CHAPTER 13
Delivery Systems for Gene Therapy

INTRODUCTION

Gene therapy is an approach to the treatment of human diseases based upon the transfer of genetic material into somatic cells of an individual. Gene transfer can be achieved directly in vivo by administration of gene-bearing viral or nonviral vectors into blood or tissues, or indirectly ex vivo through the introduction of genetic material into cells manipulated in the laboratory followed by delivery of the gene-containing cells back to the individual. In this regard, gene therapy is a set of approaches to the delivery of recombinant DNA to somatic cells, and as such is a natural progression in the application of recombinant DNA technology to human medicine. Viewed broadly, gene therapy is simply an extension of conventional medical therapies in which genetic material rather than protein is the therapeutic agent. By altering the genetic material within a cell, gene therapy may correct underlying disease pathophysiology. In principle, gene transfer should be applicable to many tissues and disease processes. It offers the potential to cure inherited disorders and/or to be used as an adjuvant to conventional therapies for many diseases for which current therapeutic approaches are ineffective or the prospects for effective therapies are low. However, gene therapy is still in its infancy, and its promise has not yet been fulfilled.

Two critical steps comprise gene therapy: delivery of the gene to appropriate cells, and gene maintenance and expression. Chapter 12 discusses vectors for somatic gene transfer, including naked or complexed DNA, RNA viruses (retroviruses), and DNA viruses (adenovirus, adeno-associated virus, and herpes virus). Each vector system has advantages and disadvantages that influence their selection for delivery to specific tissues. Unfortunately, none of the available vector systems is satisfactory for delivery to all tissues, and hence the specific characteristics of each vector must be considered for each particular clinical application.

Expression of transferred genes is essential to successful gene therapy. Although much may be known about the DNA sequences that direct high-level gene expression in tissue culture cells or transgenic mice, in practice the same principles may not apply to the expression of recombinant genes in larger-animal models of human disease. These difficulties may reflect undefined cellular mechanisms that repress virally introduced genes, a selective disadvantage of cells expressing transferred genes, a lack of appropriate positive regulatory sequences in the constructs, or other unknown factors. These and other issues must be considered when evaluating delivery and expression of transferred genes in specific tissues. Because of the complexities of interactions between vector systems and different tissues, Chapter 13 is devoted to information on gene delivery to specific tissues and organ systems in animals. Characteristics of the organ systems are presented within each unit. General considerations concerning animal models for gene delivery to the organ systems, including advantages and disadvantages of each animal model, are also discussed. Although specific hypotheses can be tested in animal models, it is important to recognize that phenotypic differences are likely to exist between animal models of disease and human patients and that the principles of disease pathogenesis may vary between species. Nonetheless, animal models provide an important link in the

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development of gene therapy approaches, especially for the elucidation of disease pathophysiology and for the design of therapeutic approaches in preclinical settings.

UNIT 13.1 describes approaches for gene delivery to normal and diseased blood vessels: catheter-mediated gene delivery is discussed in several animal models of human vascular disease. The use of naked DNA for in vivo vaccination, an area in which rapid advances have been made, is presented in *UNIT 13.2*. In this approach standard molecular biology techniques are used to construct and purify the vaccine plasmid; the DNA is then injected intramolecularly and elicits immunities similar to those elicited by infection with live microorganisms.

Protocols for ex vivo and in vivo gene delivery to the brain are described in *UNIT 13.3.* These include specialized methods to address the complexity of the central nervous system tissue and the anatomical location of different regions of the brain. Gene delivery to skeletal muscle has proven to be an important vehicle for vaccination and generation of recombinant proteins which are secreted in the circulation for treatment of systemic disorders; these protocols are discussed in *UNIT 13.4*.

Also included are several approaches to the genetic treatment of cancer. In *UNIT 13.5*, the in vivo delivery of genes encoding viral or bacterial enzymes capable of converting nontoxic prodrugs to active molecules is presented. An example is the thymidine kinase gene from herpes simplex virus, which renders cells susceptible to the drug ganciclovir. The area of gene therapy for AIDS is discussed in *UNIT 13.6*. Human immunodeficiency virus (HIV) has received much attention as an infectious disease potentially amenable to gene therapy; approaches to expressing genes that render target cells unable to support HIV replication or be infected are presented. Another cancer gene therapy technique, discussed in *UNIT 13.7*, is the transfer of genes for cytokines or other immunomodulatory products to cancer cells in vivo or ex vivo in an attempt to stimulate immune recognition of gene-modified and other cancer cells. Hematopoietic stem cells are an important vehicle for gene delivery to treat malignancies, systemic disorders, and possibly AIDS. Techniques for gene delivery and expression in hematopoietic stem cells are provided in *UNIT 13.8*.

Gene delivery to the airway is described in *UNIT 13.9*, which presents the preparation and transduction of commonly used airway models including primary and polarized airway epithelial cells, human bronchial xenografts, and rodent lung.

Protocols for optimized in vivo hepatic gene transfer are described in *UNIT 13.10*. Hepatic gene transfer has proven extremely effective in animal models and is currently being evaluated in clinical trials for a variety of metabolic disorders. In rodents, a single tail vein injection of an adenoviral vector can transduce most hepatocytes in vivo. This provides a convenient model for assessing vector design as well as for evaluating the effects of specific transgenes in genetic mouse models of human disease.

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