Significant advances have been made over the past two decades in our understanding of the basic biology of the nervous system and the molecular basis of neurologic disease. Identification of the complex regulatory mechanisms involved in the maintenance of normal cellular function and the roles assumed by different genes in the pathogenesis of specific diseases have paved the way for a new therapeutic approach based on the alteration of cellular phenotype by genotype manipulation. This new modality, designated gene therapy, has raised high expectations as a potential solution for a large spectrum of currently untreatable conditions. Unfortunately, the rapid transfer from in vitro studies to clinical trials has so far yielded only anecdotal reports of success.

In this chapter, the current status of gene therapy as a regenerative tool for neurologic disease will be reviewed. The principles that underlie the methodology, the main gene delivery vehicle types (vectors), and the different therapeutic approaches adopted for the nervous system will be outlined. This will be followed by a review of several examples of gene therapy use for neurologic diseases. We conclude this chapter by a detailed analysis of those obstacles, technical and conceptual, that still hinder the translation of in vitro efficacy to clinical cure.

Gene Therapy to the Nervous System
Hillel Haim and Israel Steiner

Gene Therapy, a Novel Therapeutic Approach

Gene therapy is defined as the introduction of specific nucleic acid sequences into selected target cells for the treatment or prevention of disease. The method was originally contemplated as a potential solution for the wide array of monogenic inherited disorders, such as cystic fibrosis and Duchenne muscular dystrophy, for which conventional pharmacotherapy is unable to provide any adequate response. Restoration of the function of a defective or missing gene product by the introduction of a correct copy of the gene seemed like a simple and feasible concept. It was soon to be appreciated that the approach should not be restricted to single-gene defect replacement therapy. Indeed, alteration of the cellular transcriptional status and in vivo production of a therapeutic protein may be implemented to achieve phenotypic changes in a wide spectrum of disorders. The list of potential applications thus expanded to encompass most neurologic disease states, acute and chronic, inherited and acquired, infectious and neoplastic (see Table 9.1).

Transition into clinical testing was then only a matter of time. Since the initiation of the first gene therapy clinical trial in 1989, nearly 1000 such trials have been approved worldwide. This time period has seen drastic changes in the types of diseases addressed. Most formidable is the slow drift from monogenic inherited disorders to neoplastic disease (in 2003, more than 60% of all gene therapy clinical trials were aimed at neoplastic disease). Two reasons underlie this change. First, the more immediately life-threatening nature of many of these conditions and the lack of efficient alternative therapies have facilitated their transition into clinical testing. Second and perhaps more fundamental, is the current limited ability to achieve efficient gene
expression in the desired cell population in vivo, to reverse disease phenotypes without causing unwanted side effects.

Thus, while experimentation in the field of gene replacement therapy goes on, the “fast lane” to the clinic has been taken up by cancer gene therapy trials. Accordingly, the number of tumor-destructive approaches increased, to include more cytotoxic genes and replication competent oncolytic viruses.13–15 Although this class of diseases lies outside the scope of this review, the principles of vector formation and the limitations imposed on the success of in vivo gene transfer similarly apply.

**Table 9.1. Gene therapy for neurodegenerative disorders: therapeutic approaches**

<table>
<thead>
<tr>
<th>Strategy</th>
<th>Aim of therapy</th>
<th>Targeted pathology</th>
<th>Example disease</th>
<th>Therapeutic gene</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction of phenotypic changes restricted to the target cell</td>
<td>Replacement of a missing gene function in monogenic disorders</td>
<td>Enzyme deficiency</td>
<td>MPS VII, Canavan’s disease</td>
<td>β-Glucuronidase, Aspartoacylase</td>
<td>1, 2, 3</td>
</tr>
<tr>
<td>Production of a secreted therapeutic protein to achieve a localized or systemic effect</td>
<td>Alteration of cellular phenotype in non-monogenic disorders</td>
<td>Enzyme deficiency</td>
<td>Parkinson’s disease</td>
<td>Tyrosine hydroxylase</td>
<td>4–6</td>
</tr>
<tr>
<td></td>
<td>Decreased neuronal viability</td>
<td></td>
<td>Parkinson’s, Alzheimer’s, Huntington, ALS, ischemia, traumatic injury</td>
<td>GDNF, NGF,CNTF</td>
<td>7–9</td>
</tr>
<tr>
<td></td>
<td>Neuronal hyperexcitability</td>
<td>Seizures</td>
<td>GAD</td>
<td>Potassium channels</td>
<td>10, 11</td>
</tr>
</tbody>
</table>

ALS, amyotrophic lateral sclerosis; CNTF, ciliary neurotrophic factor; GAD, glutamic acid decarboxylase; GDNF, glial cell line-derived neurotrophic factor; MPS, mucopolysaccharidosis; NGF, nerve growth factor.

**Methods of Gene Delivery**

Depending on the application, two modes of delivery may be used to transfer a chosen gene to the target tissue, the ex vivo and in vivo approaches. The ex vivo approach is based on the isolation and in vitro culture of selected cells, where they are manipulated and subsequently returned to the host. Although mainly applied for gene therapy of the immune system where isolation of a specific group of cells is possible,16 the approach may also be used to generate “mini-factories” that produce and locally secrete desired proteins. The genetically modified cells may thus serve as delivery platforms of neurotrophic factors or neurotransmitter-forming enzymes into the central nervous system (CNS).17,18

In contrast, the in vivo approach involves the direct administration of the gene-carrying vector to the host. Delivery may be via simple localized application (such as stereotactic injection into the brain parenchyma), or by the vascular (systemic) route. The main drawback of this approach, and probably the most significant problem currently encountered in gene therapy to the nervous system, is the difficulty in targeting the vector to the selected cell type. To date, this remains the most prominently unsurmounted obstacle.

**The Gene-Carrying Vectors**

The efficient delivery of the gene to its nuclear target, where it may be expressed, serves as the most basic requirement in gene therapy. Two general types of vectors are used to package and deliver genes: Virus-based and synthetic gene delivery systems. In synthetic systems, the transgene forms part of a plasmid, which is replicated and subsequently purified from bacteria. The only genetic material available for transcription in the target cells is therefore the transgene itself. Plasmid DNA may be delivered