Current and Future Gene Therapy for Malignant Gliomas

Takao Kanzawa, Hideaki Ito, Yasuko Kondo, and Seiji Kondo*

Department of Neurosurgery, Mount Sinai School of Medicine, New York, NY 10029, USA
Department of Neurosurgery, The University of Texas M. D. Anderson Cancer Center, Houston, TX 77030, USA

Malignant gliomas are the most common neoplasm in the central nervous system. When treated with conventional treatments including surgery, irradiation, and chemotherapy, the average life expectancy of the most malignant type, glioblastoma multiforme is usually less than 1 year. Therefore, gene therapy is expected to be an effective and possibly curative treatment. Many gene therapeutic approaches have demonstrated efficacy in experimental animal models. However, the current clinical trials are disappointing. This review focuses on current therapeutic genes/vectors/delivery systems/targeting strategies in order to introduce updated trends and hopefully indicate prospective gene therapy for malignant gliomas.

INTRODUCTION

Malignant gliomas are the most common primary tumors arising in the human brain [1]. The most malignant type of them, the glioblastoma multiforme, represents 29% of all primary brain tumors or 5,000 new cases per year in the United States [2]. Despite surgery, chemotherapy, and radiotherapy, glioblastomas are almost always fatal, with a median survival rate of less than a year and a 5-year survival rate of 5% or less [1, 2, 3]. No therapeutic modality has substantially changed the outcome of patients with glioblastoma [2, 3]. Therefore, it is no wonder that one of the earliest targets of cancer gene therapy was malignant glioma.

The epoch-making human trial of herpes simplex virus *thymidine kinase* gene/ganciclovir (HSV-*tk*/GCV) using retroviral vector started in early 1990s [4]. Although the antitumor effect of HSV-*tk*/GCV therapy had looked very promising in the animal model, the effect on human patients was disappointing. To augment the effect of gene therapy, adenoviral vectors were developed and advanced to human trials [5]. Adenoviral vectors significantly improved transduction efficacy but raised other problems, as discussed later in this review. Additionally, there was a death of a patient who received gene therapy using an adenoviral vector [6]. This incident raised a nationwide debate especially on the safety of gene therapy using viral vectors. Most of clinical trials were put on hold for several months to make sure that safety guidelines are strictly followed. However, hopes for gene therapy have not been quenched out. Researchers have been exploring many candidate genes, developing improved viral and nonviral vectors, trying different methods to deliver genes, and combining gene therapies with other modalities such as immunotherapy. In this review, we focus on animal and human studies of gene therapy for malignant gliomas. We have collected relatively recent references to introduce updated trends and hopefully to indicate future directions in this field.

THERAPEUTIC GENES

Many therapeutic genes have shown efficacy in experimental models and been divided into three categories. First, therapeutic genes are used to induce direct killing effect (Table 1). In this category, HSV-*tk*/GCV [5, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16], toxin [17], tumor suppressor genes [18, 19, 20, 21, 22, 23, 24, 25, 26], apoptosis-inducers [27, 28, 29, 30, 31, 32], antisense against telomerase [25, 33, 34, 35], and oncolytic viruses [36, 37, 38, 39, 40] are included. Second, immunomodulation has been performed to elicit immune response against malignant gliomas [41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54]. Third, angiogenesis inhibitors [55, 56, 57, 58] or neural stem cells [59, 60, 61] are used to induce antitumor effect, although direct killing effect or direct immunoreaction may be unrelated.

Direct killing effect

HSV-tk/GCV

Herpes simplex virus *thymidine kinase* gene/ganciclovir (HSV-*tk*/GCV) therapy is a two-step strategy [4]. First, HSV-*tk* gene is transduced into tumor cells. Second, GCV is administered systematically. GCV is harmless to normal cells without HSV-*tk*. When tumor

TABLE 1. Therapeutic genes.

Direct killing effect	References
HSV-*tk*/GCV	[5, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16]
Toxin	[17]
Tumor suppresor gene	[18, 19, 20, 21, 22, 23, 24, 25, 26]
Apoptosis inducer	[27, 28, 29, 30, 31, 32]
Antisense therapy for telomerase	[25, 33, 34, 35]
Oncolytic virus	[36, 37, 38, 39, 40]
Immunomodulation	**References**
IL-2	[41, 42, 43]
IL-4	[44, 45, 46]
IL-12	[47, 48]
IFN	[49, 50]
TNF-α	[51, 52, 53, 54]
Others	**References**
Angiogenesis inhibitor	[55, 56, 57, 58]
Neural stem cell	[59, 60, 61]

cells express HSV-*tk*, this enzyme converts GCV into a cytotoxic molecule, resulting in cell death. This HSV-*tk*/GCV therapy is also called suicide gene therapy. When HSV-*tk* gene is transduced with retroviral vectors, these vectors are selectively incorporated into dividing cells, predominantly into brain tumor cells. However, the original scheme using retroviral vectors turned out to be not potent enough. In vivo studies showed that effective tumor-cell killing depends on bystander effect and transduction efficacy [7, 13]. Bystander effect refers to killing cells that do not express HSV-*tk*, but closely located with the cells expressing the enzyme. On the other hand, to improve transduction efficacy, retroviral-vector producing cells or adenoviral vectors were developed. Many human trials of HSV-*tk*/GCV therapy were carried out using either retroviral-vector producing cells or adenoviral vectors. Some phase I/II studies reported significant therapeutic responses in small groups of patients [10, 14], but others claimed only marginal benefit [5, 11, 15]; overall, results were disappointing. The main reasons of failure are assumed to be low transduction efficacy and lack of bystander effect. Additionally, there was a report warning that adenoviral vector induced long-term active inflammation in the animal model [12]. More recently, HSV-*tk*/GCV therapy is used in in vivo experiments with adeno-associated virus [9] or combined with other therapy [16].

Toxin

Martin et al constructed retroviral vectors with a toxin gene (the Pseudomonas exotoxin or the Ricinus toxin, ricin) placed under the control of the thyroid hormone (T3) regulatable promoter of the myelin basic protein

(MBP) [17]. They showed that malignant glioma cells, stably transduced with the vector, failed to establish a tumor or regressed the tumor mass in the rat brain.

Tumor suppressor genes

Tumor suppressor genes are often mutated or deleted in malignant gliomas and the lack of function of these genes is supposed to cause tumorigenicity. Therefore, it is quite reasonable to replace them. The most extensively studied is the p53 tumor suppressor gene. Since alterations in the p53 gene occur in 35–60% of human malignant glioma [62, 63], p53 gene therapy is logically appropriate for these tumors. Accumulating evidences show that replacement of p53 significantly inhibits tumor growth in the subcutaneous [19] or intracranial [18, 22, 24] tumor model. However, many gliomas are mixture of cells with mutated p53 and wild-type p53 (wt-p53), and p53 gene transfer is known to be ineffective for the cells with wt-p53 [23, 25]. That is, p53 gene therapy is supposed to be effective only for part of malignant gliomas. There is a conflicting report claiming that p53 gene therapy induces apoptosis in glioma cells with wt-p53 [24]. New studies show that the combination of p53 gene therapy and irradiation is effective for malignant gliomas with heterogenous p53 status [20, 21]. Other tumor suppressor genes (p21, p16, and p27) belong to cyclin-dependent kinase inhibitors (CDKIs) and they are involved in the regulation of cell cycle. Wang et al showed that retroviral transfer of p16 and p21 inhibited tumor growth [26].

Apoptosis-inducers

Apoptosis, also called programmed cell death, is a genetically-encoded program to get rid of unwanted cells. It is well known that apoptotic pathways are suppressed in malignancies including malignant gliomas [64]. The rationale to use apoptosis-inducers is to activate apoptotic pathways and induce cell death effectively in malignant glioma cells. The genes used in the studies so far are caspase-1/interleukin-1β-converting enzyme (ICE) [27], caspase-3/CPP32β [28], caspase-6 [32], caspase-8 [30], Fas associated protein with death domain (FADD) [29], and Bax [31]. The main concern of these strategies is about safety, what if apoptosis is also induced in normal brain cells surrounding the tumor? It is necessary to regulate the induction of apoptosis to occur only in malignant cells as described below.

Antisense therapy for telomerase

Our group developed a system to inhibit human telomerase RNA (hTER) with 2-5A-linked antisense [25, 33, 35]. 2-5A or $2^{'}, 5^{'}$-oligoadenylate is a pathway of interferon actions [65, 66]. It activates RNase L that is ubiquitous in the cells and degrades RNA randomly. Chimeric combination of an antisense and 2-5A enables us to

degrade specific RNA [67]. We designed an antisense and synthesized 2-5A antisense molecule to target hTER (2-5A-anti-hTER) [33]. It degrades hTER specifically and effectively, resulting in the inhibition of telomerase. Interestingly, 2-5A-anti-hTER induced apoptosis massively and inhibited tumor growth in the subcutaneous and intracranial tumor models [33, 35]. 2-5A-anti-hTER induced apoptosis unexpectedly early (within 4 days, in vitro) compared to the treatment with the cDNA vector for hTER (about one month) [34].

Oncolytic viruses

Oncolytic viruses are designed to replicate selectively in and lyse tumor cells. These viruses are more effective in infecting tumor cells compared to the viruses that are constructed as replication-defective. Oncolytic viruses increase in number in tumor cells and lyse the cells directly, not by transducing specific genes. A recombinant herpes simplex virus with some deletions, designated DM33, inhibited growth of intracranial tumors and prolonged the survival of tumor-bearing animals [38]. The tumor-killing effect was even better with the following injection of ganciclovir [40]. Adenovirus ONYX-015 targets tumors with mutant p53 and its clinical studies are ongoing for head and neck cancer. Recently, it was shown that ONYX-015 is effective in malignant gliomas regardless of their p53 status [39]. Fueyo et al constructed Delta 24, a tumor-selective adenovirus with a deletion in the EIA region responsible for binding Rb protein [36]. This virus targets tumor cells with Rb alteration. They showed that Delta 24 inhibited the growth of glioma cells implanted subcutaneously. Ansardi et al constructed oncolytic RNA-based vectors derived from poliovirus and termed replicons [37]. They showed that replicons suppressed tumor growth and extended the survival of the animals with intracranial tumors.

Immunomodulation

Central nervous system (CNS) had been considered as immune-privileged site. The important factors of tolerance for activated host immune are the presence of the blood-brain barrier and the absence of a lymphatic drainage system [68]. However, lymphocytic infiltration has been observed frequently in malignant gliomas and the degree of infiltration seems to correlate with survival [69]. Moreover, disability to elicit the immune reaction implies that the oncogenesis of primary glioma cells occurs without interaction to immune cells. Therefore, glioma cells derived from brain parenchyma may be more immunogenic than tumors derived from peripheral system and the immunogene therapy is very attractive for therapy of malignant glioma.

IL-2

Interleukin (IL)-2, a cytokine produced by activated T cells, can promote immune reactions. Fibroblasts, genet-ically engineered to secrete IL-2, suppress tumor growth and induce antitumor immunity to murine gliomas in vivo [41]. Clinically, patients with malignant glioma were subcutaneously immunized with autologous glioma cells and received IL-2 secreting fibroblasts [42]. Posttreatment with MRI revealed the marked tumor necrosis, but the tumor did not disappear. As shown in vivo immunotherapy model using IL-2, the combination of immunization in peripheral tissues and intracerebral transplantation of IL-2-producing cells is necessary to eliminate established brain tumors [43].

IL-4

IL-4 is a multifunctional lymphokine produced by helper T cells and has a broad range of activities on both B and T lymphocytes. When IL-4-transduced glioma cells were injected into animals, eosinophil infiltration and inhibition of tumor angiogenesis were observed in athymic mice [45] and T-cell infiltration and humoral response were shown in immunocompetent rats [46]. Furthermore, retroviral packaging cells producing IL-4 were produced [44]. When these cells were injected into established intracranial tumors, tumors were completely eradicated and inhibition of tumor angiogenesis and infiltration of T cells and macrophages were revealed.

IL-12

IL-12 is secreted by antigen-presenting cells such as dendritic cells, macrophages or microglia [70]. Among cytokines, it has been demonstrated that IL-12 possesses particularly potent antitumor properties [71]. It is because IL-12 plays a critical role in mediating inflammatory and immune responses in host defense, IL-12 exerts a variety of biological effects on T cells and natural killer (NK) cells [72], including induction of interferon (IFN)-γ production [71], enhancement of proliferation and cytolytic function of T cells and NK cells [73], and promotion of the Th1-type immune response [74]. In addition to stimulatory effects on the immune system, IL-12 is also a potent antiangiogenic factor [75]. Local delivery of IL-12 by genetically engineered cells significantly prolongs the survival time in animals with brain tumor [47]. Moreover, a single intratumoral treatment of nude mice with a vaccinia virus (VV) expressing IL-12 induced significant tumor growth inhibition [48]. However, most animals injected with high doses of recombinant viruses (10^5 to 10^7 pfu) showed signs of cytokine toxicity.

Interferon (IFN)

IFNs are produced by activated immune cells including T cells, NK cells, and monocyte lineage cells. Glioma cells transfected with the human β-interferon gene by liposomes, elicit systemic immune reactions and inhibit the tumor growth [49]. In addition, IFNs directly stimulate cell differentiation and apoptosis via signals from interferon receptors on glioma cells [50].

TNF-α

Tumor necrosis factor (TNF)-α was initially supposed to be a promising cancer therapeutic reagent. However, recent investigation shows that TNF-α does not kill most types of cancer cells partly due to the activation of an anti-apoptotic gene, NF-κB [76]. Therefore, suppressing NF-κB is expected to potentiate TNF-α-induced apoptosis. Recently, it has been demonstrated that combination of TNF-α with other cytokines [51], radiation [52], chemotherapy [53], or hyperthermia [54] is more effective in therapy for glioma models than single treatments.

Others

Angiogenesis inhibitors

Based on the observation that gliomas are among the most angiogenic tumors, therapeutic strategies aimed to inhibit angiogenesis are theoretically attractive. Angiostatin, an internal peptide fragment of plasminogen, has recently been shown to potently inhibit endothelial proliferation in vitro and tumor growth in vivo [77]. The AAV (adeno-associated virus) vector has been able to deliver sustained and high-level gene expression in vivo. Intratumoral [55] or intramuscular [56] injection of a high-titer AAV-angiostatin vector has rendered efficacious tumor suppression and resulted in long-term survival. However, recombinant angiostatin, a peptide fragment, might be unstable in vivo. A tricistronic retroviral vector, expressing natural antiangiogenic factors, inhibits angiogenesis in vitro, but is not able to block tumor progression in vivo [57]. A sustained in vivo protein delivery is required to achieve the therapeutic effects. Endostatin, internal peptide fragment of 18 collagen, is also an angiogenic inhibitor. Engineered C6 cells that endogenously express mouse endostatin reduced tumor growth in vivo [58]. However, complete tumor inhibition was not observed in either the athymic or immunocompetent tumor models. Antiangiogenetic therapy using these peptides might be developed as an adjuvant gene therapy for the effective treatment of malignant gliomas.

Neural stem cells

Gene therapy of glioblastomas is limited because viral vectors usually are unable to survive for long time, continue to express proteins, and hardly reach glioblastoma cells infiltrating the brain parenchyma. Neural stem cells, implanted distant from brain tumor lesion into experimental intracranial gliomas in vivo, migrated brain parenchyma towards brain tumor site, chasing the infiltrating tumors [59]. This migratory cell delivery method has the potential to expand the range of delivery of HSV-1 vectors to tumor cells in the brain [60]. Genetically engineering neural stem cells expressing IL-4 elicited the systemic immune response and inhibited tumor growth [61]. Moreover, supernatant of neural stem cells inhibited the proliferation of glioma cells [61]. Therefore, neural stem

TABLE 2. Vectors.

Viral vectors	References
Retrovirus	[8, 13, 15, 44, 57]
Adenovirus	[5, 14]
Adeno-associated virus	[9, 56]
Nonviral vectors	References
Antisense oligonucleotide	[25, 33, 34, 80, 81]
Naked DNA plasmid	[32, 49, 82, 83]

cells may be an ideal vehicle to overcome the above difficulties in gene therapy of malignant gliomas.

VECTORS

Vector development is an important field of study, because efficacy of gene transfer depends mostly on the ability of vectors to be incorporated into tumor cells. Vectors for gene therapy can be divided into classes of viral and nonviral systems (Table 2). Viruses are effective vehicles for gene delivery as they can enter human cells and express their genes specifically and efficiently. The main device for viral vector development is improving the targeting efficiency of viruses, while abrogating their ability to cause disease. Modifying the viral genome to remove sequences necessary for viral replication and pathogenecity makes it possible to achieve these goals. The removed viral coding sequence can be replaced with exogenous therapeutic genes. Such genetically engineered viruses theoretically keep wild-type viral cellular tropism and ensure transgene expression in the target cell population without causing harmful diseases. Efforts to alter the natural tropism of viruses by manipulating the viral components that mediate cell binding and internalization represent a means of leading viruses specifically to chosen target cells.

Viral vectors

Retrovirus

Retrovirus and adenovirus have been used for a wide variety of gene therapy applications. Retrovirus is a single strand RNA virus. Retrovirus enters cells by binding surface envelope protein, encoded by the *env* gene, to specific cellular receptors. After entering cells, the viral enzyme reverse transcriptase, encoded by the *pol* gene, transcribes the viral genome into a double-strand DNA copy. Double-strand DNA can enter the nucleus of dividing cells and become integrated randomly into the host genome. This event occurs preferentially in dividing cells, meaning the virus does not enter neurons or other normal brain cells. Retroviruses used in gene therapy protocols are designed to be replication-deficient by removing their *gag*, *pol*, and *env* genes. Therefore, infectious but replication-deficient retrovirus particles are produced in packaging cells that express retrovirus *gag*, *pol*, and *env* genes from plasmids lacking the packaging sequence. A great variety of in vivo

experiments [13, 44, 57] and clinical trials [8, 15] using retroviral vectors for gene therapy for malignant glioma have been performed. There are some drawbacks to use retroviruses as vectors. Retrovirus packaging cells produce relatively low titers. The retrovirus genome is small, which limits the size of genetic constructs they can carry. Random integration into the host genome may disrupt cellular genes by insertional mutagenesis.

Adenovirus

Adenovirus is a double-strand DNA virus. Adenovirus enters cells by binding to the adenoviral receptor, which promotes interaction of viral arginine-glycine-aspartate sequences with cellular integrins. After internalization, the virus escapes from cellular endosomes, partially disassembles and translocates to the nucleus, where viral gene expression begins. They can produce in high titers and infect non-dividing cells. Gene expression occurs without integration into the host genome and gutless adenovirus offers the opportunity to develop vectors with expanded capacity for therapeutic transgenes. Transduction efficiency of adenovirus is better than that of retrovirus. One of the drawbacks is that the induced genomes decrease by cell divisions. The administration is limited only once, as it possesses high antigenicity. Sandmair et al compared the effect of HSV-*tk* gene therapy combined with ganciclovir medication in malignant gliomas between retrovirus-packaging cell and adenovirus gene therapy [14]. Although the results are from a small number of patients and should be interpreted cautiously, adenoviral HSV-*tk* gene therapy seems to be beneficial as shown by magnetic resonance imaging (MRI)-determined tumor regrowth, 3 months after gene therapy, and the survival of patients [14].

Adeno-associated virus

Enhanced gene delivery has been demonstrated in preclinical studies using adeno-associated viruses (AAV) vectors targeting malignant glioma cells [9, 56]. AAV is a native human parvovirus that does not cause any known human disease. They enter cells after binding to heparan sulfate but need coinfection with a helper virus (adenovirus or herpes virus) to replicate. Without coinfection of helper virus, AAV infection leads to latency, in which the viral genome remains in an integrated form or as episomal DNA. Subsequent infection of the cell with a virus capable of providing the necessary helper functions allows replication to proceed. AAV vectors have a number of potential advantages over retroviral and adenoviral vectors. They can infect a wide range of host cells independent of their cell cycling status and are stably integrated and maintained in the host genome in which transient transgene expression may be adequate. The drawbacks of AAV as vectors for gene therapy are limited packaging capacity of approximately 5 kbp and gene expression that may

be slow to reach its peak. In addition, production requires helper viruses, which may contaminate preparations for preclinical and clinical use.

Nonviral vectors

Gene transfer using nucleic acid therapeutics or nonviral vectors is much less immunogenic and cytotoxic than viral-vector systems. Although major weakness of nonviral vectors is that transduction efficiency is significantly lower than viral vectors, it can be overcome by frequent intratumoral injection or application of osmotic minipumps or polymer/microsphere system as described below.

Antisense oligonucleotide

Over the last two decades, cloning and sequencing of the critical genes in tumorigenecity have made progress remarkably. Many target genes attractive for antisense therapy have been identified. Antisense oligonucleotides are designed to bind to a certain sequence of specific mRNA and degrade it, providing a powerful tool for the cancer therapy. We have recently selected telomerase, a ribonucleoprotein enzyme, as a target for the therapy of malignant gliomas [25, 33, 34]. Telomerase is considered as a potential target of cancer therapy because malignant gliomas are predominantly telomerase-positive, while normal brain tissues do not express the enzyme [78, 79]. We have shown that a 19-mer antisense oligonucleotide against human telomerase RNA linked to a 2′, 5′-oligoadenylate (2-5A) inhibited malignant tumors growth in subcutaneous or brain tumor models in mice [25, 33, 34]. Interestingly, inhibition of telomerase activity resulted in apoptotic cell death. Angiogenic factors are also potentially optimal targets for antisense oligonucleotide because malignant gliomas are highly angiogenic. The antisense therapy against vascular endothelial growth factor (VEGF) was useful in down-regulation of VEGF expression, resulting in inhibition of growth of malignant glioma cells in vivo [80, 81].

Naked DNA plasmid

Gene transfer with naked DNA plasmid in the presence of the modified liposomes such as cationic liposome or the hemagglutinating virus of Japan (HVJ) liposome has been developed and applied to the treatment of malignant tumors. Multiple intratumoral administrations of liposomes containing the murine IFN-β gene resulted in the reduction of tumors in the brains of mice and elicited cytotoxic T lymphocytes without side effects [49]. Intramuscular injection of DNA plasmid encoding murine IFN-α leads to potent antitumor effects in mice bearing subcutaneous glioma cells [82]. Moreover, this gene transfer system has been applied to the treatment using the suicide gene [83], apoptosis inducible-genes [32], because this delivery system can be repeated.
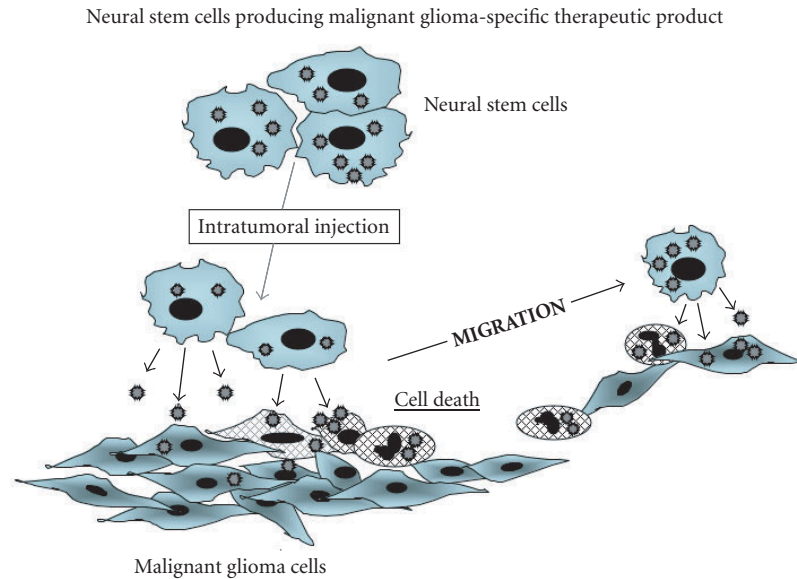
Neural stem cells producing malignant glioma-specific therapeutic product

FIGURE 1. Prospective gene therapy for malignant gliomas.

TABLE 3. Delivery systems.

	References
Direct intratumoral injection	[5, 8, 9, 13, 14, 15, 25, 32, 33, 34, 36, 37, 38, 39, 40, 44, 47, 49, 56, 57, 80, 81, 82]
Osmotic minipump	[83]
Polymer/microsphere	[84, 85, 86]
Neural stem cell	[59, 60, 61]

TABLE 4. Targeting.

	References
HTERT	[32, 87, 88]
MBP	[30, 31]
EGFR	[90, 91]
Stress (hyperthermia, hypoxia)	[92, 93]

DELIVERY SYSTEMS

The transfer of therapeutic genes into malignant brain tumors is the subject of experimental models and clinical trial of gene therapy (Table 3). Most approaches have used direct intratumoral placement of a variety of vectors and genes, such as retroviruses [8, 13, 15, 44, 57], replication-defective adenoviruses [5, 14], replication-competent or-conditioned oncolytic viruses [36, 37, 38, 39, 40], antisense oligonucleotides [25, 33, 34, 80, 81], and naked plasmid vectors [32, 49, 82, 83]. These approaches have shown the efficiency of gene therapy. However, these approaches need to repeat injections or are unable to keep continuous gene expression.

Using a minipump combined with stereotaxic techniques allows continuous delivery of therapeutic genetic materials into the brain. Continuous intracerebral delivery of liposome-mediated HSV-*tk* gene complexes using an osmotic minipump led to tumor regression in the treated animal [83]. Polymer microspheres can encapsule antisense oligonucleotide, naked DNA plasmid, viral particle, or monoclonal antibody, and release them. This

system not only decreases treatment frequency, but also reduces the potent immune response by sequestering the content from antibody exposure, leading to improvement of in vivo efficacy [84, 85, 86]. However, the above systems are unable to effectively distribute the genetic materials into the target cell population. As described above (Table 1), neural stem cells may have the potential to chase the infiltrating tumor cells [59, 60, 61].

TARGETING

Gene transfer vectors will dramatically increase the safety and effectiveness of cancer gene therapy, if they can restrict expression of the therapeutic products to the target tumors. Substances that are overexpressed in tumor cells but not in normal cells are good targets for gene therapy (Table 4). Although no specific marker is known for malignant gliomas, four targets including us came up with expression regulating system using specific promoters. First, we used the promoter of the human telomerase reverse transcriptase (hTERT) gene and developed expression vectors of caspase-6 [32], caspase-8 [87], or FADD [88] under the control of the promoter, respectively. The activity of telomerase is tightly regulated at the

transcriptional level of the hTERT gene [89]. Since about 75% of malignant gliomas have telomerase activity while normal brain tissues do not have the enzyme [78, 79], we can restrict the expression of apoptosis-inducing proteins to malignant glioma cells. We showed that the growth of subcutaneous tumors was inhibited due to induction of apoptosis after the treatment [32, 87, 88]. Second, Shinoura et al used the myelin basic protein (MBP) promoter to regulate the expression of Bax and caspase-8 [30, 31]. They showed that the growth of tumors in the animal model was suppressed. Third, epidermal growth factor receptor (EGFR) is often highly expressed in tumor but not in normal brain. EGFR may be a good target to increase the selectivity of delivering genes to tumor cells [90, 91]. Forth, stress can also be a target for tumor specific expression of therapeutic genes. The presence of hypoxic cells in human brain tumor is an important factor leading to resistance to radiation therapy. However, this physiological difference between tumor and normal tissues also provides the potential for designing cancer-specific gene therapy [92]. When the gene expression is triggered by heat stress, combined therapeutic effects of hyperthermia and gene therapy may be promising [93].

CONCLUSIONS

In recent years, many neuro-oncologists have focused on evolving gene therapy as a new therapeutic modality for malignant gliomas. However, clinical success has been limited due to insufficient gene transfer or limited spread of therapeutic genes. These obstacles may be overcome by neural stem cell-guided gene therapy (Figure 1). Neural stem cells are expected to migrate extensively into malignant gliomas in the brain, although further investigation is necessary. Therefore, the application of neural stem cells producing tumor-specific therapeutic product will offer a means of accessing invasive tumor cells. If we can engineer neural stem cells to produce oncolytic virus as tumor-specific therapeutic product, this approach may be significantly enhanced. It is expected that replication-conditional or competent oncolytic virus will significantly increase the extent of gene transfer into tumors compared to replication-defective virus. However, issues such as controlling virus replication and keeping normal cells intact should be confirmed. The use of tumor-specific promoter such as hTERT promoter system in the brain will increase the safety of oncolytic virus produced by neural stem cells.

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* Corresponding author.
E-mail: seikondo@mdanderson.org
Fax: +1 713 794 5514; Tel: +1 713 792 3526