Gene Therapy

The brain and spinal cord are subject to diverse pathologies that hitherto have been difficult to treat because of an incomplete understanding of the aberrant cellular processes as well as a lack of suitable tools to change the natural course of the diseases. Repairing damaged or genetically deficient cells in the brain and spinal cord is an important goal, given the amount of disability and death caused by CNS disease, but remains problematic because of the complexity, heterogeneity, and relative inaccessibility of these tissues. Furthermore, neurons often do not respond favorably after injury, and once lost, they cannot regenerate on their own.

Many neurological conditions, both inherited and acquired, have been correlated with specific etiological genes, opening the door to rational clinical intervention. Neurodegeneration, stroke, autoimmune disorders, cancer, infection, and even psychiatric or behavioral disorders are being approached, using the methods of gene therapy to replace a defective gene, overexpress a beneficial gene, inactivate a misfunctioning gene, or selectively activate a drug. Currently there are almost 400 FDA-approved gene therapy clinical protocols, and a growing number of these are for disorders involving the brain and spinal cord.

An important advance in CNS gene therapy was the discovery that naturally occurring neurotropic viruses can be gutted of viral genes and modified to deliver human DNA, using an in vitro system to package human genes inside a viral capsid. Current production methods allow for high titers of ultrapure recombinant vectors that are completely free of helper virus.

Different types of viruses differ in their neurotropism, which can be modified by adding or deleting viral surface proteins and using targeted regulatory elements, or promoters, to drive expression. For instance, adeno-associated virus is taken up preferentially by neurons, and its natural tropism can be accentuated by using a strong neural promoter such as that for neuron-specific enolase. Alternatively, glial tropism can be introduced by using a promoter such as those for glial fibrillary acidic protein or myelin basic protein.

There is a basic dichotomy between in vivo and ex vivo gene transfer approaches, both of which have been tried successfully in the CNS, mainly in animal models of human disease. In vivo gene transfer employs recombinant DNA vectors, either viral or plasmid-based, to transduce somatic cells with a therapeutic gene. Other in vivo techniques involve antisense and ribozyme approaches to modify or regulate the organism's own DNA or RNA.

Ex vivo gene therapy uses cells engineered to contain a therapeutic gene, which are introduced as a graft at the site of interest in the CNS. Many types of cells have been used as transgenic vehicles, including bone marrow-derived and brain-derived progenitor cells as well as cells of a more differentiated lineage such as fibroblasts and oligodendrocytes.

Nonviral delivery techniques have also been widely studied but in general afford less efficient long-term expression than other methods. Thus, the armamentarium for gene therapy is already substantial, and recent improvements in vector design and delivery techniques for both in vivo and ex vivo approaches will be crucial in bringing CNS gene therapy from the laboratory to the clinic.

Gene therapy in the central nervous system poses unique technical problems, notably the choice of the gene vector and the route of administration. Because mature neurons are postmitotic, in vivo delivery is restricted to those neurotropic viral vectors that can transduce and express their foreign recombinant DNA in nondividing cells. Adeno-associated virus, lentivirus, and herpes simplex virus are examples. This limitation does not hold for ex vivo gene therapies, which often employ retroviruses such as the Moloney murine leukemia virus as vehicles for engineering graft cells.

The brain and spinal cord are surrounded by multiple physical barriers, so that delivery of a therapeutic gene requires neurosurgery to place the vector or engineered cells directly into the brain using a sterotactic frame and injection cannula. Intraparenchymal and intraventricular delivery are both used for delivery to the brain, and intrathecal delivery has been used in the spinal cord.

Global brain delivery with sustained high levels of transgene expression is necessary for most nonfocal CNS diseases but remains an elusive goal because of limited tissue penetration and far-field axonal transport. Intravascular delivery is in theory the best method because of the large surface area of brain capillaries, but passage into the brain is ordinarily blocked by the blood-brain barrier, a layer of closely apposed capillary endothelial cells joined by tight junctions. Although methods have

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been devised to circumvent this barrier, including hyperosmolar shock and receptor-mediated transcytosis, passing adequate amounts of gene vector across the brain vasculature remains a formidable problem. Other, less invasive delivery routes have been studied, including intranasal and intraocular delivery.

Our laboratory concentrates on translational research on neurodegeneration, using in vivo adeno-associated virus transduction of brain cells as a primary method. One advantage of adeno-associated virus is that unlike most viruses it is naturally nonpathogenic and does not elicit a toxic response. However, it can evoke an immune response, mainly mediated by B lymphocytes against the viral capsid, that can eventually down-regulate the transgene in peripheral organs, especially after repeated delivery. First-generation AAV vectors, which used viral promoters, also tended to be down-regulated as a result of transcriptional silencing by CpG methylation or local cytokine release, but this problem is partly avoided by the choice of strong mammalian promoters.

On the bright side, while robust immune responses can arise in the CNS from T cells and microglia, the CNS is less subject to routine immune surveillance than sites in the periphery, which means that immune responses to vector delivery, especially the B lymphocyte response, tend to be attenuated in the CNS.

Many neurodegenerative diseases are candidates for gene therapy. In particular, the leukodystrophies and lysosomal storage diseases are appealing model systems to test CNS gene therapy necause of their well-defined single-gene defects and severe neurological features. We currently have a phase I clinical trial under way of in vivo aspartylacylase replacement for Canavan's disease, and others are working on Tay-Sachs disease (α-hexose-aminidase deficiency), Krabbe's disease (galacto-cerebrosidase), Gaucher's disease (glucocerebrosidase), Sly syndrome (β-glucuronidase), and Fabry's disease (α-galactosidase).

Other diseases currently being studied in our laboratory are Parkinson's disease and amyotrophic lateral sclerosis. The motor symptoms characteristic of Parkinson's disease result from loss of dopaminergic cells and gradual degeneration of the substantia nigra pars compacta. Other projecting neuronal populations containing glutamate, GABA, and neuropeptides are also involved.

Five years ago, we showed that tyrosine hydrolase, the rate-limiting enzyme in dopamine synthesis, could be neurosurgically delivered to the striatum of parkinsonian rats and primates using a herpes virus vector, resulting in long-term functional recovery. Since that time, other relevant genes have been studied, including the vesicular monoamine transporter and the transcription factor Nurr-1, and it appears that a multi-gene approach to Parkinson's disease may be beneficial for human clinical trials. In addition, new neurosurgical treatments for Parkinson's disease using focal ablation and high-frequency stimulation of deep brain nuclei have defined other anatomical regions of the basal ganglia that may be targeted through gene therapy.

In the case of ALS, a number of candidate genes have been defined. Linkage studies originally suggested an autosomal dominant inheritance pattern with superoxide dismutase as the candidate gene, but subsequent studies showed that 95% of cases are sporadic and that SOD is involved in 25% of familial cases at most, or about 1% of all cases.

Aberrant RNA processing of the high-affinity glutamate uptake transporter in glial cells, called GLT-1 or EAAT-2, has now been implicated as the most common cause of the disease, and studies are currently underway to test adeno-associated viral vectors carrying GLT-1 in a mouse knockout model, leading up to human clinical trials in the coming year. Other approaches have been tried in animal models, mainly involving delivery of neurotrophic factors, but a phase III human trial using intrathecal brain-derived neurotrophic factor recently reported disappointing results. It is hoped that this targeted approach to blocking glutamate excitotoxicity with a transporter delivered by recombinant AAV will be of some benefit to ALS patients.

Our hope is that advances in gene transfer technology will be readily applicable to many other neurological diseases in the future, even those with a multi-gene basis, as more is learned about the relevant biology. For instance, Alzheimer's disease, which is still poorly understood although at least five disease-related genes have been identified, imposes terrible human and economic costs: thousands of lives and billions of dollars are lost every year to this disease.

Gene therapy to arrest the course of CNS diseases such as Alzheimer's or Parkinson's disease will translate into better quality of life for millions of affected people and their families. In the future, many more refinements are possible in terms of eliciting high-level expression and control of transgene vectors, perhaps using DNA elements such as inducible activator-repressors and enhancer-response sequences, or co-expressed genes for regulatory factors, such as cytokines and transcription factors. Such approaches have the potential to cure even complex multi-gene disorders of the CNS.

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