NEUROLOGY Discovery

Gene Therapy for Diseases of the Nervous System

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Advances in molecular biology and recombinant DNA technologies have contributed to our understanding of the molecular basis of many diseases. Now the possibility of gene transfer into normal cells to produce a gene product of therapeutic potential, or into diseased cells to correct the pathologic alteration, promises to revolutionize medical practice. In contemporary medicine, many therapeutic strategies focus on the link between a biochemical deficiency and the ensuing disorder. The treatment of noninfectious disease is often based on replacement therapy; medication is given to compensate for biochemical defects and to prevent or reverse the progression of disease. Although conventional therapies seldom alter the fundamental cause of a disease, gene therapy potentially could correct, at a molecular level, the genetic abnormalities contributing to its pathogenesis. Treatment directed at specific molecular alterations associated with the development of neurologic disease provides expectations of more effective and less toxic therapy. The development of gene therapy for nervous system tumors has progressed rapidly and may be prototypical in the development of therapies for inherited and acquired disorders of the nervous system. We describe possible strategies for using gene therapy to treat nervous system disorders, and we review recent advances in gene therapy for nervous system tumors.

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Three decades ago, scientists developed techniques that enabled them to manipulate the genome of bacteria. Soon afterwards, advances permitted researchers to clone and then transfer genes into animal cells. It was during that time that progress in research laboratories fueled speculation that gene transfer might one day be used to treat disease in human patients. Gene transfer techniques have now established a foothold in modern clinical medicine,[1-8] and investigational gene therapy trials are under way for a variety of human diseases.[9-17]

Disease in the nervous system can have dire consequences for a stricken patient; those afflicted can be left immobile without sight, speech, or memory. This devastation occurs over the spectrum of neurologic disease, from the degenerative to the neoplastic forms. Distressingly, these particular diseases have defied modern therapeutics. For all of those reasons, gene therapy for disease of the nervous system has drawn the attention of researchers and clinicians alike.

Gene Therapy for Inherited Disease

The ideal design of a gene therapy strategy would first take into account the molecular basis of a disease process and then effectively tailor gene transfer techniques to mitigate toxicities and improve the effectiveness of existing therapies. A successful example of this strategy is provided by the efforts of investigators at the National Institutes of Health who developed a somatic cell gene therapy approach for the treatment of the severe combined immunodeficiency syndrome resulting from adenosine deaminase (ADA) deficiency.[4,10-13,17] Patients afflicted with ADA deficiency can be cured by matched bone marrow transplants providing immune cells with normal ADA levels. On that basis, it was proposed that the transfer of the human ADA gene into T lymphocytes from ADA-deficient patients might have the same effect.[4] An investigational approach was then designed in which mononuclear cells were isolated from the blood of patients with ADA deficiency, stimulated to proliferate in tissue culture, infected with a retroviral vector carrying the human ADA gene, and then returned to the patient.[10,12] Current reports of these patients' status indicate that this treatment approach may be efficacious.[17]

Key to the success of that strategy was the identification of a disease that resulted from a mutation in a single gene encoding an enzyme vital to the function of a recog-

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nizable class of cells. The isolation of T lymphocytes from a patient, the growth of those cells in tissue culture where conditions favoring efficient gene transfer could be maintained, and the return of cells expressing the desired gene back to the patient where they would resume their normal functions were all required for this strategy to succeed. Unfortunately, the use of this approach as a model of gene therapy for nervous system disease is, for many reasons, a formidable task.

Among currently recognized neurologic disorders, variations in the age of onset, the severity of disease at presentation, and the time course of disease progression suggest that the causes of disease may be multiple. It is also possible that several genetic alterations may contribute to a specific disease, and the effects of those changes may be independent of each other. Before effective gene therapy strategies can be designed to treat these diseases, the genes contributing to their development and the metabolic pathways mediated by the products of those genes will have to be better understood.

The challenge in the study of Huntington's disease is a case in point. Last year the Huntington's Disease Collaborative Research Group reported the cloning of a new gene, named *IT15*, from patients with this neurodegenerative disorder.[18] An expanded, unstable CAG trinucleotide repeat sequence was found in the 5′ coding region of the gene, and the length of the repeated sequence was found to correlate with the age of disease onset. Research is now focused on establishing the relationship between this trinucleotide repeat expansion, the function of the *IT15* gene, and the characteristic degeneration of neurons found only in the caudate and putamen in patients with Huntington's disease.[18] Treatment of the disease may have to address why neuronal degeneration occurs only in a particular location in the brain despite widespread expression of the protein encoded by the *IT15* gene. Although the neurons in the basal ganglia would be a logical target for gene therapy, gene transfer techniques cannot yet selectively target a class of neurons in a defined location; as specific cells in the nervous system have discrete functions, nonselective gene transfer could result in a certain class of cells gaining an inappropriate new characteristic that has adverse effects on their normal function.

Investigators pursuing approaches focused on developing new vectors for gene transfer to cells of the nervous system have turned to adenoviruses and neurotropic viruses, such as the herpes simplex virus.[19-23] In each case, it has been possible to show that foreign genes can be delivered to a variety of cell types in rodent brains, but advances in gene delivery techniques and knowledge about the mechanisms regulating the expression of transgenes in host tissue will be required before selected populations of cells in the nervous system can be targeted for gene therapy.

Somatic Cell Gene Therapy for Neurodegenerative Disease

Creative somatic cell therapies have been developed to circumvent the limitations of gene transfer into nervous system cells. These strategies have been used in experimental models, and they show promise for the management of some neurodegenerative diseases. Fibroblasts genetically modified to secrete nerve growth factor have been shown to prevent the death of cholinergic neurons when implanted into the brain after a surgical lesion was placed in the fimbria-fornix.[24] A pilot case in which nerve growth factor was infused into the lateral ventricle in a patient with Alzheimer's disease showed results that included increased cortical blood flow, normalization of electroencephalographic abnormalities, and improvement in some psychological tests.[25] These findings suggest that somatic cell approaches to gene therapy that lead to increased delivery of nerve growth factor may play an important role in therapeutic strategies designed to limit the loss of basal forebrain cholinergic neurons in patients with Alzheimer's disease.[26] A similar approach has been shown to be effective in reducing abnormal neurologic activity resulting from the destruction of nigrostriatal pathways in rodent models of Parkinson's disease after transplantation of cells modified to produce levodopa.[27]

Although these therapies do not address the mechanisms that initiate neurodegenerative disorders, they provide experimental evidence that the delivery of trophic factors and substrates by genetically modified somatic cells can alter disease progression and open new avenues for further clinical investigation. A gene therapy strategy modeled after the ADA protocol could play a role in delivering neuronal growth factors to the nervous system of patients with neurodegenerative disease. Cells from various tissues could be removed from a patient, be grown in tissue culture where they could be stimulated to replicate, be genetically modified with retroviral vectors carrying a gene of therapeutic importance, and then be implanted into a brain with the intent to increase the local delivery of biologically active molecules.[28,29]

Gene Therapy for Brain Tumors

The challenges that must be addressed in the design of gene therapy protocols for nervous system disease include the lack of knowledge about the genetic changes that lead to disease, the complexity of the structure and function of the nervous system, the limitations of effective techniques to deliver genes to the nervous system, and the possible toxicities of available vectors. For several reasons, these limitations have had less effect on the development of gene therapy strategies to treat tumors of the nervous system. First, most brain tumors are localized masses of proliferating cells surrounded by normal, postmitotic brain tissue; therefore, retroviral vector-mediated gene transfer can be selective for replicating tumor cells and can spare normal brain. Second, insertional mutagenesis is less of a concern when neoplastic cells are the target for gene transfer. A gene therapy strategy known as viral-directed enzyme prodrug therapy has used retroviral vectors to eliminate some types of experimental brain tumors in rodent models.[30-36] The basis of this approach is straightforward: a gene encoding an enzyme that converts an inactive prodrug into a therapeutically active metabo-

lite is transferred selectively into tumor cells. This enzyme can then mediate a cytotoxic effect in the infected cell. A key feature of this therapy is that retroviral vector-mediated gene transfer requires dividing cells,[37-39] and in the nervous system the most actively dividing cells are tumor cells.

To date, the most extensively studied strategy of viral-directed enzyme prodrug therapy for brain tumors has used a recombinant retrovirus vector into which the gene encoding the enzyme thymidine kinase from the herpes simplex virus *(HSVtk)* has been inserted. Following retroviral vector infection of a tumor cell, the *HSVtk* gene is incorporated into the host cell genome. The thymidine kinase of mammalian cells does not have a high affinity for certain nucleoside analogues, such as the antiviral drug ganciclovir, that the *HSVtk* enzyme will avidly phosphorylate. These phosphorylated analogues can be incorporated into the cellular genome during DNA replication, leading to tumor cell death. Because ganciclovir is not a substrate for the mammalian thymidine kinase, this drug does not harm normal cells when given in therapeutic doses.

The efficiency of retroviral-mediated gene transfer into tumor cells following the direct inoculation of retroviral vectors into intracerebral tumors in rodents is rarely greater than 1%. When xenografted tumors are injected with a murine fibroblast cell line producing recombinant retroviral vectors containing a marker gene, the percentage of tumor cells expressing the marker gene can be increased to 10%.[40] Although this suggests that only 10% of a tumor could be killed with ganciclovir therapy, it has been shown that, in some tumor models, cell death after infection with the *HSVtk*-containing retrovirus and treatment with ganciclovir far exceeds the number of cells that express the *HSVtk* gene. It has been shown that tumors in rodents could be eliminated after treatment with ganciclovir when as few as 10% of the cells expressed the *HSVtk* gene.[31] This is referred to as the "bystander effect," but the mechanism of this phenomenon is not known.[41-43] Evidence for the bystander effect has also been observed by several groups using the *HSVtk* and ganciclovir gene therapy strategy to treat a variety of tumor types in animal models.[44-48] To date, no evidence of toxicity to the normal brain in animals treated with this gene therapy strategy has been reported, and this suggests that the toxicity from the bystander effect is tumor-specific. Also, toxicity to organs outside the nervous system has not been detected, and no retroviral vector-induced secondary malignant neoplasms have occurred.[35]

When it is successful in animal models, the viral-directed enzyme prodrug therapy approach for treating experimental brain tumors appears to offer several possible advantages over the conventional therapies for brain tumors in human patients. The median survival of a patient with the most malignant type of glioma, glioblastoma multiforme, has remained at about a year over the past two decades, despite the development of treatment strategies that combine surgical therapy, irradiation, and chemotherapy.[49] For a patient with a newly diagnosed malignant glioma, the long-term prognosis is bleak because the natural history of this disease is one of unrelenting tumor growth with invasion of the surrounding brain that can be accompanied by symptoms of increased intracranial pressure and loss of neurologic function. Treatment is hampered by the invasive nature of this tumor, which makes it difficult to achieve a complete surgical resection and limits the radiation dose that can be used to treat residual tumor in infiltrated but normally functioning brain. The effectiveness of chemotherapy for patients who have brain tumors can be reduced by acquired drug resistance during therapy and the associated bone marrow suppression and organ toxicity. Also, reductions in the dose and frequency of drug administration may be required because of the age or medical condition of the patient. In contrast, retroviral-mediated gene transfer is selective for tumor cells in animal models, and there is no systemic toxicity associated with the administration of the prodrug ganciclovir. Although the bystander effect results in the death of a greater number of tumor cells than would be predicted by the number of cells that express the *HSVtk* gene, this toxicity appears to remain tumor-specific. Whether the success of this approach can be duplicated in human patients remains an open question.

Summary

In this review we have focused on the challenges of designing gene therapy protocols for the treatment of neurologic disease. An important goal of gene therapy will be to correct the phenotype of diseased tissue by altering the mutated genotype. Initially most gene therapy protocols may be designed to augment conventional therapies, as gene therapy could be most useful in conjunction with other therapies of proven efficacy. This will certainly be the case for the treatment of cancer of the nervous system as multimodality approaches predominate in current practice. Some gene therapy approaches may prove to be superior to standard therapy, whereas others may provide insights into mechanisms that could be exploited in the design of new therapies. The viral-directed enzyme prodrug gene therapy strategy has been shown to eliminate experimental brain tumors in animals. Although the mechanisms responsible for tumor cell death are not completely understood, this toxicity appears to be restricted to the tumor without effect on normal brain tissue. That capability has previously eluded researchers and suggests that knowledge regarding the molecular mechanisms that mediate both the pathogenesis of nervous system disease and the available therapeutic tools could provide key opportunities to devise more effective and less toxic therapies.

REFERENCES

1. Friedmann T: Progress toward human gene therapy. Science 1989; 244: 1275-1281

2. Sikora K: Gene therapy for cancer. Eur J Cancer 1991; 27:1069-1070

3. Miller AD: Human gene therapy comes of age. Nature 1992; 357:455-460

4. Anderson WF: Human gene therapy. Science 1992; 256:808-813

5. Thompson L: At age 2, gene therapy enters a growth phase. Science 1992; 258:744-746

6. Mulligan RC: The basic science of gene therapy. Science 1993; 260:926-932

7. Karp JE, Broder S: New directions in molecular medicine. Cancer Res 1994; 54:653-665

8. Fox JL: NIHRAC okays nine gene-therapy proposals. Biotechnology 1994; 12:24-25

9. Rosenberg SA, Aebersold P, Cornetta K, et al: Gene transfer into humans—Immunotherapy of patients with advanced melanoma, using tumor-infiltrating lymphocytes modified by retroviral gene transduction. N Engl J Med 1990; 323:570-578

10. The ADA human gene therapy clinical protocol. Hum Gene Ther 1990; 1:327-362

11. Culver K, Cornetta K, Morgan R, et al: Lymphocytes as cellular vehicles for gene therapy in mouse and man. Proc Natl Acad Sci USA 1991; 88:3155-3159

12. Blaese RM, Culver KW, Chang L, et al: Treatment of severe combined immunodeficiency disease (SCID) due to adenosine deaminase deficiency with $CD34^+$ selected autologous peripheral blood cells transduced with a human ADA gene—Amendment to clinical research project, Project 90-C-195, January 10, 1992. Hum Gene Ther 1993; 4:521-527

13. Blaese RM, Culver KW: Gene therapy for primary immunodeficiency disease. Immunodefic Rev 1992; 3:329-349

14. Brenner MK, Rill DR, Moen RC, et al: Gene-marking to trace origin of relapse after autologous bone-marrow transplantation. Lancet 1993; 341:85-86

15. Rosenberg SA, Anderson WF, Blaese RM, et al: The development of gene therapy for the treatment of cancer. Ann Surg 1993; 218:455-463

16. Oldfield EH, Ram Z, Culver KW, Blaese RM, DeVroom HL, Anderson WF: Gene therapy for the treatment of brain tumors using intra-tumoral transduction with the thymidine kinase gene and intravenous ganciclovir. Hum Gene Ther 1993; 4:39-69

17. Culver KW, Anderson WF, Blaese RM: Lymphocyte gene therapy. Hum Gene Ther 1991; 2:107-109

18. Huntington's Disease Collaborative Research Group: A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. Cell 1993; 72:971-983

19. Breakefeld XO: Gene delivery into the brain using viral vectors. Nat Genet 1993; 3:187-189

20. Chiocca EA, Choi BB, Cai W, et al: Transfer and expression of the lacZ gene in rat brain neurons mediated by herpes simplex virus mutants. New Biol 1990; 2:739-746

21. Davidson BL, Allen ED, Kozarsky KF, Wilson JM, Roessler BJ: A model system for in vivo gene transfer into the central nervous system using an adenoviral vector. Nat Genet 1993; 3:219-223

22. Akli S, Caillaud C, Vigne E, et al: Transfer of a foreign gene into the brain using adenovirus vectors. Nat Genet 1993; 3:224-228

23. Bajocchi G, Feldman SH, Crystal RG, Mastrangeli A: Direct in vivo gene transfer to ependymal cells in the central nervous system using recombinant adenovirus vectors. Nat Genet 1993; 3:229-234

24. Rosenberg MB, Friedmann T, Robertson RC, et al: Grafting genetically modified cells to the damaged brain: Restorative effects of NGF [nerve growth factor] expression. Science 1988; 242:1575-1578

25. Olson L: Nerve growth factor and the treatment of Alzheimer's disease. Exp Neurol 1993; 124:5-15

26. Ad Hoc Working Group on Nerve Growth Factor and Alzheimer's Disease: Potential use of nerve growth factor to treat Alzheimer's disease. Science 1989; 243:11

27. Jiao S, Gurevich V, Wolff JA: Long-term correction of rat model Parkinson's disease by gene therapy. Nature 1993; 362:450-453

28. Gage FH, Wolff JA, Rosenberg MB, et al: Grafting genetically modified cells to the brain: Possibilities for the future. Neuroscience 1987; 23:795-807

29. Gage FH, Kawaja MD, Fisher LJ: Genetically modified cells: Applications for intracerebral grafting. Trends Neurosci 1991; 14:328-333

30. Ezzeddine ZD, Martuza RL, Platika D, et al: Selective killing of glioma cells in culture and in vivo by retrovirus transfer of the herpes simplex thymidine kinase gene. New Biol 1991; 3:608-614

31. Culver KW, Ram Z, Wallbridge S, Ishii H, Oldfield EH, Blaese RM: In vivo gene transfer with retroviral vector-producer cells for treatment of experimental brain tumors. Science 1992; 256:1550-1552

32. Takamiya Y, Short MP, Ezzeddine ZD, Moolten FL, Breakefield XO, Martuza RL: Gene therapy of malignant brain tumors: A rat glioma line bearing the herpes simplex virus type 1-thymidine kinase gene and wild type retrovirus kills other tumor cells. J Neurosci Res 1992; 33:493-503

33. Ram Z, Culver KW, Walbridge S, Blaese RM, Oldfield EH: In situ retroviral-mediated gene transfer for the treatment of brain tumors in rats. Cancer Res 1993; 53:83-88

34. Takamiya Y, Short MP, Moolten FL, et al: An experimental model of retrovirus gene therapy for malignant brain tumors. J Neurosurg 1993; 79:104-110

35. Ram Z, Culver KW, Walbridge S, Frank JA, Blaese RM, Oldfield EH: Toxicity studies of retroviral-mediated gene transfer for the treatment of brain tumors. J Neurosurg 1993; 79:400-407

36. Barba D, Hardin J, Ray J, et al: Thymidine kinase-mediated killing of rat brain tumors. J Neurosurg 1993; 79:729-735

37. Springett GM, Moen RC, Anderson S, Blaese RM, Anderson WF: Infection efficiency of T lymphocytes with amphotropic retroviral vectors is cell cycle dependent. J Virol 1989; 63:3865-3869

38. Miller DG, Adam MA, Miller AD: Gene transfer by retrovirus vectors occurs only in cells that are actively replicating at the time of infection. Mol Cell Biol 1990; 10:4239-4242

39. Roe T, Reynolds TC, Yu G, Brown PO: Integration of murine leukemia virus DNA depends on mitosis. EMBO J 1993; 12:2099-2108

40. Short MP, Choi BC, Lee JK, Malick A, Breakefield XO, Martuza RL: Gene delivery to glioma cells in rat brain by grafting of a retrovirus packaging cell line. J Neurosci Res 1990; 27:427-439

41. Kolberg R: The bystander effect in gene therapy: Great, but how does it work? J Natl Inst Health Res 1994; 6:62-64

42. Bi WL, Parysek LM, Warnick R, et al: In vitro evidence that metabolic cooperation is responsible for the bystander effect observed with HSV-tk retroviral gene therapy. Hum Gene Ther 1993; 4:725-731

43. Freeman SM, Whartenby KA, Koeplin DS, et al: Tumor regression when a fraction of tumor mass contains the HSV-tk gene. J Cell Biochem 1992; 16F:47

44. Moolten FL: Tumor chemosensitivity conferred by inserted herpes thymidine kinase genes: Paradigm for a prospective cancer control strategy. Cancer Res 1986; 46:5276-5281

45. Moolten FL, Wells JM: Curability of tumors bearing herpes thymidine kinase genes transferred by retroviral vectors. J Natl Cancer Inst 1990; 82:297-300

46. Vile RG, Hart IR: Use of tissue-specific expression of the herpes simplex virus thymidine kinase gene to inhibit growth of established murine melanomas following direct intratumoral injection of DNA. Cancer Res 1993; 53:3860-3864

47. Caruso M, Panis Y, Gagandeep S, Houssin D, Salzmann JL, Klatzmann D: Regression of established macroscopic liver metastases after in situ transduction of a suicide gene. Proc Natl Acad Sci USA 1993; 90:7024-7028

48. Freeman SM, Abboud CN, Whartenby KA, et al: The 'bystander effect': Tumor regression when a fraction of the tumor mass is genetically modified. Cancer Res 1993; 53:5274-5283

49. Mahaley MS Jr, Mettlin C, Natarajan N, Laws ER Jr, Gillespie GY: National survey of patterns of care for brain-tumor patients. J Neurosurg 1989; 71:826-836