Cancer is a disease of a series of genes. Thus, theoretically, brain tumors could be treated by targeting their fundamental molecular defects. Currently, most of the approved clinical protocols for gene therapy involve cancer patients. Several of these protocols are designed to improve the treatment of brain tumors. In this brief report, we analyze the rationale, advantages, and disadvantages of a series of gene therapy approaches against brain tumors that include transfer of tumor suppressor genes and cell-cycle modulators; suicide or prodrug strategies; immunogene therapy; antiangiogenesis; and oncolytic virus therapy. In summary, in this review, we highlight the translational advances in molecular medicine that broaden our battery of therapies for patients with brain tumors.

BRAIN TUMORS

The combined incidence of all recorded primary intracranial and spinal axis tumors is between 2 and 9 in 100,000 persons per year. Central nervous system tumors are the most prevalent solid neoplasms of childhood, the second leading cancer-related cause of death in children, and the third leading cancer-related cause of death in adolescents and adults between the ages of 15 and 34 years. The most frequent brain tumors are the gliomas, which are divided into low grade, anaplastic, and glioblastoma multiforme. The important role of certain molecules, such as tumor suppressor genes, in glioma formation and progression has been underscored by the discovery of individuals with brain tumors carrying germline mutations of the TP53 gene (Li-Fraumeni syndrome), P16 gene (melanoma-glioma syndrome), and MMAC1 (mutated in multiple advanced cancers) gene (autosomal dominant disorders that share multiple benign tumors and an increased susceptibility for malignant tumors). The relevance of these genetic abnormalities as tumor markers is a developing concept. However, screening of these genes in preclinical situations is still not routine procedure and should be individualized. The current therapies for glioblastoma multiforme are ineffective. New molecular techniques have yielded a battery of potential therapies for gliomas. Malignant gliomas are attractive targets for local gene therapy because of their absolute localization into the central nervous system and absence of remote metastases. The majority of the molecular strategies for gliomas aim at 3 major targets: (1) controlling cell cycle or inducing apoptosis; (2) use of suicide genes and enzyme-prodrug systems; and (3) enhancement of the immune system by immunogene therapy. In addition, 2 other important fields include antiangiogenesis and oncolytic viruses.

CELL CYCLE CONTROL AND APOPTOSIS IN GLIOMAS

The TP53 Gene

The wild-type TP53 is involved in several aspects of cell cycle control, and its protein suppresses transformation either by inducing apoptosis or by blocking cell cycle progression. Loss of function mutations of TP53 gene are present in more than 30% of astrocytomas and constitute the earliest detectable genetic alteration in these tumors. The ability of p53 to activate transcription...
indicates that genes induced by p53 may mediate its biological role as a tumor suppressor. The TP53 gene up-regulates the expression of at least 2 genes whose encoded products are able to regulate growth arrest or apoptosis. Thus, the p53 protein directly induces the expression of the P21 gene, which in turn results in block of the cell cycle in the G1 phase. The p53 protein also transcriptionally activates the death gene BAX, an apoptosis-promoting member of the BCL-2 family. Experimental transfer of p53 to glioma cells first induces expression of the p21 protein and growth arrest, and, subsequently, induces BAX and promotes apoptosis. In addition to the p53-direct effect, other molecules may enhance the p53-apoptotic ability. Thus, coexpression of p16 and p53 induces apoptosis in p53-resistant cells. In addition, p19ARF, the protein encoded by the alternate reading frame of the P16 locus, has recently been implicated in activation of p53. The TP53 gene is a critical target to develop gene therapy strategies for cancer, and the toxicity and anticancer effect of the transfer of TP53 to gliomas is currently being tested in a clinical trial (Table). However, in gliomas, TP53-transfer strategies may be insufficient to control tumor growth because many gliomas already express wild-type p53. In addition, the majority of mutant TP33 tumors harbor a subpopulation of cells that express wild-type p53 protein, and many gliomas overexpress p21 protein, which advances resistance to p53-mediated apoptosis.

The E2F-1 Pathway

Although the regulatory network p16/Rb/E2F is the most commonly altered pathway in gliomas, transfer of P16 or RB to gliomas merely produces a cytostatic effect. The ultimate target of any alteration in the p16/Rb/E2F pathway is the deregulation of E2F transcription factors. Among them, the best characterized is E2F-1. This protein positively influences the transcription of S-phase genes and drives cell cycle progression through the G1 checkpoint. Furthermore, the E2F-1 protein can act as an oncogene and transform normal cells. However, several lines of evidence indicate that the E2F-1 is a tumor suppressor gene. The E2F-1 protein promotes apoptosis in several systems, either alone or in association with p53. Deletion of the E2F-1 gene resulted in spontaneous development of tumors in transgenic modified animals demonstrating that E2F-1 is a bona fide tumor suppressor gene. As with TP53, transfer of E2F-1 to gliomas induces apoptosis. Preliminary studies suggest that E2F-1 may be more effective than p53 in annihilating glioma cells, because it is able to induce apoptosis in p53-resistant cells. The downside of this molecule is its potential toxic effects and possible oncogenic potential. Thus, transfer of E2F-1 is able to induce apoptosis in normal fibroblasts. However, the encouraging preliminary results obtained with the adenovirally mediated transfer of E2F-1 to glioma in vitro and in animal models should propel the development of clinical trials based on the intratumoral transfer of E2F-1, alone or combined with TP53, for glioma treatment.

The MMAC1 Pathway

One of the most frequent abnormalities of glioblastomas is deletion of chromosome 10. The MMAC1 or phosphatase and tensin homolog deleted from chromosome 10 (PTEN) is a tumor suppressor gene that has been found mutated in malignant gliomas. The MMAC1 protein apparently exerts its function by removing a phosphate from

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**USA Gene Therapy Clinical Trials for Gliomas**

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*Data from Human Gene Therapy Protocols. Bethesda, Md: National Institutes of Health; October 14, 1998. Shown is the first institution listed in the National Institutes of Health list of protocols. IGF indicates insulinlike growth factor; TGF, transforming growth factor; IL, interleukin; Tk, herpes simplex virus thymidine kinase; and CNS, central nervous system.

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a lipid in a key growth control pathway. Restoration of the MMAC1 activity to glioma cells led to suppression of their neoplastic phenotype, providing evidence that MMAC1 is a tumor suppressor gene in gliomas. Although, the MMAC1 function is still not completely understood, the data from experiments with knock-out mice indicate that MMAC1 can suppress tumorigenesis through its ability to regulate cellular differentiation and anchorage-independent growth. In addition, since MMAC1 is inactivated in the latest stages of the progression of gliomas, it may play a role in angiogenesis or invasiveness.

SUICIDE GENE THERAPIES

This approach involves the conferring of drug sensitivity by transfixing tumor cells with a gene encoding an enzyme that can metabolize a nonprotoxic drug to its toxic form (suicide genes).

The herpesvirus thymidine kinase gene converts non-toxic nucleoside analogs such as ganciclovir into phosphorylated compounds that kill dividing cells. Thus, glioma cells genetically modified to express the herpesvirus thymidine kinase gene can be killed by the administration of ganciclovir. Preliminary studies in a rat glioma model showed that marked tumor regression occurred although only a small fraction of cells were transduced by the exogenous gene. This cytotoxic effect of transduced on nontransduced cells is termed the bystander effect. The thymidine kinase–ganciclovir approach is currently used in several clinical trials (Table). Although the preliminary results of these studies are not completely satisfactory, it is expected that the substitution of retrovirus for adenovirus as a vehicle to transfer the herpesvirus gene will improve the efficiency of this strategy. In addition to the thymidine kinase–ganciclovir, the cytosine deaminase–5-fluorocytosine system is also popular among gene therapists. In this system, the cytosine deaminase is used to convert 5-fluorocytosine to 5-fluorouracil in cancer cells.

IMMUNOGENE THERAPY

The immunogene therapy is based on the use of recombinant DNA constructs to express cytokines and lymphokines. A major advantage of this approach is the potential to generate a systemic response against the tumor. This strategy refers to at least 3 different approaches.

Presentation of Tumor-Rejection Antigens by Antigen-Presenting Cells

Autologous antigen-presenting cells can be harvested by mobilization from the blood of the patient or from biopsy specimens of the brain tumor and then expanded in vitro with cytokines. To generate the antitumor immune response, antigen-presenting cells are transduced with the DNA or messenger RNA coding for the tumor antigen and then injected into the patient.

Cytokine-Expressing Tumor Cells

This approach is based on the use of ex vivo vaccination with tumor cells expressing cytokines. Disadvantages of this approach include the unavailability of tumor cells from every patient (inaccessible tumors) and the failure of the transduced cells to express the cytokine gene.

Expression of Costimulatory Molecules on the Surface of Tumor Cells

The transfer of costimulatory molecules (such as HLA-type molecules) into tumor cells aims at generating T-cell responses against the tumor. The identification of tumor rejection antigens from brain tumors and of the critical components of afferent and efferent arms of the immune response are necessary to increase the efficiency of these systems.

ANTIANGIOGENESIS

Among the most suitable targets for new therapies of gliomas are the regulators of angiogenesis. These molecules are especially important in gliomas because neovascularization is a major feature of these tumors. Indeed, the progression of an astrocytoma to anaplastic astrocytoma or glioblastoma multiforme is characterized by increased neovascularization. Angiogenesis modulators are extraordinarily important in tumor growth, as shown by the fact that neovascularization must occur for solid tumors to grow beyond a diameter of 2 to 3 mm. One of these molecules is the vascular endothelial growth factor (VEGF). This protein is efficiently secreted by tumor cells and promotes neovascularization. Several lines of evidence indicate that VEGF plays a major role in the growth of gliomas. Thus, the VEGF–messenger RNA is overexpressed in the highly vascularized glioblastoma multiforme. In addition, the transfection of VEGF complementary DNA to rat glioma cells results in hypervascularized tumors with abnormally large vessels, and the abrupt withdrawal of VEGF results in the regression of preformed tumor vessels. Importantly, it has been demonstrated that the transfection of antisense-VEGF–complementary DNA results in down-regulation of the endogenous VEGF and suppresses ability of glioma cells to form tumors in mice. In addition, a recent report shows that the transfer of antisense VEGF to glioma cells in vivo, by using adenovirus, inhibits tumor growth.

ONCOLYTIC VIRUSES

Viruses have been tested in the past in an attempt to directly kill tumor cells by lysis. The current advances of genetic engineering allow the modifications of wild-type viruses to improve their safety and efficiency. The perfect oncolytic virus must be one able to distinguish between normal tissue and abnormal cancer cells. The virus should be able to efficiently eliminate the cancer cells and propagate the anticancer effect to surrounding tumor cells. In addition, these viruses should be able to avoid the neutralizing immune response of the host. Finally, this kind of virus should be controllable pharmacologically. Among the oncolytic viruses, at least 2 have been explored with success for glioma treatment in animal models.
**Herpes Simplex Virus**

Genetically engineered herpes simplex viruses have been proved proficient in the elimination of glioma cells in vitro and in vivo. Since these viruses are highly toxic and may induce encephalitis in human beings, current research is focused on the generation of genetically altered viruses with low virulence for normal cells. The advantages of this system include the sensitivity of the herpes simplex virus to ganciclovir, which renders them manageable in case of spread to normal cells.

**Replication Competent Adenovirus**

Another viral approach is the use of replication competent adenoviruses to lyse cancer cells. However, adenoviruses are able to kill normal as well as cancer cells. To overcome this problem, Bischoff and colleagues modified a replicant competent adenovirus to have tumor-selective cytopathic effect. The rationale is based on the fact that adenovirus needs a specific protein of the virus, E1B, to replicate in host cells. The E1B protein binds and inactivates the p53 protein and allows the cell to enter into S phase and synthesize the viral proteins necessary for viral replication. Bischoff and colleagues designed and generated an E1B-mutated adenovirus that was not capable to replicate in wild-type TP53 cells but was able to replicate and kill mutant TP53 cancer cells. Injection of glioma tumors growing in mice with the mutant adenovirus led to complete regression of the tumors. The advantages of this approach include the presumably low toxicity of the adenovirus. The disadvantages include the high heterogeneity of gliomas that express wild-type and mutant TP53 cells inside the same tumor. In addition, the neutralizing immune response of the host against this adenovirus is the principal barrier to a successful therapy in humans.

**COMBINATION THERAPY**

As opposed to conventional forms of therapy, gene therapy is treatment directed against the basic neoplastic mechanism. However, effective single-gene therapy is limited and probably restricted to few tumors. Gliomas generally arise as the culmination of a multistep process that involves a variety of genetic abnormalities. Multiple gene transfer is necessary because gliomas are constituted by a heterogeneous mixture of cancer cells, some of which may already present or eventually acquire resistance against a specific gene. Several agents with different mechanisms of action (eg, apoptosis, antiangiogenic, immunogenic) would probably be necessary to kill a wide spectrum of cancer cells. In addition, coexpression of multiple genes may induce additive or synergistic effects. The multiple-gene molecular approach is similar to previous strategies of developing chemotherapeutic anticancer drugs combinations. Both the multidrug and multigene therapies are governed by the same principles: (1) combination of drugs with different phase-specific cytotoxicities; p53 triggers apoptosis; caspases are more important as a part of the executioner mechanism; p19 is important to inactivate inhibitors of the p53 protein; (2) sequential combinations in which the first drug causes an initial insult to cancer cells while the subsequent drug is lethal to already partially damaged cells: blocking the action of bcl-2 will render the cells more sensitive to the p53-related apoptotic effect; (3) combination of drugs in which one drug blocks cell cycle progression at a particular phase and the second is cytotoxic for cells in that phase: wild-type p16 expression induces senescence, which, under certain circumstances, might be necessary for the effect of other agents such as p53; p53 may be required for the E2F-1 effect. Combination therapy is not limited to gene therapies. Since the delivery of the therapeutic gene to every tumor cell is beyond the capability of the vectors currently available, gene therapy is focusing on enhancing the effect of existing therapies. In this regard, both radiotherapy and conventional chemotherapeutic agents are able to augment the antitumor effect of gene therapy strategies. Most of the studies performed with such combinations have demonstrated the superiority of this approach as compared with single therapies.

**REFERENCES**