91e Gene Therapy in Clinical Medicine

Katherine A. High

Gene transfer is a novel area of therapeutics in which the active agent is a nucleic acid sequence rather than a protein or small molecule. Because delivery of naked DNA or RNA to a cell is an inefficient process, most gene transfer is carried out using a vector, or gene delivery vehicle. These vehicles have generally been engineered from viruses by deleting some or all of the viral genome and replacing it with the therapeutic gene of interest under the control of a suitable promoter (Table 91e-1). Gene transfer strategies can thus be described in terms of three essential elements: (1) a vector; (2) a gene to be delivered, sometimes called the transgene; and (3) a physiologically relevant target cell to which the DNA or RNA is delivered. The series of steps in which the vector and donated DNA enter the target cell and express the transgene is referred to as *transduction*. Gene delivery can take place in vivo, in which the vector is directly injected into the patient, or, in the case of hematopoietic and some other target cells, ex vivo, with removal of the target cells from the patient, followed by return of the gene-modified autologous cells to the patient after manipulation in the laboratory. The latter approach effectively combines gene transfer techniques with cellular therapies (Chap. 90e).

Gene transfer is one of the most powerful concepts in modern molecular medicine and has the potential to address a host of diseases for which there are currently no available treatments. Clinical trials of gene therapy have been under way since 1990; a recent landmark in the field was the licensing, in 2012, of the first gene therapy product approved in Europe or the United States (see below). Given that vector-mediated gene therapy is arguably one of the most complex therapeutics yet developed, consisting of both a nucleic acid and a protein component, this time course from first clinical trial to licensed product is noteworthy for being similar to that seen with other novel classes of therapeutics, including monoclonal antibodies or bone marrow transplantation. Over 5000 subjects have been enrolled in gene transfer studies, and serious adverse events have been rare. Some of the initial trials were characterized by an overabundance of optimism and a failure to be appropriately critical of preclinical studies in animals; in addition, it was in some contexts not fully appreciated that animal studies are only a partial guide to safety profiles of products in humans (e.g., insertional mutagenesis). Initial exuberance was driven by many factors, including an intense desire to develop therapies for hitherto untreatable diseases, lack of understanding of risks, and, in some cases, undisclosed financial conflicts of interest. After a teenager died of complications related to vector infusion, the field underwent a retrenchment; continued efforts led to a more nuanced understanding of the risks and benefits of these new therapies and more sophisticated selection of disease targets. Currently, gene therapies are being developed for a wide variety of disease entities (Fig. 91e-1).

GENE TRANSFER FOR GENETIC DISEASE

Gene transfer strategies for genetic disease generally involve gene addition therapy, an approach characterized by transfer of the missing gene to a physiologically relevant target cell. However, other strategies are possible, including supplying a gene that achieves a similar biologic effect through an alternative pathway (e.g., factor VIIa for hemophilia A); supplying an antisense oligonucleotide to splice out a mutant exon if the sequence is not critical to the function of the protein (as has been done with the dystrophin gene in Duchenne's muscular dystrophy); or downregulating a harmful effect through a small interfering RNA (siRNA). Two distinct strategies are used to achieve long-term gene expression: one is to transduce stem cells with an integrating vector, so that all progeny cells will carry the donated gene; and the other is to transduce long-lived cells, such as skeletal muscle or neurons. In the case of long-lived cells, integration into the target cell genome is unnecessary. Instead, because the cells are nondividing, the donated DNA, if stabilized in an episomal form, will give rise to expression for the life of the cell. This approach thus avoids problems related to integration and insertional mutagenesis.

IMMUNODEFICIENCY DISORDERS: PROOF OF PRINCIPLE

Early attempts to effect gene replacement into hematopoietic stem cells (HSCs) were stymied by the relatively low transduction efficiency of retroviral vectors, which require dividing target cells for integration. Because HSCs are normally quiescent, they are a formidable transduction target. However, identification of cytokines that induced cell division without promoting differentiation of stem cells, along with technical improvements in the isolation and transduction of HSCs, led to modest but real gains in transduction efficiency.

The first convincing therapeutic effect from gene transfer occurred with X-linked severe combined immunodeficiency disease (SCID), which results from mutations in the gene (*IL2RG*) encoding the γc subunit of cytokine receptors required for normal development of

TABLE 91e-1 CHARACTERISTICS OF GENE DELIVERY VEHICLES

	Viral Vectors						
Features	**Retroviral**	**Lentiviral**	**Adenoviral**	**AAV**	**Human Foamy Virus**	**HSV-1**	**Alpha Viruses**
Viral genome	RNA	RNA	DNA	DNA	RNA	DNA	RNA
Cell division requirement	Yes	G_1 phase	No	No	No	No	No
Packaging limitation	8 kb	8 kb	8–30 kb	5 kb	8.5 kb	40–150 kb	5 kb
Immune responses to vector	Few	Few	Extensive	Few	Few	Few in recombinant virus	Few
Genome integration	Yes	Yes	Poor	Poor	Yes	No	No
Long-term expression	Yes	Yes	No	Yes	Yes	No	No
Main advantages	Persistent gene transfer in dividing cells	Persistent gene transfer in transduced tissues	Highly effective in transducing various tissues	Elicits few inflammatory responses, nonpathogenic	Persistent gene expression in both dividing and nondividing cells	Large packaging capacity with persistent gene transfer	Limited immune responses against the vector
Main disadvantages	Theoretical risk of insertional mutagenesis (occurred in multiple cases)	Might induce oncogenesis in some cases (not yet observed)	Viral capsid elicits strong immune responses	Limited packaging capacity	In need of a stable packaging system	Residual cytotoxicity with neuron specificity	Transduced gene expression is transient

Abbreviations: AAV, adeno-associated virus; HSV, herpes simplex virus; SV, sarcoma virus.