Gene Therapy of Central Nervous System Tumors

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Abstract

Recently, remarkable advances have been achieved in molecular and genetic researches of different kinds of general diseases, as well as in basic and clinical studies using gene therapy for central nervous system diseases. For brain tumors, clinical trials have been already started in more than 10 clinical protocols and more than 100 patients with malignant brain tumors. Nevertheless, there are still major issues that remain to be resolved for achieving better clinical results, such as delivery system of genetic material, regulatory methods of the intracellular expression of the transgene, antitumor efficacy, and tumor selectivity. In this paper, molecular genetic studies and the current state of gene therapy for neurological diseases, especially brain tumors, are described, and the future direction of this fascinating approach is discussed.

Key words: gene therapy, central nervous system disorders, liposome, brain tumor

Introduction

In the last 10 years, gene therapy in clinical reality has been proposed for several kinds of inherited and acquired diseases. The first authorized human gene therapy was started in the U.S.A. on September 14, 1990 on 4-year-old girl who had been born with a defective adenosine deaminase (ADA) gene which caused severe combined immunodeficiencies. With a combined treatment consisting of gene transfer of the ADA gene and administration of polyethylene glycol-ADA, she is apparently in good health without any side effects.

Since this successful treatment, a variety of human gene therapy protocols have occurred worldwide. According to the worldwide gene therapy report by TMC development in France, more than 2500 patients are enrolled in these protocols as of June 1996. Most trials are performed in the U.S.A. and European countries, with a few in Asian countries including Japan. Target diseases for this therapy are expanding from congenital metabolic disorders to encompass acquired life-threatening diseases such as cancer and acquired immunodeficiency syndrome (AIDS). Chronic benign diseases are also expected to be a focus of this therapy. At present, cancer is by far the most popular protocol, involving 848 patients. AIDS (372 patients), cystic fibrosis (152 patients), leukemia/myeloma (89 patients) are second, followed by arterial disease (16 patients) and ADA deficiency (12 patients).

Gene Therapy Approaches for General Disorders

Three different approaches are proposed for human gene therapy: 1) Genes of interest are delivered into patients in vivo or ex vivo in order to produce therapeutic materials, 2) amplification of normal gene or suppression of abnormal gene by antisense ribonucleic acid or ribozyme, and 3) replacement of abnormal gene to normal gene by homologous recombination and repaired genomic deoxyribonucleic acid (DNA). Most protocols approved by the Recombinant DNA Advisory Committee (RAC) involve the first approach, which contains three key components: a) the vector, b) the gene of interest, and c) the target cells. The vectors for gene transfer are classified into viral and non-viral. Until now several kinds of vector systems have been used for central nervous system (CNS) gene transfer, that is included retroviruses, recombinant herpes simplex virus (HSV), adenoviruses, adeno-associated viruses (AAVs), and liposome-entrapped plasmid vector (Table 1)

In the viral vector, retrovirus vectors have been studied most extensively and hence are most commonly used for clinical application. However, the retrovirus vector has a limitation because it requires only dividing cells for the target to achieve gene delivery and is rapidly inactivated in the blood. Thus most clinical applications of the retrovirus vector involve a complex ex vivo procedure whereby patient
cells are removed and the gene is delivered in vitro.

Another type of viral vector is derived from the adenovirus and AAV. The adenovirus vector is capable of delivering a gene to both dividing and non-dividing cells, so primarily clinical application of gene therapy for cystic fibrosis was started using with a vector based on a crippled adenovirus. However, adenovirus genes express proteins that induce an immune response. This immune response is thought to inhibit the length of efficient time that gene expression can be maintained in the target cell.

AAV vectors are derived from AAV, a common non-pathogenic human parvovirus. They may offer several potential advantages over other viral vectors. These advantages include efficient delivery of genes to both dividing and non-dividing target cells, potential site-specific integration of chromosome 19 and the absence of viral genes responsible for causing an undesirable immune response. A major limitation in the development of clinical application for AAV vectors has been the lack of an efficient production method, but this technical problem will be solved in near the future.

On the other hand, non-viral vectors, especially DNA/liposomes, are known to be much safer because they are non-infectious and non-immunogenic. Liposomes, artificially generated lipid vesicles that can entrap genes within their aqueous compartment or in the lipid bilayer, have been regarded as a useful gene delivery system. In 1987, Felgner et al. developed a cationic liposome with N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium (DOTMA). The authors reported the DOTMA interacts spontaneously with DNA to form a DNA-lipid complex and facilitates fusion of the complex with the cell membrane, resulting in both uptake and expression of the DNA. Since this basic work, cationic liposome mediated gene transfer has been widely used in the field of molecular biology and for gene therapy studies. Several groups have explored more efficient and less toxic cationic liposome compositions using different cationic lipids. We have also developed novel cationic liposomes with high transfection efficiency and low cytotoxicity which permit their use for in vivo gene transfer. Our liposomes are multilamellar vesicles prepared by a simple procedure with N-(a-trimethyl ammonioacetyl)-didodecyl-D-glutamate chloride (TMAG), dilauroyl phosphatidylcholine, and dioleoyl phosphatidylethanolamine in a molar ratio of 1:2:2. To transfer the gene selectively and efficiently into target cells, we coupled a monoclonal antibody (MCA) with the liposomes and made immunoliposomes. For this purpose, we developed two MCAs (G-22 and 3C10) against cell surface molecules expressed on human glioma cells. G-22 MCA operates against the standard or hematopoietic form of CD44, which is known to be overexpressed in the cell adhesion molecules on most glioma cells. 3C10 MCA operates against the truncated epidermal growth factor

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**Table 1 Vector system for delivery of genes to the central nervous system**

<table>
<thead>
<tr>
<th>Vector</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
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<tbody>
<tr>
<td>Retrovirus</td>
<td>infect only dividing cells, highly effective delivery of toxic genes to treat tumor cells</td>
<td>cannot infect non-dividing cells like neurons, low titer and low levels of viral integration, limited size of DNA insert, labile and inactivated in blood plasma, transient gene expression</td>
</tr>
<tr>
<td>Herpes simplex virus</td>
<td>infection of non-dividing cells including neurons, no insertion into host genome, high titers</td>
<td>neurotoxicity, recombination to wild type of lytic virus, transient gene expression</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>efficient transduction of both dividing and non-dividing cells, continued activation still in plasma, expression without integration in host genome, low level of neurotoxicity</td>
<td>inherent immunogenicity of the current generation, transient gene expression</td>
</tr>
<tr>
<td>Adeno-associated virus</td>
<td>efficient delivery of genes to both dividing and non-dividing cells, site-specific integration of chromosome 19. absence of viral genes responsible for causing an undesirable immune response</td>
<td>lack of efficient production methods, limited size of DNA insert</td>
</tr>
<tr>
<td>Liposome</td>
<td>no toxicity, easy delivery of plasmid DNA</td>
<td>short transient expression, cell type non-specificity</td>
</tr>
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DNA: deoxyribonucleic acid.
Concept Approaches for Gene Therapy of CNS Disorders

The basic study of gene therapy for the CNS disorders has been investigated in several directions. Until now, at least three categories of CNS diseases could be classified according to the possible mode of gene therapy: 1) Gene replacement therapy for inherited or acquired neurodegenerative disorders using transfected cells ex vivo. 2) Gene therapy of stroke or neurovascular diseases. 3) Gene therapy of brain tumors, not only solid mass but also CNS disseminated meningitis.

For neurodegenerative diseases, viral vector-mediated gene replacement therapy can use ex vivo transfected non-dividing cells. For inherited diseases due to errors of metabolism, the defective gene is relatively well-known and it is not difficult to characterize and make available for use as expression vectors. On the other hand, for acquired or late onset neurodegenerative disorders, such as Parkinson's disease, Huntington's disease, and Alzheimer's disease, different kinds of techniques and/or challenges have been applied to repair several kinds of pathogenetic cells lost because of neurodegenerative processes. As for the possible strategies for gene therapy, several clinical trials have been applied such as embryonic whole brain implantation, transplantation of genetically modified cells ex vivo, direct transfer of plasmid DNA by lipofection complex, etc.

As for protection of neurons from programmed cell death caused by ischemic injury, gene targeting or genetic transfer of components of interleukin-1 receptor antagonist protein, Bcl-2, nerve growth factor, neuronal apoptosis inhibitory protein, and glucose transporter gene have been investigated so far.

As for the control of expression of different inflammatory mediators in brain, several kinds of cytokines that are produced in response to an ischemic insult and are involved in the development of brain injury may offer novel therapeutic strategies.

Gene therapies of brain tumors will be described in the next section.

Characteristics of CNS Tumors

In the last decade, the prognosis of brain tumor patients has dramatically improved due to recent advances in microneurosurgical techniques and the development of functional neuroimaging, computer-assisted neuronavigation system, endoscopic surgery, intravascular surgery, radiosurgery, and so on. According to a report by the Committee of Brain Tumor Registry of Japan, the 5-year survival rate of patients with benign brain tumors (meningioma, neurinoma, and pituitary adenoma) is more than 95%. In contrast, patients with glioma (which constitute 33% of primary brain tumor cases) still have a poor prognosis, especially in the case of malignant types (anaplastic astrocytoma and glioblastoma) (Table 2). This poor prognosis is related to the fact that malignant glioma cells aggressively infiltrate into normal brain tissues, making total removal of the tumor impossible. The median survival time of glioblastoma patients is less than 2 years, despite multimodality treatment with extensive surgical resection and adjuvant therapies using radiotherapy and immunotheraphy.

In order to overcome this formidable neoplasm, the effectiveness of molecular biology using gene therapy has been investigated since 1992. Astrocytic gliomas are the most frequent human brain tumors. They are classified into three malignant grades (astrocytoma, anaplastic astrocytoma, and glioblastoma) on the basis of histopathological parameters. Genetic alternation is commonly encountered in as-

Table 2 Five-year relative survival rate of patients with brain tumors*

<table>
<thead>
<tr>
<th>Period</th>
<th>Astrocytoma</th>
<th>Anaplastic astrocytoma</th>
<th>Glioblastoma</th>
<th>Menigioma</th>
<th>Pituitary adenoma</th>
<th>Neurofibroma</th>
<th>Cranio-pharyngioma</th>
<th>Germ cell tumor</th>
<th>Medulloblastoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>1969–1975</td>
<td>50.9</td>
<td>21.7</td>
<td>11.9</td>
<td>99.6</td>
<td>105.6</td>
<td>91.9</td>
<td>71.7</td>
<td>63.3</td>
<td>22.2</td>
</tr>
<tr>
<td>1976–1980</td>
<td>63.2</td>
<td>25.3</td>
<td>12.0</td>
<td>105.5</td>
<td>102.8</td>
<td>102</td>
<td>77.7</td>
<td>74.6</td>
<td>32.1</td>
</tr>
<tr>
<td>1981–1985</td>
<td>61.6</td>
<td>27.4</td>
<td>9.8</td>
<td>116</td>
<td>108.1</td>
<td>111</td>
<td>88.6</td>
<td>89.0</td>
<td>36.3</td>
</tr>
<tr>
<td>1986–1990</td>
<td>69.2</td>
<td>22.2</td>
<td>8.0</td>
<td>120.4</td>
<td>110.9</td>
<td>115</td>
<td>94.9</td>
<td>—</td>
<td>40.8</td>
</tr>
</tbody>
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*From a report by the Committee of Brain Tumor Registry of Japan.

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trocytic glioma. Loss of heterozygosity (LOH) on chromosome 17p occurs in approximately half of all grades of malignancy, and most of these are mutations in the conserved region of the p53 genes.\textsuperscript{27,33} Low-grade astrocytomas carry a significant risk of malignant progression.\textsuperscript{32} LOH of chromosome 9p21 and chromosome 10 are known for potential involvement in this progression event. A multiple tumor suppressor-1 gene for the newly identified inhibitor of cell cycle-dependent kinase 4, a protein named p16, has been confirmed to be localized in the areas of 9p21.\textsuperscript{10} It was recently reported that deletion of the gene occurs frequently in anaplastic astrocytoma and glioblastoma.\textsuperscript{29} Amplification of EGFR gene is the most common genetic alteration in glioblastoma.\textsuperscript{31}

Malignant glioma (anaplastic astrocytoma and glioblastoma), in general, infiltrates aggressively into the surrounding normal brain tissue. Several factors including matrix metalloproteinase-II-IX, CD44,\textsuperscript{25} and tenasin\textsuperscript{49} were reported to be strongly correlated with the invasion of tumor cells. For these reasons, total resection of gliomas by surgery is impossible, although recent advances in microsurgical technique are remarkable. Recently, postoperative radiation has been applied to all patients with malignant glioma. The tumors do show a response to the radiation in many cases, but relatively high doses are necessary to achieve control of the tumor growth. The normal brain around the tumor can generally tolerate no more than 60 Gy, which is below the curative level for glioma.

Other approaches to malignant glioma treatment have included chemotherapy using nitrosourea derivatives either as an adjuvant to radiation\textsuperscript{48} or at the time of relapse, and immunotherapy with interferon-\(\beta\) (IFN-\(\beta\)),\textsuperscript{40} intratumoral lymphokine-activated killer cell instillation,\textsuperscript{18} intra-arterial tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)) infusion,\textsuperscript{45} and intratumoral injection of radiolabeled MCAs.\textsuperscript{46} These adjuvant therapies help to prolong survival at least for anaplastic astrocytoma. However, none of these methods are curative, and the median survival time for malignant glioma patients is less than 2 years at present. Nevertheless, this malignant glioma has important features which make it an excellent candidate for gene therapy. The brain is a closed cavity separated from the general circulation system by the blood-brain barrier, and it has been noted to be an immunologically privileged site with no lymphatic system. Normal glia and neuron are relatively quiescent compared to tumor cells. Furthermore, the glioma arising from a glia is a localized tumor in the CNS with no extra-CNS metastasis.

**Fundamental Studies of Gene Therapy for CNS Tumor**

Two gene therapy approaches were studied for the treatment of malignant CNS tumors, which have been already applied for clinical trial: 1) suicide gene therapy using the HSV thymidine kinase (HSV-tk) gene and ganciclovir (GCV) and 2) immune gene therapy using cytokine genes.

Suicide gene therapy has been shown to be an effective and relatively safe new approach for treatment of experimental brain tumors and leptomeningeal metastases. First retrovirus, then HSV, and later adenovirus vectors and AAV have been developed to deliver the HSV-tk suicide gene to brain tumor cells. As for retroviral vectors, expression of the HSV-tk renders dividing cells sensitive to GCV. HSV-tk converts GCV into a toxic phosphate, which acts as a chain terminator of DNA synthesis, which will eventually lead to cell death. Because only dividing cells are killed selectively while sparing normal surrounding healthy tissue, treatment of CNS tumors by suicide gene therapy could have advantages over current treatment modalities like radiotherapy, chemotherapy, and immunotherapy.\textsuperscript{10,26}

Adenovirus vectors have the advantage that high titers can be obtained and cell-free virus can be administered in vivo. Adenovirus does not integrate into the genome, thereby reducing the risk of insertional mutagenesis. The vector is also able to infect non-dividing quiescent tumor cells and consequently kill these cells at the time of their entry into the cell cycle when GCV is provided. Several investigators have demonstrated that treatment of experimental glioma and leptomeningeal metastases with adenovirus vectors harboring the HSV-tk gene was not associated with toxicity. Recombinant adenoviruses thus have potential to be used as vectors in suicide gene therapy of CNS tumors in humans. But when clinical use is considered, more fundamental research concerning interaction of tumor cell rate division and killing efficiency should be investigated. A team from Howard Hughes Medical Institute (Chevy Chase, Md., U.S.A.) and the Baylor College of Medicine (Houston, Tex., U.S.A.) reported the efficacy of adenovirus-mediated gene therapy to treat brain tumor.\textsuperscript{30} Tumors were generated in syngeneic rats by stereotactic implantation of 9L gliosarcoma cells into the caudate nucleus. Eight days later, the tumors were injected and transduced in situ with a replication-defective adenovirus carrying the HSV-tk gene, and the rats were treated with GCV. No tumors were detected in animals treated with adenovirus thymidine kinase and GCV.

We are developing a gene therapy using the AAV.
vector and have demonstrated the efficacy of AAV vector-based gene therapy for malignant glioma in an experimental animal model. We obtained AAV vectors containing the gene for either HSV-tk (AAV-tk) or β-galactosidase (AAV-LacZ) from Avigen Inc. (Alameda, Calif., U.S.A.). In several experiments, we confirmed that gene expression was seen in more than 30% of glioma cells by intratumor injection of the AAV-LacZ vector. Following a single injection of an AAV-tk or AAV-tk-Internal Ribosome Entry Segment (IRES)-interleukin-2 vector into human glioma implanted into the brains of nude mice, a significant reduction in tumor size was observed in all animals who also received GCV.21) Furthermore, after multiple AAV-tk injections intratumorally followed by intraperitoneal GCV administration, complete regression of the intracerebral implanted human glioma could be obtained, and the survival period of host mice was also prolonged remarkably.18)

With respect to immune gene therapy, as in our other experimental studies, we also investigated how to make transfected human glioma cells with a plasmid vector containing the human IFN-β (HuIFN-β) gene (pSV2IFN-β) by means of our novel TMAG cationic liposome, and found that HuIFN-β produced in the cells had a much stronger inhibitory effect on the growth of the tumor cells than exogenously added HuIFN-β.17) Our results suggest that the mechanism causing the growth-inhibitory effect of transfection-induced HuIFN-β is different from the exogenous one. The former process is thought to be cytotoxic to the transfected glioma cells, which can be ascribed to HuIFN-β production in the cells transfected with its gene by the process of apoptosis.17,43)

In vivo experiments using transplanted human glioma growing in the brain of nude mice clearly showed that HuIFN-β was expressed in the solid tumor and that growth of the brain tumor was inhibited by intratumoral injection of liposomes with entrapped pSV2IFN-β, while a high dose of exogenous HuIFN-β or empty liposomes did not significantly inhibit the growth of human glioma.

Interestingly, the production of HuIFN-β in the cells and its release from the transfected cells were increased by treating cells with a small dose of TNF-α before transfection. Correspondingly, the antitumor effect of the transfection-induced HuIFN-β was significantly elevated by combination with TNF-α.42) The intraperitoneal injection of a small amount of TNF-α inhibited tumor growth only slightly. On the other hand, prior treatment with TNF-α followed by intratumoral injection of liposomes with entrapped pSV2IFN-β had a remarkable effect. The subcutaneous tumors regressed completely in all nude mice tested; they were tumor free and surviving for long follow-up period. TNF-α is a cytokine that possesses a wide variety of biological and immunomodulatory properties, although the problem of dose-limiting toxicity of TNF-α was reported in its clinical trials.39) Rosenberg et al. began immune gene therapy for cancer patients using gene transfer instead of administration of TNF-α, either by adding the TNF-α gene to the tumor-infiltrating lymphocytes to make them more effective28) or by adding a TNF-α gene to the tumor cells to induce a host immune system response.39)

In our experimental studies of brain tumors, we found that human TNF-α (HuTNF-α) was produced in the glioma cells transfected with liposomes with entrapped pcDVTNF-α and that the growth-inhibitory effect of transfection induced HuTNF-α was much stronger than that of exogenously added HuTNF-α.6) In our study to analyze this mechanism, we found that it was due to transmembrane-formed TNF-α.

Using this liposomal transfection strategies to clarify the effect of glial fibrillary acidic protein (GFAP) expression in brain tumor cells, we also transferred the GFAP gene into the human medulloblastoma cell line, DAOY-1, which lacks the expression of GFAP. As a result, after GFAP gene transfection, growth inhibition and increase of sensitivity to anticancer drug (cisplatinum) were observed with GFAP expression in the cells. These results indicate that gene therapy also may become one of the adjuvant treatment methods combined with conventional chemotherapy.29)

Clinical Application of Gene Therapy for CNS Tumors

Gene therapy of CNS tumors requires specific tumoricidal effects that could be achieved by selective expression of toxic genes, leading to specific cytolysis of CNS tumor cells, or by the use of drug susceptibility or suicide genes. Either type of therapy should result in inhibition of tumor growth and ultimately should kill the tumor cells without having an effect on normal healthy brain tissue. A combined approach with surgery, radiation therapy, and gene therapy of certain types of brain tumors is expected to result in prolonged survival, in comparison to standard therapy. In addition, adoptive immunotherapy offers the potential for highly specific tumor therapy. To date, protocols of suicide gene therapy with the HSV-tk gene, antisense therapy to insulin-like growth factor-1, and immune gene therapy with interleukin-220) have been approved by an RAC meeting in the U.S.A. A therapeutic approach
Table 3  Human gene therapy clinical protocol for brain tumors approved so far by the Recombinant DNA Advisory Committee meeting8)

<p>| | | |</p>
<table>
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</table>
| 1 | i) Gene therapy for the treatment of brain tumors using intra-tumoral transduction with the thymidine kinase gene and intravenous ganciclovir  
   | ii) Oldfield EH  
   | iii) National Institute of Health, Bethesda, Md., U.S.A.  
   | iv) 8/14/92  
   | v) 12/7/92 | 2 | i) Gene therapy for the treatment of malignant brain tumors with in vivo tumor transduction with the herpes simplex thymidine kinase gene/ganciclovir system  
   | ii) Culver K  
   | iii) Iowa Methodist Medical Center, Des Moines, Iowa, U.S.A.  
   | iv) 4/16/93  
   | v) 7/13/93 | 3 | i) Gene therapy for the treatment of recurrent pediatric malignant astrocytomas with in vivo tumor transduction with the herpes simplex thymidine kinase gene  
   | ii) Raffel C  
   | iii) Children's Hospital, Los Angeles, Calif., U.S.A.  
   | iv) 9/3/93  
   | v) pending | 4 | i) Gene therapy for human brain tumors using episome-based antisense cDNA transcription of insulin-like growth factor 1  
   | ii) Ilan J  
   | iii) Case Western Reserve, Cleveland, Ohio, U.S.A.  
   | iv) 12/2/93  
   | v) 3/94 | 5 | i) Gene therapy for recurrent pediatric brain tumors  
   | ii) Kun KE  
   | iii) St Jude Children's Research Hospital, Memphis, Tenn., U.S.A. and National Institute of Health, Bethesda, Md., U.S.A.  
   | iv) 10/7/93  
   | v) pending | 6 | i) Intrathecal gene therapy for the treatment of leptomeningeal carcinomatosis  
   | ii) Oldfield EH  
   | iii) National Institute of Health, Bethesda, Md., U.S.A.  
   | iv) 1/20/94  
   | v) 8/8/94 | 7 | i) Injection of glioblastoma patients with tumor cells genetically modified to secrete interleukin-2 (IL-2): A phase I study  
   | ii) Sobol R  
   | iii) San Diego Regional Cancer Center, San Diego, Calif., U.S.A.  
   | iv) 7/12/94  
   | v) pending | 8 | i) Treatment of advanced CNS with recombinant adenovirus H5.020RSVTK: A phase I trial  
   | ii) Eck SL  
   | iii) University of Pennsylvania Medical Center, Philadelphia, Pa., U.S.A.  
   | iv) 2/2/96  
   | v) pending | 9 | i) Phase I study of adenoviral vector delivery of the HSV-TK gene and the intravenous administration of ganciclovir in adults with malignant tumor of the central nervous system  
   | ii) Grossman R, Woo S  
   | iii) Baylor College of Medicine, Houston, Tex., U.S.A.  
   | iv) 2/2/96  
   | v) pending | 10 | i) Stereotaxic injection of herpes simplex thymidine kinase vector producer cells (PA317/GITlgDvNa7) and intravenous ganciclovir for the treatment of recurrent malignant glioma  
   | ii) Fettell M, Warnick R, Yung WKA, Maria BL, Shaffrey M, Ram A  
   | iii) various institutions  
   | iv) 2/10/95  
   | v) 3/10/95 | 11 | i) A phase I study of the safety of injecting malignant glioma patients with irradiated TGF-β2 antisense gene modified autologous tumor cells  
   | ii) Black KL, Fakhrai H  
   | iii) University of California, Los Angeles, Los Angeles, Calif., U.S.A.  
   | iv) 4/2/96  
   | v) pending |

i) Title, ii) principal investigator, iii) institute, iv) date of final approval, v) date of first patient treated. cDNA: complementary deoxyribonucleic acid, CNS: central nervous system, HSV-TK: herpes simplex virus thymidine kinase, TGF-β2: transforming growth factor-β2.
to genetically modify CNS tumor cells to arrest tumor growth by either favoring genetically directed lysis of the tumor or by altering genetic material of tumor to make tumor cells susceptible to antiviral drug GCV; numerous experimental studies have been performed, and a major clinical multi-institutional trial has been initiated in patients with glioblastoma multiforme. Until now more than 100 patients have been enrolled in a similar protocol in the U.S.A. and in some European countries, and some of them were treated by direct injection of vector producer cells (VPCs) into the brain tumor or by administration of VPCs through an Ommaya reservoir into the residual tumor. At first, in 1992, Oldfield et al. started suicide gene therapy in patients with glioblastoma or metastatic brain tumor. VPCs that had been genetically engineered into NIH3T3 cells to continually produce HSV-tk recombinant retroviral vectors were injected into the brain tumors using a magnetic resonance imaging-guided stereotactic approach. On the 5th postoperative day, intravenous injection of GCV started at 5 mg/kg/dose twice daily for 14 days. With this approach, they evaluated 15 patients with progressive growth of CNS malignant tumors. As a result, antitumor activity was detected in five of the smaller sized tumors. In situ hybridization for HSV-tk demonstrated survival of VPC at 7 days but indicated limited gene transfer to tumors, suggesting that indirect bystander effects provide local antitumor activity in human tumors. They concluded that the response was detected in only very small tumors in which a high density of VPC had been placed, so that techniques to improve delivery and distribution of the therapeutic gene will need to be developed if clinical utility is to be achieved with this approach.29

Assessment of Current Status and Recommendation for the Future Direction of Gene Therapy

Since the first human gene therapy started successfully, more than 100 protocols have been approved by the RAC, and clinical application has been carried out in more than 2500 patients worldwide. As for CNS tumors, 11 protocols have been approved so far and more than 100 patients treated by gene therapeutic methods (Table 3).8 Gene therapy has been conducted mostly in university hospitals and at other U.S.A. and European academic centers, supported by the National Institute of Health (NIH) and private companies. However, an ad hoc NIH committee assessing the current state of gene therapy in 1995 found that its clinical efficacy had not been definitively demonstrated in any gene therapy protocol, because significant problems remain with all basic aspects of gene therapy. In order to confront the major outstanding obstacles to successful gene therapy, the committee recommended a greater focus on basic aspects of gene transfer and gene expression, and an emphasis on research dealing with the pathogenesis of target diseases. Furthermore, the negatives associated with the concepts of gene therapy, in addition to the delivery that probably remains a major obstacle, are transient gene expression, toxicity of viral proteins, and the problem of immune response to the transfected protein.

Advances in gene therapy in progress will really depend on the development of gene delivery systems into the target cells. As for the methods resolving this problem, Zlokovic and Apuzzo47 emphasize the development of techniques which circumvent the impermeable blood-brain barrier and ways to breach the more versatile blood-brain-tumor barrier to deliver the genetic material to the target CNS cells. These include the following: 1) Local stereotactic CNS injection/infusion of viral vectors, administration of VPCs, or cell replacement; 2) local administration of genetic material into the cerebrospinal fluid ventriculocisternal system; 3) osmotic opening of the blood-brain barrier; 4) local intra-arterial infusion; and 5) administration of blood-brain-tumor barrier permeabilizers, such as bradykinin B2 antagonist.

Furthermore, in the future, as Rutka mentioned in a comment to the article of Zlokovic and Apuzzo,47 all gene therapy strategies still have to overcome at least two major obstacles. One is the significant immune response, which is invoked in the host when viruses, bacteria, or heterologous cell types are used for gene transfer. The other is the difficulty with brain specific targeting of vectors to the CNS. Though there is still little evidence that current gene therapy in clinical use can achieve tumor regression or cure, after resolving such issues which described already, we expected that gene therapy will become one of the standard treatment choices for several kinds of diseases including CNS tumors.

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