Chapter 2
Gene-Based Therapy for Cancer: Brain Tumors

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Abstract Malignant gliomas are a devastating disease with dismal prognosis. Gene-based therapies hold the promise for more specific and efficacious intervention. Clinical experiences have revealed the limits of gene transfer by replication-deficient adenoviral or retroviral vectors. The problem has been partially improved with the bystander effect of suicide gene therapy. Replication-competent oncolytic viruses are expected to bridge the vector gap since the viruses replicate and spread the progeny to the adjacent cancer cells. The oncolytic viruses discussed in this review represent either genetically engineered (adenovirus, herpes simplex virus-1 and measles virus) or naturally occurring (reovirus and newcastle disease virus) strains of viruses that exhibit relatively selective replication in tumor cells. Clinical trials demonstrate that these viruses are well tolerated in glioma patients. Thereafter, certain challenges need to be addressed in future clinical studies to achieve desirable efficacy.

Keywords Glioma • Oncolytic virus

1 Introduction

Malignant gliomas account for approximately 70% of the 22,500 new cases of malignant primary brain tumors that are diagnosed in adults in the US each year (Wen and Kesari 2008). Although relatively uncommon, malignant gliomas are associated with disproportionately high morbidity and mortality. Despite optimal treatment, the median survival is only 12–15 months for patients with glioblastomas

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and 2–5 years for patients with anaplastic gliomas (Wen and Kesari 2008). The clinical outcome of patients with glioblastomas almost remained the same for the past decade (Furnari et al. 2007). According to the CRS Brain & CNS Cancers Workshop, June 2007, high rates of mortality convert malignant glioma into the first leading cause of cancer death in children, the third leading cause of cancer-related death among men 15–54 years of age and the fourth leading cause of death for women 15–34 years of age.

Malignant gliomas are highly resistant to conventional therapies (i.e., surgery, radiation, and chemotherapy). Tumor recidivism followed by unstoppable progression is the rule. Thus, more efficacious and specific therapies are urgently needed. As the genetic basis of brain tumors has been delineated, many molecular defects have been tagged as promising targets for therapy. Viral vectors have been used in preclinical and clinical studies to transfer therapeutic genes into cancer cells. However, experience has shown that targeting only one gene or one pathway at a time may not be an effective approach to tackle the heterogeneous glioblastomas, in which many of the genes involved in regulating cell proliferation, differentiation, and death are abnormal. Moreover, replication-deficient adenoviruses lack the ability to reach to enough number of cancer cells to induce significant anti-cancer effect. A goal is now to design strategies to target universal defects in cancer cells and develop agents with expandable cytotoxic power only in cancer cell population. Oncolytic viruses hold the promise for implementing such strategies. Oncolytic viruses represent either genetically engineered or naturally occurring strains of viruses that exhibit relatively selective replication in tumor cells. Naturally, viruses induce changes in host cells to create an environment favorable for viral replication that are similar to the processes involved in cellular transformation (Thorne et al. 2005). These include uncontrolled cellular proliferation, prevention of apoptosis, and resistance to host organism immune effector mechanisms (Thorne et al. 2005). Viral strains or mutants that are defective in these processes are exploited as oncolytic viruses.

In this review, we summarize the principle and the application of replication-deficient and replication-competent (oncolytic) viral vectors in the therapy for malignant brain tumors (Table 1). Our focus is on those therapies that have already arrived to clinical trials in glioma patients.

2 Replication-Deficient Viral Vectors

2.1 Ad-*p53*

One of the most frequent genetic alterations in cancer is p53 mutation which occurs in more than 50% of human cancers (Levine 1997). p53 is frequently inactivated in brain tumors either through mutation of the *p53* gene or posttranslational regulations (Lang et al. 2003). The inactivation of p53 is a critical event in the formation

Table 1 Clinical trials testing gene-based therapies in glioma patients

Therapy	Virus type	Targeted defects in glioma	Clinical trial status	Route of delivery	References
Replication-deficient viral vectors					
Ad-p53	Replication-deficient adenovirus	p53 pathway	Phase I	I.T. I.B.	Lang et al. (2003)
RV-HSVtk/GCV	Replication-deficient retrovirus	Cell proliferation	Phase III	Virus: I.B. GCV: I.V.	Rainov (2000)
HSVtk/IL-2	Replication-deficient retrovirus	Cell proliferation	Phase I/II	RVPC: I.T GCV: I.V.	Colombo et al. (2005)
Ad-HSVtk/GCV (Cerepro)	Replication-deficient adenovirus	Cell proliferation	Phase III	Virus: I.B. GCV: I.V.	Immonen et al. (2004)
Oncolytic viruses					
Onyx-015	Adenovirus	RNA export	Phase I	I.B.	Chiocca et al. (2004)
Delta-24-RGD	Adenovirus	RB pathway	Phase I	I.T.	N/A
G207	HSV-1	Ras pathway Cell proliferation	Phase I/Ib	I.T. I.B.	Markert et al. (2000, 2009)
HSV1716	HSV-1	Ras pathway	Phase I	I.T. I.B.	Rampling et al. (2000), Papanastassiou et al. (2002), Harrow et al. (2004)
Reolysin	Reovirus	Ras pathway	Phase I/II	I.T.	Forsyth et al. (2008)
MV-CEA	Measles virus	CD46 Ras pathway	Phase I	I.T.	N/A
HuJ	Newcastle disease virus	Ras pathway	Phase I/II	I.V.	Freeman et al. (2006)

I.T. intratumoral, *I.B.* injected into adjacent brain posttumor resection, *I.V.* intravenous, *RVPC* retroviral vector-producing cell

and progression of gliomas (Lang et al. 2003). Since wild-type p53 is a primary mediator of cell cycle arrest and apoptosis, it is rational to transfer *p53* into cancerous cells to control tumor growth and achieve therapeutic benefits.

Ad-*p53* is a type 5 replication-deficient human adenovirus in which the E1 region has been replaced with the cDNA of the wild-type *p53* gene driven by the cytomegalovirus promoter (Zhang et al. 1993; Zhang et al. 1995). Ad-*p53* has been shown to be effective against a variety of tumor types (Lang et al. 2003). Only minimal toxicity has been observed in patients with lung or head and neck cancer after direct intratumoral injection during Phase I clinical trials (Lang et al. 2003). Because of the promising preclinical results in brain tumors and the encouraging clinical results in other tumor types, the clinical potential of Ad-*p53* in the treatment of human gliomas has been tested in a Phase I clinical trial. In all 15 patients enrolled, exogenous p53 protein was detected within the nuclei of astrocytic tumor cells (Lang et al. 2003). Exogenous p53 transactivated $p21^{CIP/WAF}$ and induced apoptosis were observed (Lang et al. 2003). However, transfected cells resided on average within 5 mm of the injection site (Lang et al. 2003). Although toxicity was minimal, the widespread distribution of this agent remains a significant goal (Lang et al. 2003).

2.2 HSVtk/GCV Gene Therapy

Because tumor cells divide more rapidly than normal cells, cytotoxic nucleotide analogs are widely used in chemotherapy to reach a therapeutic index in which damage to the cancer cells is maximized while keeping the toxicity to the normal host cells acceptable. To further achieve an optimal therapeutic effect while limiting systemic toxicity, gene-directed enzyme prodrug therapy (GDEPT, suicide gene therapy) has been developed (Fillat et al. 2003). This is a two-step therapeutic approach for cancer gene therapy. In the first step, the transgene is delivered into the tumor and expressed. In the second step, a prodrug is administered and is selectively activated by the expressed enzyme. The first GDEPT system described was the thymidine kinase gene of the herpes simplex virus (HSVtk) in combination with the prodrug Ganciclovir (GCV). The thymidine kinase enzyme (tk), produced by the herpes simplex virus (HSV), is harmless to humans since it lacks a substrate in the human body. However, in cells that express tk, the tk can metabolize intravenously administered GCV to produce a cytotoxic GCV triphosphate, which is selective for dividing cells (Fillat et al. 2003). This is of particular significance in the brain where the normal neurons surrounding the tumor are nonproliferative and therefore not susceptible to toxic metabolites. The treatment effect is further strengthened by a "bystander effect" (Fillat et al. 2003).

Using the retrovirus (RV)-mediated transduction of glioblastoma cells with HSVtk gene and subsequent systemic treatment with GCV, a Phase III, multicenter, randomized, open-label, parallel-group, controlled trial has been performed in the treatment of 248 patients with newly diagnosed, previously untreated

glioblastoma (Rainov 2000). The results revealed that the adjuvant treatment improved neither time to tumor progression nor overall survival time, although the feasibility and good biosafety profile of this gene therapy strategy were further supported. The failure of this specific protocol may be due mainly to the presumably poor rate of delivery of the HSVtk gene to tumor cells.

Since antitumor immune response was observed during HSVtk/GCV therapy (Barba et al. 1994), to amplify this antitumor immune response, a strategy was developed based on the combined delivery of a cytokine gene (human interleukin-2, *IL-2*) together with *HSV-TK* (Pizzato et al. 1998; Barzon et al. 2002; Barzon et al. 2003). In preclinical experimental models, it demonstrated not only the efficient killing of transduced cancer cells but also the growth inhibition of distant nontransduced tumor masses (Barzon et al. 2003). In a Phase I/II clinical study, a total of 12 patients with recurrent glioblastoma multiforme received intratumor injection of retroviral vector-producing cells (RVPCs), followed by intravenous GCV (Colombo et al. 2005). The results demonstrated that the intratumor injection of RVPCs was safe, provided effective transduction of the therapeutic genes to target tumor cells, and activated a systemic cytokine cascade, with tumor responses in 50% of cases.

In another randomized, controlled study, HSVtk gene was transduced by a replication-deficient adenoviral vector (type 5) (Immonen et al. 2004). AdvHSV-tk treatment produced a clinically and statistically significant increase in median survival time of 36 patients with operable primary or recurrent malignant glioma. Six patients had increased anti-adenovirus antibody titers, without adverse effects. The treatment was well tolerated. It is concluded that AdvHSV-tk gene therapy and GCV is a potential new treatment for operable primary or recurrent high-grade glioma. Recently, a Phase III multicentre, standard care-controlled, pivotal trial in 236 patients with operable high grade glioma revealed results that are consistent with those previously reported. This therapeutic regimen (Cerepro), developed by Ark Therapeutics Group plc, works by harnessing healthy cells to produce GCV triphosphate that is either incorporated into DNA or inhibits the polymerase to destroy newly growing cancer cells (bystander effect) (Fillat et al. 2003). It has been granted Orphan Drug Status by the European Committee for Orphan Medicinal Products and by the Office of Orphan Products Development, FDA. Approvals for named patient supply of Cerepro have been given by the French Medicines Control Agency (AFSSAPS) in February 2009 and also by the Finnish Medicines Authorities (NAM) in May 2009.

3 Oncolytic Viruses

3.1 Adenovirus

Adenoviruses are a nonenveloped virus with a single, linear, double-stranded DNA genome of approximately 36–38 kpb in size (Fields et al. 2007). Wild-type adenoviruses induce cell death through replicating in and lysing infected cells.

This cytotoxic capacity, together with the efficiency with which viruses can spread from one cell to another, inspired the notion that replication-competent viruses could be a solution for the problems encountered in delivering gene therapy for cancer. Because adenoviruses require host factors for replication, they express a series of genes immediately after infection to reprogram the host cell to facilitate replication. These so-called early genes encode proteins that bind and inactivate cellular regulators of cell cycle and apoptosis. The first adenoviral gene expressed after infection is called E1A. The E1A products bind to and inactivate critical cellular proteins, such as the RB family of pocket proteins (Jiang et al. 2006). Inactivation of these proteins propels the cell into unscheduled DNA synthesis, which creates a favorable environment for adenoviral replication. However, forced entry into S phase may trigger p53-mediated apoptosis during the early stage of viral infection, aborting the process of replication. To prevent the p53-induced apoptotic response, adenovirus encodes other early proteins, such as E1B-55K, E4orf6 and E1B-19K, to bind and inactivate p53 and Bax, resulting in a prolonged cell life that ensures the propagation of virions (Jiang et al. 2006). Such biological logic allows the design of replication-competent adenoviruses unable to express proteins that interact with cell cycle regulators or apoptosis inducers. Thus, these mutant viruses can propagate selectively in cancer cells, but are unable to acquire a replication phenotype in normal cells.

3.1.1 ONYX-015

Based on the knowledge of virus–host cell interactions, a mutant adenovirus *dl*1520/ONYX-015, which does not express the E1B-55K protein to inactivate p53, was constructed and tested for anticancer effect (Bischoff et al. 1996). ONYX-015 was demonstrated to have preferential replication as well as anti-tumor efficacy in some p53-deficient human tumor cells (Bischoff et al. 1996; Heise et al. 1997). The work on ONYX-015 pioneered the field of oncolytic adenoviral research and application. Further studies showed that loss of E1B-55K-mediated late viral RNA export, rather than p53 degradation, restricts ONYX-015 replication in primary cells (O'Shea et al. 2004). In contrast, tumor cells, which have altered mechanisms for RNA export to complement the RNA export function of E1B-55K, support ONYX-015 replication (O'Shea et al. 2004).

ONYX-015 has undergone extensive testing in the clinic and has proved safe at the doses up to 2×10^{12} viral particles (McCormick 2003). A dose-escalation trial of intracerebral injections of ONYX-015 for patients with recurrent malignant glioma, conducted by the National Cancer Institute's New Approaches to Brain Tumor Therapy (NABTT) CNS Consortium, showed that injection of ONYX-015 into the tumor cavity after glioma resection is well tolerated at doses up to 10^{10} plaque-forming units of the virus (Chiocca et al. 2004). ONYX-015 has been administered to over 250 cancer patients in roughly 15 clinical trials in a variety of tumor types involving intratumoral, intravenous, intraperitoneal, and hepatic arterial administration (Wildner 2005). Controlled clinical trials using the mutant as an oncolytic agent have led to important insights into the use of this virus as an

anticancer strategy (Wildner 2005). ONYX-015 as a single-agent treatment has disappointing efficacy, but in combination with chemotherapy shows encouraging antineoplastic activity (Wildner 2005).

3.1.2 Delta-24-RGD

The RB pathway is disrupted in virtually all human cancers. After adenovirus infection, in normal cells, E1A protein binds and inactivates RB protein. This function of E1A is not required in Rb-deficient cancer cells (Whyte et al. 1989; Pelicano et al. 2006). At M. D. Anderson Cancer Center, we have tested an oncolytic adenovirus Delta-24 with a mutant E1A protein that is unable to bind RB (Fueyo et al. 2000). The Delta-24 adenovirus replicates in and lyses cancer cells with great efficiency. In vivo, one dose of the virus induced a dramatic inhibition of tumor growth in nude mice. However, normal fibroblasts or cancer cells with restored RB activity were resistant to Delta-24. These findings suggest that Delta-24 may be therapeutically useful against gliomas, and also possibly against other cancers with a disrupted RB pathway (Fueyo et al. 2000). After the Delta-24 strategy was reported, this virus was tested in other laboratories, and it is currently being used at our center and others as a platform for the development of combined tumor-targeting approaches.

Because glioma cells consist of low levels of CAR (a native adenovirus receptor) and high levels of RGD-related integrins (Fueyo et al. 2003), adenoviruses retargeted to bind integrins should be able to circumvent the lack of CAR expression on the glioma cell surface, thus improving the ability of adenovirus to enter into cancer cells to achieve higher efficacy. In the first report of an oncolytic adenovirus (Delta-24) modified by the genetic introduction of an RGD sequence in the fiber HI loop, Suzuki and colleagues (2001) showed that the fiber-knob protein modification fostered CAR-independent transduction, enhancing viral propagation and an oncolytic effect in vitro and in vivo in prostate and lung cancers. When this RGD-modified vector Delta-24-RGD was later tested in gliomas, it was more cytopathic to both low- and high-CAR-expressing glioma lines than was Delta-24, and it replicated more efficiently in both types of cell lines (Fueyo et al. 2003). Comparing the results of intratumoral injection of Delta-24 and Delta-24-RGD in mice-bearing glioma xenografts, Delta-24-RGD was associated with an improved regression of the glioma xenografts and with longer survival (Fueyo et al. 2003). In addition, when combined with agents currently used in glioma therapy, such as RAD001 and temozolomide, Delta-24-RGD synergistically enhanced the therapeutic effect (Alonso et al. 2008; Alonso et al. 2007). In a recent study, Delta-24-RGD showed robust efficacy against brain tumor stem cells (BTSCs) that are responsible for cancer initiation and resistance to conventional chemo- and radiotherapy (Jiang et al. 2007). Delta-24-RGD significantly improved the survival of the mice-bearing gliomas derived from BTSCs (Jiang et al. 2007). The virus-induced autophagic cell death in BTSCs and the drastic upregulation of autphagic protein ATG5 can be used as surrogate markers to monitor the therapeutic effect of Delta-24-RGD in the future clinical trials (Jiang et al. 2007). In December, 2008, a Phase I clinical trial of

Delta-24-RGD started in patients with recurrent malignant gliomas at the Brain Tumor Center, UT M. D. Anderson Cancer Center.

3.2 Herpes Simplex Virus-1

HSV-1 is a well-characterized enveloped, double-stranded DNA virus whose genome is ~152 kb (Roizman 1996). This virus is especially attractive for the development of oncolytic vectors because it is highly infectious, replicates rapidly, and can be readily modified to achieve vector attenuation in normal brain tissue (Grandi et al. 2009). Tumor specificity can be achieved by deleting viral genes that are only required for virus replication in normal cells and permit mutant virus replication selectively in tumor cells (Grandi et al. 2009). G207 is an attenuated replication-competent HSV-1 with deletions of both copies of neurovirulence gene γ34.5 (encoding ICP34.5) and an inactivating mutation of U_L39, which encodes ICP6, the large subunit of HSV ribonucleotide reductase (RR) (Mineta et al. 1995). Loss of ICP6 expression restricts the replication of the virus to cells with elevated RR activity, presumably because of RB pathway abnormality that is common in glioma cells (Furnari et al. 2007). During viral infection, a stress response occurs in the host cell. Protein kinase R (PKR) activation shuts down translation in the infected cell as an anti-viral protective mechanism by phosphorylating and inactivating eukaryotic initiation factor-2α (eIF-2α) (He et al. 1997). The γ134.5 protein product ICP34.5 recruits protein phosphatase-1a in order to dephosphorylate eIF-2α and allow protein synthesis to proceed (He et al. 1997). Thus, defects in the ICP34.5 function restrict replication to cancer cells that possess decreased PKR activity, possibly due to the expression of a constitutively active form of RAS through mutations in upstream receptor tyrosine kinases (RTKs) or mutations of Ras itself (Farassati et al. 2001). In malignant gliomas, Ras mutations can be rare (Guha et al. 1997). However, the RTKs epidermal growth factor receptor (EGFR) and platelet-derived growth factor receptor (PDGFR) are commonly overexpressed in malignant glioma (Furnari et al. 2007), leading to overexpression of Ras and upregulation of the Ras signaling pathway.

G207 has been tested in Phase I/Ib trial in patients with malignant glioma (Markert et al. 2000; Markert et al. 2009). Administration was carried out by direct stereotactic injection into the tumor or the brain surrounding the resection cavity. Doses of up to 3×10^9 infectious units were well tolerated and a maximum tolerated dose was not achieved. The trials showed that the inoculation of an attenuated HSV, the wild-type counterpart's main pathogenic effect of which is encephalitis, remained relatively safe in a human brain. Recently, it was reported that temozolomide exhibited strong synergy with G207 through the induction of GADD34 and RR expression in malignant glioma cells and mice with intracranial gliomas (Aghi et al. 2006). These findings unveil the potential of HSV to target cells that survive temozolomide treatment.

Another HSV-1 mutant HSV1716 bears a deletion of 759 bp in each copy of the γI34.5 gene, resulting in the null expression of the PKR inhibitor ICP34.5

(Kesari et al. 1995). Due to this mutant's preferential proliferation in malignant cells, several studies have employed HSV1716 to treat glioma or brain tumors. Three Phase I clinical trials have been carried out to evaluate the safety of HSV1716. In one of these trials, patients with high-grade glioma were treated with the virus. HSV1716 DNA was detected by PCR at the sites of inoculation. In 5 patients (of 12 total), an immune response to the virus was detected. Although it remains unclear whether the immune response to the virus contributes to the eradication of cancer cells infected by the virus, a significant increase in long-term survival following surgery was also observed. These trials demonstrated that HSV1716 can replicate selectively in high-grade glioma without severe adverse effects in patients (Rampling et al. 2000; Papanastassiou et al. 2002; Harrow et al. 2004). To enhance tumor cytotoxicity induced by HSV1716, noradrenaline transporter gene (NAT) was inserted into its backbone (Quigg et al. 2005). The resultant new construct HSV1716/NAT enabled active uptake of the radiopharmaceutical [131I] MIBG and resulted in significantly enhanced cytotoxicity compared to either agent alone. These studies show that the combination of oncolytic HSV therapy with targeted radiotherapy has the potential for effective tumor cell kill and warrants further investigation as a treatment for malignant glioma.

3.3 Reovirus

Reovirus is a nonenveloped virus with a segmented, double-stranded RNA genome and can be associated with mild respiratory or gastrointestinal tract symptoms although infections tend to be asymptomatic (Fields et al. 2007). RNA viruses are attractive for cancer therapy because during their life cycle, double-stranded RNA activates type I interferon system that is crippled in tumor cells, providing a more permissive environment for the propagation of the viruses than in normal cells (Stojdl et al. 2000). When reovirus infects cells, double-stranded RNA can activate the host interferon-induced PKR which shuts down protein synthesis to protect the cell from viral infection (Strong et al. 1998). Activated Ras (or an activated element of the Ras pathway) inhibits (or reverses) PKR activation and allows viral protein synthesis and a lytic infection to occur (Strong et al. 1998). Since Ras-activated pathways are present in the majority of malignant gliomas (Guha et al. 1997; Libermann et al. 1985; Shamah et al. 1993; Helseth et al. 1988), the virus was tested in glioma cell cultures and in human gliomas growing in mice (Wilcox et al. 2001). When tested against cell cultures, live reovirus killed 20 of 24 established glioma cell lines. It also killed all nine primary cell cultures from gliomas removed from patients, but none of the cultured meningiomas. The reovirus was also effective against human gliomas established in the hind flank of mice. A single injection of live reovirus into these well-established tumors led to a statistically significant decrease in tumor size. In a Phase I clinical trial, reovirus was administered intratumorally stereotactically at up to 1×10^9 pfu in 12 patients with recurrent malignant gliomas (Forsyth et al. 2008). Maximum tolerated dose was not reached and the

treatment was well tolerated. In July 2006, Oncolytics Biotech Inc. started a Phase I/II trial of reovirus (Reolycin) in patients with recurrent malignant gliomas. The primary objective of the study is to determine the maximum tolerated dose, dose limiting toxicity, and safety profile of Reolycin. Secondary objectives include the evaluation of viral replication, immune response to the virus, and any evidence of antitumor activity. Patients will be treated with Reolycin through infusion delivery. An additional group of patients will be treated at the maximum tolerated dose.

3.4 Measles Virus

Measles virus is a negative strand RNA virus with a nonsegmented genome of 15,894 nucleotides in size (Fields et al. 2007; Blechacz and Russell 2008). The enveloped virions are pleomorphic and range in size from 100 to 300 nm (Fields et al. 2007). Measles virus causes cell–cell fusion and apoptotic cell death (Fields et al. 2007). Wild-type measles virus is a virulent pathogen, causing deadly infection in children (Fields et al. 2007). Attenuated strains have been developed as vaccines. One of them is Edmonston B strain (MV-Edm) which demonstrates tumor selectivity (Blechacz and Russell 2008). One factor contributes to the tumor selectivity is that this strain enters cell preferentially using CD46 which is overexpressed in a variety of human cancers (Blechacz and Russell 2008; Fishelson et al. 2003). Another factor is that, in normal cells, MV-Edm is unable to inhibit innate immune response pathways that are defective in tumor cells (Blechacz and Russell 2008). These two factors determine that MV-Edm propagates efficiently in tumor cells but not in normal cells.

In a preclinical study, MV-CEA, a genetically engineered MV-Edm that produces carcinoembryonic antigen (CEA), has been tested in glioblastomas (Phuong et al. 2003). The results reveal that MV-CEA has potent antitumor activity against gliomas in vitro, as well as in both subcutaneous and orthotopic U87 animal models. Monitoring CEA levels in the serum can serve as a low-risk method of detecting viral gene expression during treatment, and could allow dose optimization and individualization of treatment. Recently, the same group reported that a combination of MV-CEA and radiotherapy showed synergistic effect against glioblastoma in vitro and in vivo (Liu et al. 2007). The synergistic effect of the combination seems to be due to increase in viral burst size and apoptotic cell death. These studies have just been translated into a Phase I clinical trial in patients with recurrent glioblastoma multiforme, starting from July 2009 at Mayo Clinic.

3.5 Newcastle Disease Virus

Newcastle disease virus (NDV) is an enveloped avian RNA virus which is potentially fatal to birds, but only causes minor illness in humans (Alexander and Allan 1974). The use of NDV as an anti-tumor agent dates back to a 1964 study by Wheelock

and Dingle (Wheelock and Dingle 1964), who published their observations of a patient who was provided repeated injections of NDV in an attempt to treat his acute leukemia. NDV exploits cancer selective replication through defected interferon system in tumor cells as mentioned previously (Stojdl et al. 2000). Certain NDV strains can replicate up to 10,000 times better in tumor cells than in normal cells (Schirrmacher et al. 1999). In addition, NDV-infected cancer cells exhibit an enhanced recruitment and activation of natural killer cells and cytotoxic T cells compared to their uninfected counterparts (Haas et al. 1998). Thus, NDV facilitates the recognition of tumors cells as "foreign" (Zorn et al. 1994), which should enhance the efficacy of the virus in patients.

The distinct strains of NDV can be either lytic or nonlytic (Shah et al. 2003). Several lytic strains of NDV have undergone preclinical animal studies for safety and efficacy against human cancers (Shah et al. 2003). MTH-68/H, a live attenuated strain of NDV, had been used in the treatment of different malignancies. It was tested in a small number of patients with glioblastoma multiforme (Csatary and Bakacs 1999; Csatary et al. 2004; Wagner et al. 2006). Anecdotal responses to MTH68/H in the patients have been reported. In a Phase I/II trial, HuJ, a lentogenic strain of NDV that has a selective cytopathogenicity for human and animal cancer cell lines, has been tested in patients with apparent recurrent glioblastoma multiforme (Freeman et al. 2006). The study was based on imaging studies to determine the safety and tumor response of repetitive intravenous administration of HuJ. Maximum tolerated dose was not achieved. Anti-NDV hemagglutinin antibodies appeared within 5–29 days. HuJ was recovered from blood, saliva, and urine samples and one tumor biopsy. One patient achieved a complete response. Intravenous HuJ is well tolerated.

4 Future Perspectives

Gene-based cancer therapies reflect the achievements of cancer research. Defects in cancers are targeted specifically at molecular levels. Therefore, compared to conventional surgery, chemo- and radio-therapy, this type of therapies is expected to optimize the therapeutic index in cancer patients. However, despite the success in cultured cancer cells and laboratory animal models, gene-based cancer therapies in human patients encountered difficulties such as vector safety, transfer efficiency, vector targeting, host immune response, efficacy, and specificity of the therapeutic genes, etc. During the last decade, tremendous efforts have been made to address these issues.

Since brain tumors are localized diseases that are suitable for gene-based therapies (Table 2), stereotactic intratumoral injection and injection into tumor cavity wall postresection are desirable delivery modes. Due to the infiltrative nature of gliomas, complete remission of the tumor largely depends on the efficient delivery of the therapeutics to the cancerous cells infiltrated into normal brain tissue. Clinical experience with replication-deficient viral vectors revealed minimal delivery

Table 2 Specific conditions of malignant gliomas

- Normally only one single tumor mass
- No metastasis
- Easy assessed through stereotactic or localized surgery
- Immune privileged (absence of a lymphatic drainage pathway; paucity of professional antigen-presenting cells)
- Lack of optima therapy
- Blood-brain barrier diminishes the efficacy of systemic interventions (e.g., chemotherapy)
- Clinical trials have shown lack of toxicity for approaches using intratumoral delivery of vectors and viruses

with these agents (Lang et al. 2003; Rainov 2000). The problem has been partially improved with the bystander effect of suicide gene therapy (Immonen et al. 2004). Replication-competent oncolytic viruses are promising to bridge the vector gap in cancer gene therapies since the viruses replicate and spread the progeny to the adjacent cancer cells. To improve efficacy in glioma therapy, oncolytic virus was combined with suicide gene therapy by using the virus to carry the suicide gene (Conrad et al. 2005). The preclinical study is promising in glioma cell lines and intracranial mouse model.

Further challenges will be the anatomic barriers of the tumor and the clearance of the viruses by the host initial innate immune responses (Jiang et al. 2006; Chiocca 2008). The anatomic barriers can be address by stereotactic techniques and protease expression by the viruses (Jiang et al. 2006). As to the host immunity, it might comprise the viral replication by the innate immune responses at the beginning (Chiocca 2008). Using cyclophosphamide to modulate host immune response, prior oncolytic virus inoculation has been shown to significantly improve the efficacy of the virus in glioma-bearing mice (Kambara et al. 2005). However, the development of antitumor immunity later during gene therapy should help to eliminate remnant malignant cells (Barba et al. 1994). For example, oncolytic viruses expressing immune enhancing gene IL-12 demonstrates increased efficacy in part because of antitumor actions of immune-related infiltrating cells (Hellums et al. 2005; Parker et al. 2000). Thus, a better understanding of the interaction between the oncolytic viruses and the host immune system will help to modulate the immune response to minimize antiviral immunity, while at the same time maximizing antitumor immunity.

References

Wen PY, Kesari S. Malignant gliomas in adults. N Engl J Med 2008;359(5):492–507.

Furnari FB, Fenton T, Bachoo RM, Mukasa A, Stommel JM, Stegh A, et al. Malignant astrocytic glioma: genetics, biology, and paths to treatment. Genes Dev 2007;21(21):2683–710.

Thorne SH, Hermiston T, Kirn D. Oncolytic virotherapy: approaches to tumor targeting and enhancing antitumor effects. Semin Oncol 2005;32(6):537–48.

Levine AJ. p53, the cellular gatekeeper for growth and division. Cell 1997;88(3):323–31.

Lang FF, Bruner JM, Fuller GN, Aldape K, Prados MD, Chang S, et al. Phase I trial of adenovirus-mediated p53 gene therapy for recurrent glioma: biological and clinical results. J Clin Oncol 2003;21(13):2508–18.

Zhang WW, Fang X, Branch CD, Mazur W, French BA, Roth JA. Generation and identification of recombinant adenovirus by liposome-mediated transfection and PCR analysis. Biotechniques 1993;15(5):868–72.

Zhang WW, Alemany R, Wang J, Koch PE, Ordonez NG, Roth JA. Safety evaluation of Ad5CMV-p53 in vitro and in vivo. Hum Gene Ther 1995;6(2):155–64.

Fillat C, Carrio M, Cascante A, Sangro B. Suicide gene therapy mediated by the Herpes Simplex virus thymidine kinase gene/Ganciclovir system: fifteen years of application. Curr Gene Ther 2003;3(1):13–26.

Rainov NG. A phase III clinical evaluation of herpes simplex virus type 1 thymidine kinase and ganciclovir gene therapy as an adjuvant to surgical resection and radiation in adults with previously untreated glioblastoma multiforme. Hum Gene Ther 2000;11(17):2389–401.

Barba D, Hardin J, Sadelain M, Gage FH. Development of anti-tumor immunity following thymidine kinase-mediated killing of experimental brain tumors. Proc Natl Acad Sci U S A 1994; 91(10):4348–52.

Pizzato M, Franchin E, Calvi P, Boschetto R, Colombo M, Ferrini S, et al. Production and characterization of a bicistronic Moloney-based retroviral vector expressing human interleukin 2 and herpes simplex virus thymidine kinase for gene therapy of cancer. Gene Ther 1998; 5(7):1003–7.

Barzon L, Bonaguro R, Castagliuolo I, Chilosi M, Gnatta E, Parolin C, et al. Transcriptionally targeted retroviral vector for combined suicide and immunomodulating gene therapy of thyroid cancer. J Clin Endocrinol Metab 2002;87(11):5304–11.

Barzon L, Bonaguro R, Castagliuolo I, Chilosi M, Franchin E, Del Vecchio C, et al. Gene therapy of thyroid cancer via retrovirally-driven combined expression of human interleukin-2 and herpes simplex virus thymidine kinase. Eur J Endocrinol 2003;148(1):73–80.

Colombo F, Barzon L, Franchin E, Pacenti M, Pinna V, Danieli D, et al. Combined HSV-TK/IL-2 gene therapy in patients with recurrent glioblastoma multiforme: biological and clinical results. Cancer Gene Ther 2005;12(10):835–48.

Immonen A, Vapalahti M, Tyynela K, Hurskainen H, Sandmair A, Vanninen R, et al. AdvHSV-tk gene therapy with intravenous ganciclovir improves survival in human malignant glioma: a randomised, controlled study. Mol Ther 2004;10(5):967–72.

Fields BN, Knipe DM, Howley PM. 5th ed. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins; 2007.

Jiang H, McCormick F, Lang FF, Gomez-Manzano C, Fueyo J. Oncolytic adenoviruses as anti-glioma agents. Expert Rev Anticancer Ther 2006;6(5):697–708.

Bischoff JR, Kirn DH, Williams A, Heise C, Horn S, Muna M, et al. An adenovirus mutant that replicates selectively in p53-deficient human tumor cells. Science 1996;274(5286):373–6.

Heise C, Sampson-Johannes A, Williams A, McCormick F, Von Hoff DD, Kirn DH. ONYX-015, an E1B gene-attenuated adenovirus, causes tumor-specific cytolysis and antitumoral efficacy that can be augmented by standard chemotherapeutic agents [see comment]. Nat Med 1997; 3(6):639–45.

O'Shea CC, Johnson L, Bagus B, Choi S, Nicholas C, Shen A, et al. Late viral RNA export, rather than p53 inactivation, determines ONYX-015 tumor selectivity. Cancer Cell 2004;6(6): 611–23.

McCormick F. Cancer-specific viruses and the development of ONYX-015. Cancer Biol Ther 2003;2(4 Suppl 1):S157–60.

Chiocca EA, Abbed KM, Tatter S, Louis DN, Hochberg FH, Barker F, et al. A phase I open-label, dose-escalation, multi-institutional trial of injection with an E1B-Attenuated adenovirus, ONYX-015, into the peritumoral region of recurrent malignant gliomas, in the adjuvant setting. Mol Ther 2004;10(5):958–66.

Wildner O. Clinical trials: the sensitizing side of ONYX-015. Gene Ther 2005;12(5):386–7.

Whyte P, Williamson NM, Harlow E. Cellular targets for transformation by the adenovirus E1A proteins. Cell 1989;56(1):67–75.

Pelicano H, Martin DS, Xu RH, Huang P. Glycolysis inhibition for anticancer treatment. Oncogene 2006;25(34):4633–46.

Fueyo J, Gomez-Manzano C, Alemany R, Lee PS, McDonnell TJ, Mitlianga P, et al. A mutant oncolytic adenovirus targeting the Rb pathway produces anti-glioma effect in vivo. Oncogene 2000;19(1):2–12.

Fueyo J, Alemany R, Gomez-Manzano C, Fuller GN, Khan A, Conrad CA, et al. Preclinical characterization of the antiglioma activity of a tropism-enhanced adenovirus targeted to the retinoblastoma pathway. J Natl Cancer Inst 2003;95(9):652–60.

Suzuki K, Fueyo J, Krasnykh V, Reynolds PN, Curiel DT, Alemany R. A conditionally replicative adenovirus with enhanced infectivity shows improved oncolytic potency. Clin Cancer Res 2001;7(1):120–6.

Alonso MM, Jiang H, Yokoyama T, Xu J, Bekele NB, Lang FF, et al. Delta-24-RGD in combination with RAD001 induces enhanced anti-glioma effect via autophagic cell death. Mol Ther 2008;16(3):487–93.

Alonso MM, Gomez-Manzano C, Bekele BN, Yung WK, Fueyo J. Adenovirus-based strategies overcome temozolomide resistance by silencing the O6-methylguanine-DNA methyltransferase promoter. Cancer Res 2007;67(24):11499–504.

Jiang H, Gomez-Manzano C, Aoki H, Alonso MM, Kondo S, McCormick F, et al. Examination of the therapeutic potential of Delta-24-RGD in brain tumor stem cells: role of autophagic cell death. J Natl Cancer Inst 2007;99(18):1410–4.

Roizman B. The function of herpes simplex virus genes: a primer for genetic engineering of novel vectors. Proc Natl Acad Sci U S A 1996;93(21):11307–12.

Grandi P, Peruzzi P, Reinhart B, Cohen JB, Chiocca EA, Glorioso JC. Design and application of oncolytic HSV vectors for glioblastoma therapy. Expert Rev Neurother 2009;9(4):505–17.

Mineta T, Rabkin SD, Yazaki T, Hunter WD, Martuza RL. Attenuated multi-mutated herpes simplex virus-1 for the treatment of malignant gliomas. Nat Med 1995;1(9):938–43.

He B, Gross M, Roizman B. The gamma(1)34.5 protein of herpes simplex virus 1 complexes with protein phosphatase 1alpha to dephosphorylate the alpha subunit of the eukaryotic translation initiation factor 2 and preclude the shutoff of protein synthesis by double-stranded RNA-activated protein kinase. Proc Natl Acad Sci U S A 1997;94(3):843–8.

Farassati F, Yang AD, Lee PW. Oncogenes in Ras signalling pathway dictate host-cell permissiveness to herpes simplex virus 1. Nat Cell Biol 2001;3(8):745–50.

Guha A, Feldkamp MM, Lau N, Boss G, Pawson A. Proliferation of human malignant astrocytomas is dependent on Ras activation. Oncogene 1997;15(23):2755–65.

Markert JM, Medlock MD, Rabkin SD, Gillespie GY, Todo T, Hunter WD, et al. Conditionally replicating herpes simplex virus mutant, G207 for the treatment of malignant glioma: results of a phase I trial. Gene Ther 2000;7(10):867–74.

Markert JM, Liechty PG, Wang W, Gaston S, Braz E, Karrasch M, et al. Phase Ib trial of mutant herpes simplex virus G207 inoculated pre-and post-tumor resection for recurrent GBM. Mol Ther 2009;17(1):199–207.

Aghi M, Rabkin S, Martuza RL. Effect of chemotherapy-induced DNA repair on oncolytic herpes simplex viral replication. J Natl Cancer Inst 2006;98(1):38–50.

Kesari S, Randazzo BP, Valyi-Nagy T, Huang QS, Brown SM, MacLean AR, et al. Therapy of experimental human brain tumors using a neuroattenuated herpes simplex virus mutant. Lab Invest 1995;73(5):636–48.

Rampling R, Cruickshank G, Papanastassiou V, Nicoll J, Hadley D, Brennan D, et al. Toxicity evaluation of replication-competent herpes simplex virus (ICP 34.5 null mutant 1716) in patients with recurrent malignant glioma. Gene Ther 2000;7(10):859–66.

Papanastassiou V, Rampling R, Fraser M, Petty R, Hadley D, Nicoll J, et al. The potential for efficacy of the modified (ICP 34.5(-)) herpes simplex virus HSV1716 following intratumoural injection into human malignant glioma: a proof of principle study. Gene Ther 2002;9(6):398–406.

Harrow S, Papanastassiou V, Harland J, Mabbs R, Petty R, Fraser M, et al. HSV1716 injection into the brain adjacent to tumour following surgical resection of high-grade glioma: safety data and long-term survival. Gene Ther 2004;11(22):1648–58.

Quigg M, Mairs RJ, Brown SM, Harland J, Dunn P, Rampling R, et al. Assessment in vitro of a novel therapeutic strategy for glioma, combining herpes simplex virus HSV1716-mediated oncolysis with gene transfer and targeted radiotherapy. Med Chem 2005;1(5):423–9.

Stojdl DF, Lichty B, Knowles S, Marius R, Atkins H, Sonenberg N, et al. Exploiting tumor-specific defects in the interferon pathway with a previously unknown oncolytic virus. Nat Med 2000;6(7):821–5.

Strong JE, Coffey MC, Tang D, Sabinin P, Lee PW. The molecular basis of viral oncolysis: usurpation of the Ras signaling pathway by reovirus. EMBO J 1998;17(12):3351–62.

Libermann TA, Nusbaum HR, Razon N, Kris R, Lax I, Soreq H, et al. Amplification, enhanced expression and possible rearrangement of EGF receptor gene in primary human brain tumours of glial origin. Nature 1985;313(5998):144–7.

Shamah SM, Stiles CD, Guha A. Dominant-negative mutants of platelet-derived growth factor revert the transformed phenotype of human astrocytoma cells. Mol Cell Biol 1993;13(12): 7203–12.

Helseth E, Unsgaard G, Dalen A, Fure H, Skandsen T, Odegaard A, et al. Amplification of the epidermal growth factor receptor gene in biopsy specimens from human intracranial tumours. Br J Neurosurg 1988;2(2):217–25.

Wilcox ME, Yang W, Senger D, Rewcastle NB, Morris DG, Brasher PM, et al. Reovirus as an oncolytic agent against experimental human malignant gliomas. J Natl Cancer Inst 2001; 93(12):903–12.

Forsyth P, Roldan G, George D, Wallace C, Palmer CA, Morris D, et al. A phase I trial of intratumoral administration of reovirus in patients with histologically confirmed recurrent malignant gliomas. Mol Ther 2008;16(3):627–32.

Blechacz B, Russell SJ. Measles virus as an oncolytic vector platform. Curr Gene Ther 2008; 8(3):162–75.

Fishelson Z, Donin N, Zell S, Schultz S, Kirschfink M. Obstacles to cancer immunotherapy: expression of membrane complement regulatory proteins (mCRPs) in tumors. Mol Immunol 2003;40(2–4):109–23.

Phuong LK, Allen C, Peng KW, Giannini C, Greiner S, TenEyck CJ, et al. Use of a vaccine strain of measles virus genetically engineered to produce carcinoembryonic antigen as a novel therapeutic agent against glioblastoma multiforme. Cancer Res 2003;63(10):2462–9.

Liu C, Sarkaria JN, Petell CA, Paraskevakou G, Zollman PJ, Schroeder M, et al. Combination of measles virus virotherapy and radiation therapy has synergistic activity in the treatment of glioblastoma multiforme. Clin Cancer Res 2007;13(23):7155–65.

Alexander DJ, Allan WH. Newcastle disease virus pathotypes. Avian Pathol 1974;3(4):269–78.

Wheelock EF, Dingle JH. Observations on the repeated administration of viruses to a patient with acute leukemia. A preliminary report. N Engl J Med 1964;271:645–51.

Schirrmacher V, Haas C, Bonifer R, Ahlert T, Gerhards R, Ertel C. Human tumor cell modification by virus infection: an efficient and safe way to produce cancer vaccine with pleiotropic immune stimulatory properties when using Newcastle disease virus. Gene Ther 1999;6(1):63–73.

Haas C, Ertel C, Gerhards R, Schirrmacher V. Introduction of adhesive and costimulatory immune functions into tumor cells by infection with Newcastle Disease Virus. Int J Oncol 1998; 13(6):1105–15.

Zorn U, Dallmann I, Grosse J, Kirchner H, Poliwoda H, Atzpodien J. Induction of cytokines and cytotoxicity against tumor cells by Newcastle disease virus. Cancer Biother 1994;9(3):225–35.

Shah AC, Benos D, Gillespie GY, Markert JM. Oncolytic viruses: clinical applications as vectors for the treatment of malignant gliomas. J Neurooncol 2003;65(3):203–26.

Csatary LK, Bakacs T. Use of Newcastle disease virus vaccine (MTH-68/H) in a patient with high-grade glioblastoma. JAMA 1999;281(17):1588–9.

Csatary LK, Gosztonyi G, Szeberenyi J, Fabian Z, Liszka V, Bodey B, et al. MTH-68/H oncolytic viral treatment in human high-grade gliomas. J Neurooncol 2004;67(1–2):83–93.

Wagner S, Csatary CM, Gosztonyi G, Koch HC, Hartmann C, Peters O, et al. Combined treatment of pediatric high-grade glioma with the oncolytic viral strain MTH-68/H and oral valproic acid. APMIS 2006;114(10):731–43.

Freeman AI, Zakay-Rones Z, Gomori JM, Linetsky E, Rasooly L, Greenbaum E, et al. Phase I/II trial of intravenous NDV-HUJ oncolytic virus in recurrent glioblastoma multiforme. Mol Ther 2006;13(1):221–8.

Conrad C, Miller CR, Ji Y, Gomez-Manzano C, Bharara S, McMurray JS, et al. Delta24-hyCD adenovirus suppresses glioma growth in vivo by combining oncolysis and chemosensitization. Cancer Gene Ther 2005;12(3):284–94.

Chiocca EA. The host response to cancer virotherapy. Curr Opin Mol Ther 2008;10(1):38–45.

Kambara H, Saeki Y, Chiocca EA. Cyclophosphamide allows for in vivo dose reduction of a potent oncolytic virus. Cancer Res 2005;65(24):11255–8.

Hellums EK, Markert JM, Parker JN, He B, Perbal B, Roizman B, et al. Increased efficacy of an interleukin-12-secreting herpes simplex virus in a syngeneic intracranial murine glioma model. Neuro Oncol 2005;7(3):213–24.

Parker JN, Gillespie GY, Love CE, Randall S, Whitley RJ, Markert JM. Engineered herpes simplex virus expressing IL-12 in the treatment of experimental murine brain tumors. Proc Natl Acad Sci U S A 2000;97(5):2208–13.

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