APPROACHES TO GENE THERAPY IN THE CNS: INTRACEREBRAL GRAFTING OF FIBROBLASTS GENETICALLY MODIFIED TO SECRETE NERVE GROWTH FACTOR

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INTRODUCTION

Considerable effort in recent years has been applied towards the development of methods for the genetic modification of mammalian cells to correct disease phenotypes in vivo, an approach that has been named gene therapy (1). In an ideal gene therapy system, the new genetic information would be applied directly to the affected tissue. This direct approach has not yet been attempted, because the current methods of gene transfer are limited to replicating cells. Because of this and other technical limitations, approaches to gene therapy in animal models of human disease have relied on removing mitotic cells from the target tissue, genetically modifying them in culture, and then returning the cells to the animal. Most of these studies have used genetic transducing vectors derived from murine retroviruses to introduce foreign genes (transgenes) into target cells, because retrovirus vectors offer several advantages over other current methods of gene transfer (2): 1) infection by retrovirus vectors is extremely efficient for a broad range of cell types and species, with up to 100% of the target cells expressing the transgene; 2) the viral genomes have a relatively large capacity for foreign DNA; and 3) infection generally causes little or no genetic or metabolic damage to recipient cells. Other methods of gene transfer, which utilize biochemical or physical means to introduce transgenes into cells, suffer from serious limitations in comparison. The first methods developed involve incubating cells with DNA complexed with DEAE-dextran (3) or calcium phosphate (4). More recent methods use direct microinjection (5), electric fields (electroporation) (6), liposomes (lipofection) (7), and tungsten microprojectiles (8).

Because of their accessibility and the presence of suitable replicating cell populations, the bone marrow (9-11) and skin (12-15) have been studied most extensively for gene therapy applications, and more recently, the liver has been actively investigated (16,17). Because of its relative inaccessibility and the lack of neuronal stem cell populations in adults, another potentially very important target organ, the brain, has not been pursued in gene therapy models. This is not meant to imply that neurons will always be the target cells for CNS gene therapy. There are certainly cases in which other cell types must be treated, such as oligodendrocytes in the demyelinating diseases. Neurons, however, will certainly be the target cells in the vast majority of disorders, and methods developed to treat neurons should be applicable to other cell types. The remainder of this chapter, therefore, will concentrate on neuronal therapy.
Several approaches, both genetic and non-genetic, are theoretically available to restore missing functions to neurons (Fig. 1). The most direct approach would entail introducing the transgene directly into neurons using an appropriate vector (Fig. 1A). As discussed above, there are presently no available methods for gene transfer into post-mitotic cells. Suitable vectors may soon be developed, however. Herpes simplex virus (HSV) normally infects post-mitotic sensory neurons, in which it is capable of establishing a life-long latent, non-destructive infection. Several groups are developing vectors based on HSV (18,19), and such vectors may prove valuable for introducing transgenes into neurons and other post-mitotic cells, both in vitro and in vivo.

If effective treatment of a specific disease requires that the transgene be expressed in the target cell itself, direct vector application will be the only possible approach. In many disorders, however, it will not be necessary to treat neurons directly but instead it will be possible to allow the target cells to take up exogenously applied factors. For example, minipumps (20-23) and implanted solid polymers (24) have been investigated for delivery of substances to the CNS (Fig. 1B). Another approach that has received considerable attention is the grafting of donor cells or tissues that produce the needed substance, which can then enter the target cells by direct transfer through tight junctions or diffusion across the membrane (Fig. 1C) or by secretion and re-uptake via specific receptors or transport systems (Fig. 1D). This method has been widely applied to provide dopamine in Parkinson's disease patients and animal models, using both neural and non-neural tissue, i.e., fetal substantia nigra and adult adrenal gland (25-27). For this approach to be useful in treating a particular CNS disorder, several criteria must be met. A suitable donor tissue must be available that produces sufficient quantities of the desired factor, and this tissue must survive and continue to function when grafted to the brain. Ideally, the donor tissue should come from the patient himself, as the use of autologous grafts will avoid problems of histocompatibility. However, for most disorders there will not be a suitable non-neural tissue, eliminating the possibility of autografts. The use of fetal tissue poses serious ethical problems in addition to immunological problems.

As an alternative approach, we have been developing a model in which cells are genetically modified in culture to express the required function and then grafted to the brain (Fig. 1E) (28,29). This approach combines neural grafting with methods previously used for gene therapy models in bone marrow and skin. An advantage to this approach is donor cells need not be CNS-derived, nor do they have to express the desired function naturally. Instead,