system will vary depending on the disorder in question. For example in the case of cancer therapy it may be sufficient to induce high level expression of a 'suicide gene' to effectively kill a cancerous cell. In this scenario transient high level expression of a delivered gene is required. In contrast, many genetic abnormalities will require long-term, and in some cases regulatable expression of a therapeutic gene product.

### ● Methods of gene transfer

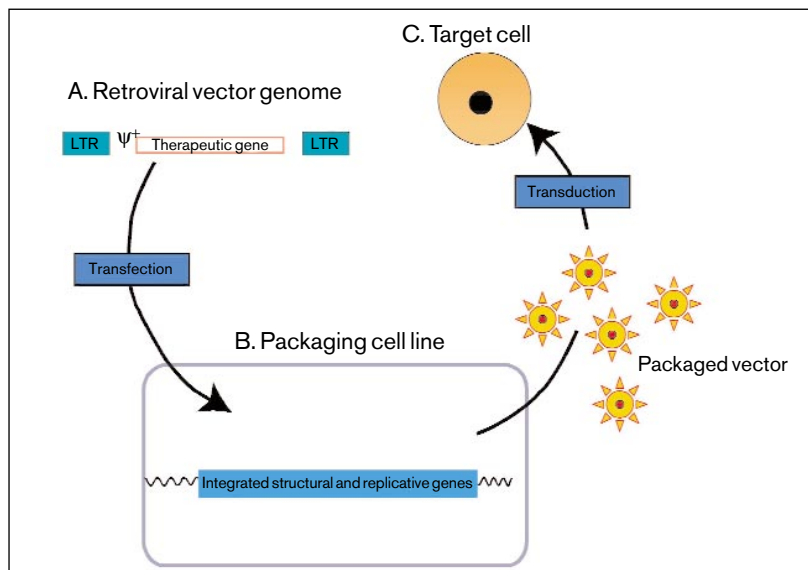
Delivery of nucleic acid to cells is not straightforward and the most efficient and reliable methodologies involve one of a number of replication-defective virus vectors, each with its own particular set of attributes

(Table 1). At the moment viruses have the edge over non-viral, liposome-based or naked DNA delivery systems and advantage is being taken of the fact that viruses have had millions of years to evolve highly efficient mechanisms of cell entry and delivery of nucleic acids to mammalian cells. Of course before a virus can be used with gene therapy in mind it must be made replication-defective, such that it can efficiently transduce a recipient cell with new genetic material in the absence of virus replication

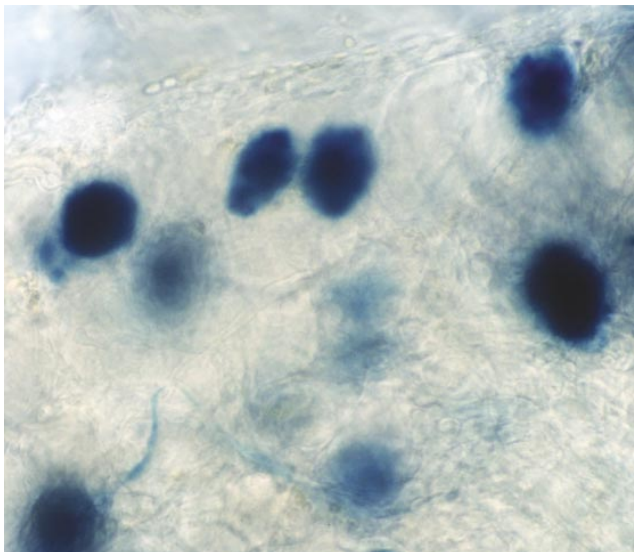
**Table 1. Properties of commonly utilized virus vectors**

Virus	Advantages	Disadvantages
Murine retrovirus	Stably integrates Straightforward to produce	Can only transduce dividing cells Risk of insertional mutagenesis?
Adenovirus	Can transduce many cell types and incorporate large inserts	Immunogenicity Long-term expression difficulties
Adeno-associated virus	Can transduce many cell types Stably integrates	Can only accommodate small inserts Production difficulties
Herpes simplex virus	Can transduce many cell types and incorporate large inserts Stable retention in neurones	Immunogenic Long-term expression difficulties
Lentiviruses	Stably integrates into both dividing and non-dividing cells Efficient transduction of neurones	Difficult to produce Risk of insertional mutagenesis?

RIGHT:  
**Fig. 1.** Basic principle of gene delivery using a retrovirus vector. (A) A therapeutic gene flanked by replication and packaging sequences is transfected into a mammalian cell line. (B) The helper or packaging cell line expresses structural genes and replication enzymes necessary to replicate and package the incoming virus genome. (C) The packaged vector constitutes a fully formed replication-defective virus, which can deliver or transduce the therapeutic gene into a target cell. COURTESY S. EFSTATHIOU



or the expression of potentially toxic virus-encoded gene products. Perhaps the most widely utilized vector systems are based on simple murine retroviruses, which can be readily engineered to lack all virus-gene-encoded functions. These virus genes can be replaced by a therapeutic gene and, so long as the gene of interest is flanked by *cis*-acting packaging/replication sequences, virus particles can be generated in helper cell lines (Fig. 1). These helper cell lines provide virus replication enzymes and structural proteins *in trans* and facilitate the production of high titres of the vector in question. Viruses released from such helper cell lines merely constitute packages of nucleic acid, which are morphologically identical to wild-type virus and therefore retain normal infectivity. However, when delivered to non-complementing target cells, transduction rather than



infection ensues. Thus following entry to the cell, reverse transcription of the virus RNA to DNA is followed by stable integration into the host genome where transcription of the delivered gene can take place. The target cell is now stably transduced with foreign genetic material and there is no possibility of generating wild-type virus. Integration is particularly relevant when considering gene delivery to dividing cells and is a particularly useful feature of all retrovirus and adeno-associated virus vector systems. In contrast, the lack of integration as part of the normal life cycles of herpes simplex and adenoviruses would indicate that vectors based on these viruses are best suited to the delivery of genes to non-dividing cells and strategies of cancer immuno- or cytotoxic therapy.

### ● The status of clinical trials

Clinical trials of therapeutic strategies based on gene therapy currently involve in the region of 3,500 patients worldwide with the majority of trials taking place in the USA and Europe. At present the focus is very heavily

directed towards cancer therapy which accounts for almost 70 % of all patients with the remainder largely enrolled in trials concerned with single gene defects, infection and vascular disease. Since the majority of trials are in their early stages it is difficult to know just how successful they will be, although, with one or two notable exceptions, there have been few reports of undesirable side effects. A useful source of information regarding the current status of gene therapy and ongoing clinical trials can be located on the *J Gene Med* website (<http://www.wiley.co.uk/genetherapy/>).

### ● The challenge ahead

The recent promising results obtained in the correction of the recessive X-linked Severe Combined Immunodeficiency Syndrome (SCID-X1) in humans using retrovirus-based vectors and the increasing success rate in the correction of a variety of both genetic and acquired disorders in animal model systems is a reason to be optimistic. Yet, as has been extensively publicized – things can go wrong. There has been one human death associated with the administration of an adenovirus-based vector and the development of leukaemia in a child treated for SCID-X1 is thought to be linked to insertional mutagenesis of the retroviral vector used in this gene therapy trial. There is clearly much to learn and at present many of the current methodologies for achieving effective gene transfer remain unpredictable. Improvements in the safety profile of viral delivery systems are necessary to reduce vector toxicity and immunogenicity. In the case of those vectors that mediate gene delivery by integration a greater understanding of the risks of insertional mutagenesis are required and methods of targeting the site of integration need to be explored. In addition to fully understanding and overcoming these safety issues, further advances towards the design of delivery systems which can target a given cell type and express a therapeutic gene product in a regulatable manner are necessary. Given the rate of progress in the fields of cellular and molecular biology one cannot help but feel that given time, effort and the necessary level of funding, gene therapy will come of age.

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**Fig. 2.** Herpes simplex virus (HSV)-mediated gene expression in sensory neurones. A recombinant HSV engineered to express the reporter gene  $\beta$ -galactosidase was used to establish a latent infection within sensory neurones. A sensory ganglion stained for  $\beta$ -galactosidase gene expression is shown. The 'transduced' cells, which express the delivered gene, are visualized as blue cells. The ability of HSV to establish life-long latency in neurones has stimulated interest in the development of this virus as a neuronal gene delivery vector.

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### Further reading

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