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· Review ·

Current status of gene therapy for gliomas

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[**ABSTRACT**] Presently glioma remains one of the most difficult tumors to treat. In the past 20 years, although with a series of progressive adjuvant therapies, the prognosis for patients with malignant glioma is still poor. With the development of medical molecular biological techniques, some inspiring and valuable results from a few experimental researches for gene therapy of gliomas had stimulated some phase I clinical trials. However, some problems about the effectiveness of gene therapy had been met in the animal models and clinical trials. How to overcome these barriers of gene therapy for gliomas and develop the biological treatment at the greatest possibility is still one of the most important research topics. To understand current status in gene therapy for gliomas, this paper reviews the basic categories of the gene therapy strategies and its obstacles.

[**KEY WORDS**] gliomas; gene therapy; review literature

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Malignant gliomas remain one of the most difficult tumors to treat and affect approximately 90 000 individuals per year in China, with extremely high mortality. The current treatments for malignant glioma include surgical resection, radiation therapy, and conventional chemotherapy. These measures only increased the average lifespan by a few months and the prognosis is still very poor in the past 25 years, suggesting that modern advances in surgical resection, radiation therapy, and conventional chemotherapy had little effect on the behavior of these tumors. Therefore, development of novel therapies is of great importance. Experimental gene therapy for brain tumors is becoming a hot topic in the field of neurosurgery.

1 BASIC CATEGORIES OF GENE THERAPY STRATEGIES

The gene therapy strategies can be divided into 5 basic categories[1]: (1) gene-directed enzyme prodrug ("suicide gene") therapy (GDEPT); (2) gene therapy designed to boost the activity of the immune system against cancer cells; (3) oncolytic virus therapy; (4) transfer of potentially therapeutic genes into cancer cells; (5) antisense therapy. The first 3 approaches are designed to destroy cancer cells. In the last 2 approaches, cancer cells may be destroyed, but the primary objective is to alter the biological behavior of the target cells.

1.1 Gene-directed enzyme-prodrug therapy

Malignant gliomas show minimal responces to currently available chemotherapy. This is, in part, due to the difficulty in delivering high levels of chemotherapeutic agents across the blood-brain barrier without significant systemic toxicity. This can be potentially overcome by producing high levels of chemotherapy within the tumor "itself". A variety of prodrug-activating enzymes and prodrugs have been tested for this so-called "suicide gene" therapy. Importantly, the benefit of enzyme-prodrug gene therapy extends to tumor cells in the tumor mass that have not taken up the therapeutic gene. This so-called "bystander effect" allows a potentially inefficient gene transfer process (transduction of a small percentage of the tumor cells) to have a large therapeutic effect[2]. The activated drug is exported to adjacent cells through gap junction connections between adjacent cells. Gap junctions appear to be important in the HSV-tk/Ganciclovir system, in which the active metabolites are not very stable outside of the tumor cell. Tumor cell death, as a result of the activated drug, may release more active drug that is taken up by neighboring tumor cells.

Oldfield *et al*[3] was the first to apply enzyme-

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prodrug gene therapy to patients with malignant gliomas. Fifteen patients with progressive malignant brain tumors were treated in the initial clinical trial. The therapy was well tolerated, although seizures and focal hemorrhages were observed in some patients. Two patients with glioblastoma multiforme had durable responses lasting more than 4 years. Shand *et al*[4] treated 48 patients with recurrent glioblastoma also using intracerebral injection of retroviral vector-producing cells immediately after tumor resection; patients were treated with daily infusions of ganciclovir 14 to 27 d after surgery. Although the treatment was found to be reasonably safe, median survival time was only 8.6 months, with 27% of the patients alive at 12 months. Klatzmann *et al*[5] treated 12 patients with recurrent glioblastoma with retrovirus-mediated HSV-TK gene transfer. Median survival was less than 7 months, with 25% of patients living longer than 12 months. Also, a similar phase I clinical trial, in which 25 patients with primary or secondary malignant gliomas were treated by HSV-TK/Ganciclovir system, was finished in our institute from 1997 to 2000. We have demonstrated that the HSV-TK/Ganciclovir system therapy was effective and safe, with median survival time being (470±93.17) d. The survival time of 33% of patients were more than 18 months[6].

These studies also highlighted some of the limitations of retroviral gene therapy. Producer cells needed to be injected because the virus could not be concentrated sufficiently. The handling of producer cells, which must remain viable, is cumbersome and difficult to apply in general clinical practice. Because the virus only transduces dividing tumor cells, tumor cells that are mitotically arrested (a significant proportion) do not express the therapeutic gene. Some authors have sought to overcome these problems using a replication defective adenovirus vector[7].

1.2 Immuno-gene therapy Of the gene therapy strategies used clinically to treat cancer, immunomodulatory trials have been the most frequently used. Cancer immunogene therapy can be defined as the transfer of immunostimulatory genes to human cells to stimulate antitumor immune responses and to activate cell-mediated immunity. Many cancer immunotherapy approaches have been proposed, including nonspecific immune adjuvants (cytokines), antitumor antibodies (serotherapy), antitumor lymphocytes (adoptive immunotherapy), and antitumor vaccines (active immunotherapy).

Recent advances in molecular biology and immunology have suggested novel methods to stimulate antitumor immunity. Many cancer immunogene therapy studies have focused on genetically engineered cancer vaccines. Tumor cells from a given patient can be cultured *in vitro* and genetically modified to increase their immunogenicity. Genetically modified tumor cells are attenuated by irradiation to prevent further cell division and readministered subcutaneously to the patients from whom they were derived. Numerous proinflammatory genes are potentially useful for immunogene therapy. These include genes encoding proinflammatory cytokines such as IFN-γ, granulocyte-macrophage colony-stimulating factor (GM-CSF), or IL-12, T cell costimulatory molecules (B7-1, B7-2), and MHC molecules[8].

Glioma immunogene therapy is not limited to the transfer of proinflammatory genes. Vaccination with irradiated autologous glioma cells expressing antisense molecules that block glioma-derived immunosuppressive factor expression has also been successful in several models. Insulin-like growth factor (IGF)-1 has been a target for glioma antisense immunogene therapy. Vaccination with rat C6 glioma cells transfected with IGF-1 or IGF-1 receptor antisense suppressed C6 glioma growth subcutaneously and intracranially in a $CD8^+$T cell-dependent manner.

1.3 Oncolytic virus therapy As noted in the discussion of enzyme-drug gene therapy, the spread of virus within the tumor may be quite limited when replication-defective viruses are used. This may be potentially overcome by employing a replication conditional virus (*i.e.*, a virus that selectively replicates only in the tumor and not in the normal brain parenchyma). In theory this should provide better tumor penetration as the virus grad-

ually spreads through the tumor. In the oncolytic virus strategy, the viruses are allowed to remain replication-competent. Viral replication within cancer cells results in lysis of the cell, and the production of progeny virions, which have the ability to infect and destroy adjacent cancer cells.

1.4 Therapeutic gene transfer-Replacement gene strategy The replacement gene strategy seeks to introduce a functional gene, such as a tumor suppressor gene, into the patient's cells because the gene is defective or absent. Tumor suppressor gene therapy is being developed for several cancers including gliomas. The overexpression of wild-type p53 can suppress tumor growth even in cells that express endogenous wild-type p53. Multicenter clinical trials to evaluate intratumoral injections of the tumor suppressor gene protein p53 are currently underway for pateints with malignant glioma[1]. p53 has been found to be functionally inactivated in about 40% of malignant gliomas. Although treatment with p53 has been the prototype for this strategy, other gene therapies have been tested in experimental animals in which the object is to express other beneficial proteins.

1.5 Antisense therapy Antisense-mediated gene inhibition has also been considered a type of gene therapy. Undesirable genes in the tumor cells are being "targeted", therefore, this strategy could be called "gene targeted therapy".

Two main antisense strategies have been employed: (1) transfection of cells with antisense cDNA; (2) treatment of cells with antisense oligodeoxynucleotides (ODNs). The former strategy has been successfully used against glioma cells *in vitro* and in animal models.

The stability and subsequent half-life of these compounds is mainly determined by whether or not they have undergone chemical modification. Direct ODN infusion into the brains of animals has shown extensive penetration and minimal toxicity. In animal models, transcription of a variety of genes has been successfully blocked within the brain, using antisense ODN infusions. Clinical trials with ODNs are now proceeding for a variety of cancers[9].

2 OBSTACLES TO GENE THERAPY OF BRAIN TUMORS BY PHYSICAL BARRIERS

As described previously, most brain tumor gene therapy strategies require viral delivery to targeted tissue within the CNS. Regardless of the gene transfer vector or the use of lytic viruses, all these approaches are associated with several barriers including the BBB, blood-brain-tumor-barrier (BBTB), innate immunity, and other factors that neutralize the viruses before they reach the targeted tissue. Innate immunity is the ability of a naive animal or human to inhibit infectivity of a virus without prior sensitization. In animals or humans who have been exposed to the virus previously, there is a full immune response and significant inhibition of the injected virus.

2.1 Blood-Brain Barrier The normal BBB restricts trans- and peri-cellular movement of blood-born molecules, effectively filtering most ionized, water-soluble molecules greater than 180 in mass[10]. In the case of brain tumors, the BBB is frequently not intact in the center of the malignancy as demonstrated by computerized tomography and MR imaging. However, the presence of an intact BBB at the proliferating edge of the tumor has been suggested to be one of the major contributing factors to the failure of chemotherapy in the treatment of CNS neoplasms[10]. To achieve therapeutic levels of chemotherapeutic drugs within the brain, different properties of the BBB have been exploited to disrupt the BBB, including hyperosmotic disruption and receptor-induced BBB permeability modification.

Osmotic BBB disruption has been reported to occur in a number of animal systems[11] and is currently being used clinically to increase delivery of chemotherapeutic agents for the treatment of brain tumors in humans. It is believed that osmotic shock disruption of the BBB results in the shrinkage of endothelial cells, opening the tight junctions and allowing passage of larger molecules including antibodies and viral particles. The most investigated hypertonic solution is mannitol which is approved for administration in patients. Permeability of the

BBB is increased significantly (5-15 min) after the infusion of mannitol, and it normalizes within 2 h.

Attempts to modulate the permeability of the BBB pharmacologically have also been undertaken. In model systems in which investigators have tested the efficacy of the titration of either bradykinin or RMP-7, increased permeability has been demonstrated in selected tumor models (RG-2 glioma in rats) without altering the permeability of vessels in surrounding brain. This change in permeability was translated to a 2.8-fold increase for intravenously administered low-molecular weight molecules and greater than a 10-fold increase for high-molecular weight dextran. In the RG-2 glioma tumor model rats treated with RMP-7 and carboplatin survived significantly longer than those treated with carboplatin alone[12]. Additionally RMP-7 has been shown to increase brain tumor permeability to ganciclovir, an observation that may be important for the TK/Ganciclovir gene therapy strategy[13].

2.2 Innate barriers A common problem in achieving therapeutic levels of many molecules injected into the circulation is the "first-pass" effect of the liver in which active molecules are significantly removed from the circulation. This is also true with virus, and as much as 35% of injected HSV can be found in the liver soon after intravenous injection[14]. Regional delivery through the arterial blood supply may provide better delivery of the virus to the brain by eliminating first-pass effects caused by the liver. In an effort to further augment the delivery of virus to the brain, the addition of BBB disrupting agents such as mannitol or disrupters of the BBTB, such as bradykinin or its analog RMP-7, have been shown to increase infection of brain tumors after intra-arterial administration of HSV or adenovirus[15]. Despite these efforts, viral infectivity of brain tumors after intraarterial administration remains relatively poor.

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