9 Novel Therapeutic Approaches for High-Grade Gliomas

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9.1 INTRODUCTION

The incidence of primary brain tumors is increasing. Approximately 18,000 new cases were expected in the U.S. in 2003. High-grade gliomas (HGGs) including glioblastoma multiforme (GBM) and anaplastic astrocytoma (AA) are the most common primary tumors of the central nervous system (CNS). HGGs remain refractory to treatment and have dismal prognoses. The median survival for AA patients is approximately 2 years, the median is 9 months for GBM patients. Neurosurgeons remain intimately involved in the care of patients with HGG and with research into new treatments for this deadly disease. This stems in part from the fact that surgical resection continues to be an important treatment for HGG.

The hypothesis of this chapter is that improved understanding of the biology of gliomas and the discovery of novel cancer treatment modalities will lead to therapies that will significantly improve the prognosis for patients with HGG. The avenues of ongoing research into novel mechanisms of cancer therapy that may eventually lead to new treatments for HGGs are vast. It is, of course, impossible to predict which of many ongoing areas of research will lead to successful therapies for brain tumors and coverage of all potential areas of future treatment is beyond the scope of this chapter.

We have chosen to limit our discussion to three brain tumor therapies that are the most promising experimental treatments involving local delivery of agents to the brain. The treatment modalities to be discussed include oncolytic viruses, gene therapy, and convection-enhanced delivery (CED) of targeted toxins and other agents. Many neurosurgeons have been and continue to be involved in the development of these novel therapies for brain tumors, and the fact that they involve local delivery of agents to the brain makes them of interest to all neurosurgeons who treat brain tumors.

Many promising areas of cancer research that will not be covered in this chapter may, of course, lead to new treatments for brain tumors. These include development of new chemotherapeutic treatments, molecular therapies, immunologic therapies, and therapies targeted at blood–brain barrier disruption. In addition, developments in intraoperative imaging to guide surgical resections will not be covered. Please see cited references for further reading on these topics.

9.2 ONCOLYTIC VIRUSES FOR BRAIN TUMOR THERAPY

9.2.1 INTRODUCTION

The revolution in molecular biology that culminated in completion of the Human Genome Project spurred an explosion of interest in various forms of gene therapy for brain tumors over the past decade. In fact, brain tumors were some of the first tumors tested in experimental gene therapy models. Unfortunately, this early enthusiasm has not yet led to the development of any tangible treatment modalities (see Section 9.3 on gene therapy). However, work with the viruses used as gene therapy vectors demonstrated that they may be powerful oncolytic tools in and of themselves.
Initial viral vector gene therapy strategies utilized viruses rendered replication-defective to reduce their neurovirulence. These studies led to the hypothesis that replication-competent viruses might serve as treatment agents for tumors, not by delivering specific genes into tumors (as is the case with gene therapy), but by directly infecting and lysing the tumor cells. The neurovirulence of the virus, however, must be reduced by creating a neuro-attenuated mutant that can replicate in tumor cells but not in normal brain. Viral replication within targeted brain tumor cells results in production of viral progeny and lysing the infected cells in the process. The progeny may then infect neighboring cells to extend the effects of the virus beyond the initially infected cells. Although the use of viruses as oncolytic agents was explored many years ago, the hypothesis that mutated viruses might selectively replicate in tumor cells was validated by Martuza et al in 1991. Their initial study led to the development of a new field of research based on the idea of using replication-competent viruses as cancer therapies.

9.2.2 Herpes Simplex Virus Type-1 (HSV-1) Vectors

To better appreciate where oncolytic viral therapy may go in the next decade, it is perhaps instructional to examine just how far science has taken us over the past decade in this exciting area of brain tumor therapy. As one of the first oncolytic viruses used in the treatment of HGG, HSV-1 has remained one of the most widely studied because:

1. It has affinity for numerous cell types including a natural neurotropism.
2. It is naturally cytolysic during its replication and virion production life cycle.
3. It contains nonessential viral genes that can be replaced with large transgenes (up to 30 kb).
4. The HSV genome contains several known genetic determinants of neurovirulence encoded by nonessential genes that may be deleted or replaced.
5. The viral genome remains as an episome in the target cell, eliminating the possibility of insertional mutagenesis.
6. It is susceptible to several antiviral medications such as acyclovir and gancyclovir.

The susceptibility of HSV to medications provides a safeguard against uncontrollable infection during attempted therapeutic uses of the virus.

Oncolytic HSV-1 viral therapy has gone through several modifications since the initial viral vectors were engineered in the early 1990s. The first generation versions involved mutations of viral enzymes involved in nucleotide metabolism, namely thymidine kinase (TK) and ribonucleotide reductase. These viral enzymes possess cellular homologues that are upregulated in actively dividing tumor cells but not in nondividing cells. DLsptk was one such HSV-1 virus with a mutated TK gene. This virus was efficacious against mice inoculated with human HGGs, but its neurovirulence at high titers and resistance to antiviral drugs hampered enthusiasm for clinical use.
The second generation HSV-1 vectors were mutated in selected genes to maximize safety as well as attempt to provide improved specificity and efficacy. The investigations found that R3616, an HSV-1 mutant in the $\gamma 34.5$ gene, could act as an oncolytic virus with greatly attenuated neurovirulence as well as maintained susceptibility to antiviral medications. $\gamma 34.5$ is present in two copies on the HSV genome and encodes a major determinant of neurovirulence. The discovery that R3616, a virus with a deletion in both $\gamma 34.5$ genes that makes it highly neuro-attenuated, could still replicate in and kill brain tumors increased excitement about this potential therapeutic modality.

The next advance in oncolytic viruses came with the development of a multimutated HSV-1 virus, G207. G207 is an oncolytic viral vector derived from R3616 by insertion of the $E. coli$ lacZ gene in the $UL39$ gene. The $UL39$ gene encodes the large subunit of ribonucleotide reductase, a key enzyme in nucleotide metabolism and viral DNA synthesis in nondividing cells but not in dividing cells. Mutation of the $UL39$ gene results in a virus that is both neuro-attenuated and hypersensitive to antiviral medications. Combining the $\gamma 34.5$ and $UL39$ gene mutations in one virus resulted in G207, an oncolytic HSV-1 mutant with multiple desirable properties:

1. An infinitely small chance of reversion to wild type, particularly as the $\gamma 34.5$ mutation is a gene deletion.
2. Highly attenuated neurovirulence, in fact, no neurotoxic dose of the virus could be attained in a highly HSV-sensitive primate model.
3. Hypersensitivity to antiviral medications in case an HSV infection occurred during cancer therapy.
4. A method of tracking viral spread through chemical detection of the inserted lacZ gene, a common marker enzyme.

The combination of these properties of G207 led to its becoming the first replication-competent HSV-1 mutant used in human clinical trials for brain tumors. Shortly thereafter, a $\gamma 34.5$ mutant vector similar to R3616 was also approved for clinical trials in glioma patients (see next).

The original studies with G207 utilized human brain tumor implants into nude mice in order to avoid immunologic rejection of the foreign tumors. These models had the disadvantage that the effects of the immune systems of the host animals on the tumors could not be assessed. Further experiments investigated inoculation of G207 into immunocompetent mice and yielded very interesting results. The injection of G207 induced a systemic immunity to the tumor that resulted in regression of distant tumors and resistance to rechallenge with tumor cells in inoculated animals. This introduced the novel concept of a viral vector acting as a tumor vaccine therapy, eliciting a CD8+ T cell-mediated immunity in this particular case.

Further advances in replication-competent HSV-1 mutants for use in tumor therapy are ongoing. HSV-1 mutants have been engineered to utilize gene promoters or enhancer sequences to specifically express an essential viral gene in tumor cells but not in normal brain. This approach relies on the tumor specificity of the gene promoter to create a virus that can replicate only in tumor cells. Such HSV-1 and
Adenovirus vectors have been created using tissue- or tumor-specific promoters such as α-fetoprotein, kallikrein, L-plastin, midkine, prostate-specific antigen, tyrosinase, and calponin; however, more work must be done to identify specific glioma promoter/enhancer sequences before this concept can be applied to brain tumors. To date, two oncolytic HSV-1 vectors have been approved for use in clinical trials for HGGs G207 and 1716. Data from a Phase I trial using G207 showed encouraging results in patients with recurrent HGGs refractory to chemotherapy and radiation. No toxicity or serious adverse events attributable to the virus were noted in 21 patients injected with G207 in escalating doses. Eight of 21 patients showed reduced tumor volumes and two patients survived more than 4 years. G207 is now in a Phase Ib trial and is being inoculated into the tumor bed, followed by tumor resection 2 days later and subsequent inoculation in the resection cavity. The clinical trial using the γ34.5 mutant 1716 also showed encouraging results in a small number of patients with recurrent HGGs. These trials require future expansion and, importantly, must include patients with newly diagnosed HGGs.

9.2.3 Adenovirus (Ad) Vectors

Adenovirus (Ad) vectors have gained popularity as gene therapy tools for several fundamental reasons:

1. They are unaffected by the complement system.
2. They remain episomal and therefore lack the risk of insertional mutagenesis.
3. Large viral titers can be generated more easily than with HSV.
4. They demonstrate broad infectivity of dividing and nondividing cells.
5. They generate high levels of transgene expression.
6. They are less neurotoxic than HSV vectors.

The smaller genome of the adenovirus confers the advantage that recombinant vectors are more easily generated. However, one disadvantage is that the size of the potential gene insert is less than the size of the HSV vector. A significant drawback to the use of Ad vectors for gene therapy is that they induce potent inflammatory and specific immune responses that may damage infected tissues and limit repeated use. In fact, such a response may have been responsible for the death of a patient in a gene therapy clinical trial using an Ad vector.

While Ad vectors have been used more commonly than HSV vectors in gene therapy applications, their use in oncolytic viral therapy has been more limited despite the fact that Ad was tested as an oncolytic agent shortly after its isolation in 1953 because of its ability to grow in epithelial cells. These early studies showed initial tumor regression that was only transient and the idea of using Ad as an oncolytic agent was abandoned until recently.

As with HSV, two basic strategies have been employed to attempt to develop Ad mutants that can selectively replicate in and lyse tumor cells. The first strategy was to delete viral genes unnecessary for replication in tumor cells. The first example of such a mutant was the E1b 55-kDa deleted Ad dl1520 (also known as Onyx-015)
designed to replicate better in p53 negative cells — a common mutation in tumors.\textsuperscript{46} Wild type Ad requires E1b binding to p53 to permit viral replication, and therefore E1b-deleted Ad is theoretically only able to replicate in p53-deficient cells.

Unfortunately, further studies found a lack of correlation between p53 expression in the host cell and replication of dl1520.\textsuperscript{47,48} These studies were extended to p53 negative gliomas, with similar disappointing results.\textsuperscript{49} However, dl1520 does replicate well in tumor cells, and has a low level of toxicity that allowed its approval for clinical trials in head and neck, colorectal, lung, and other cancers.\textsuperscript{50} In a similar strategy, an Ad mutant with deletion of the E1a gene, a retinoblastoma tumor suppressor binding site, was developed and studied for its ability to lyse glioblastoma cells.\textsuperscript{51} Further work must be done to determine whether this Ad mutant has sufficiently low toxicity to be appropriate for testing in clinical trials of brain tumor patients.

As mentioned above and as with HSV vectors, a second strategy has been tested for development of oncolytic Ad mutants. It employs tumor-specific promoter or enhancer gene sequences to drive expression of an essential viral gene product in tumor cells but not normal tissues. Typically this work has used putative tumor-specific promoter sequences such as $\alpha$-fetoprotein, prostate specific antigen, and others to drive expression of E1a, an Ad gene product essential for replication of the virus.\textsuperscript{52,53} Unfortunately, very low levels of E1a are adequate for replication of Ad, and thus tumor-specific promoter vectors are not as tumor-specific as was hoped. Neither HSV-1 nor Ad vectors designed with this strategy have reached clinical trials to date, and further work is needed to create a truly tumor-specific virus by this method.

\textbf{9.2.4 Other Oncolytic Viruses}

Although most work in the area of oncolytic viral therapy has focused on the use of HSV-1 or Ad vectors, several other viruses have also been investigated for oncolytic potential. They include vesicular stomatitis virus,\textsuperscript{54} reovirus,\textsuperscript{55} and poliovirus.\textsuperscript{56} Interestingly, the highly neurotoxic poliovirus may be among the most promising oncolytic viruses for glioma therapy. The neurotoxicity of poliovirus can be greatly attenuated by replacement of the internal ribosome entry site element with that of a human rhinovirus.\textsuperscript{57} The safety of the attenuated virus has been extended to studies in nonhuman primates.\textsuperscript{58} Meanwhile, increased expression of the cellular receptor for poliovirus on glioma cells may make the tumor particularly susceptible to poliovirus infection. Gromeier et al. demonstrated that a neuro-attenuated poliovirus can replicate in and lyse human gliomas \textit{in vitro} and \textit{in vivo}.\textsuperscript{56} Further work is ongoing to begin clinical trials with attenuated poliovirus in human glioma patients.

\textbf{9.2.5 Future Potential of Oncolytic Viral Therapy}

There are numerous ways that oncolytic viral therapy may be improved upon over the next decade in a quest for the ideal viral vector and hopefully a cure for malignant brain tumors. One potential strategy for improved targeting of tumor cells may be
through the use of cell surface molecular markers unique to tumors that may be exploited to create virus mutants able only to infect tumor cells. Modification of viral knob, fiber, or coat proteins can alter viral tropism and enhance tumor transduction in Ad vectors. Unfortunately, HSV-1 utilizes multiple cell surface glycoproteins for viral–cell interactions, and it will be more difficult to restrict viral infection only to tumor cells.

Another potential strategy to increase the effectiveness of oncolytic viruses is to combine the use of replication-competent vectors with gene therapy strategies. In this scenario, an oncolytic virus has a gene inserted in its genome that directly or indirectly enhances tumor cell killing. An example of this strategy proved efficacious when the antiviral drug sensitizing gene TK was inserted in the E1b mutant Ad virus dl1520. The virus is then used to infect a tumor and the lytic effect of the viral therapy is enhanced by administration of gancyclovir.

Another example of the potential of combining gene therapy with oncolytic viral therapy is through addition of immunomodulatory gene products in the oncolytic virus. For example, HSV-1 vectors have been engineered to express immunostimulatory cytokines such as interleukin-2, interleukin-12, or soluble B7.1-Ig. Other vectors have been designed to inhibit viral-induced down-regulation of major histocompatibility complex (MHC) class I in an effort to increase immune-mediated tumor cell killing.

Suboptimal viral spread within tumors has been a challenge because of many physical and antiviral immune barriers. Extracellular matrix proteins, tight gap junctions, fibrosis, necrosis, neutralizing antibodies, and cell-mediated immunity are all significant impediments to the spread of even replication-competent viruses within a tumor. Viruses that spread via cell-to-cell transmission may prove less affected by neutralizing antibody and other extracellular factors.

Another factor effecting virus spread is, of course, method of delivery. To date, oncolytic viruses have been primarily delivered locally via injection into the tumor or tumor resection cavity. Other routes of delivery, such as intravenous, intra-arterial, lymphatic, intraperitoneal, and local vascular perfusion, have proven successful in animal models with oncolytic HSV-1 vectors and may proceed to clinical trials in the near future.

For systemic administration to be an effective delivery method for oncolytic viruses, additional obstacles such as viral inactivation from instability, absorption, homing to nonspecific cells, clearance by the reticuloendothelial system, innate immunity, preexisting immunity with antibodies, and complement-mediated inactivation must be addressed. It is possible that armored vectors able to avoid clearance by the reticuloendothelial and immune systems could be tested systemically.

Viral vectors coated with polyethylene glycol to avoid interaction with macrophages are already under investigation. Other nonviral vectors that avoid neutralizing antibodies and allow repeated administration are in development. Undoubtedly, the next decade will witness the design of several “trojan horse” viral vectors with cell carriers, chemical coatings, and other ways of bypassing the immune system.

The past decade has witnessed the birth of a novel therapy for treating malignant brain tumors with oncolytic viral therapy, and hopefully, the next decade will demonstrate its full therapeutic potential. Clinical trials have demonstrated that
replication-competent viruses can be administered safely in humans. The next decade will see the continued refinement and clinical testing of oncolytic viruses for use in brain tumor therapy.

9.3 GENE THERAPY FOR BRAIN TUMORS

9.3.1 INTRODUCTION

Gene therapy is broadly defined as the transfer of genetic material into a patient’s cells for therapeutic purposes. It is an elegant conceptual approach for the treatment of many diseases that are largely due to genetic aberrations, including brain tumors. The resistance of gliomas to current treatment modalities has stimulated interest in new therapeutic approaches, and raises the prospect of gene therapy as a novel component of multimodal therapy for these extremely aggressive tumors.

The scientific progress of gene therapy and its ultimate translation into clinical benefit depend upon four key steps. First, genes encoding products that specifically destroy or inhibit the growth of tumor cells must be discovered. Second, vectors that deliver one or more genes effectively to tumors must be developed. Third, methods of reliably delivering vectors to target cells with minimal toxicity must be designed. Finally, preclinical data must be translated into well-designed clinical trials in order to test the safety and efficacy of this emerging technology. Recent developments and ongoing research related to these four steps will be discussed next.

9.3.2 GENE TARGETING: TUMOR SUPPRESSION, APOPTOSIS, AND DRUG SUSCEPTIBILITY

Recent advances in molecular biology techniques and the completion of the Human Genome Project provided a wealth of potential targets for gene therapy. One method to conceptualize the strategies being developed is to group potential therapeutic genes into those that induce one of three effects on targeted cells, tumor growth suppression, cellular suicide (apoptosis), or drug susceptibility. There are several mechanisms by which gene therapy may induce suppression of tumor growth. One paradigm aims to directly suppress genetic alterations responsible for the molecular progression of normal cells to HGG cells. Other indirect mechanisms for inhibiting tumor growth via gene therapy, for example, by inducing host immunity to the tumor or inhibiting angiogenesis will not be discussed here.

The specific genetic mutations that occur in brain tumors correlate with tumor type and may be utilized for further subclassifications of tumors. For example, primary malignant astrocytomas develop via molecular pathways distinct from secondary HGGs that develop from low-grade lesions. The most frequent genetic alteration in primary malignant astrocytomas is in the gene for the epidermal growth factor receptor (EGFR) located on chromosome 7.71,72 The mutated form of EGFR (most often a truncated product known as EGFR-Viii) has a high level of tyrosine kinase activity in the absence of receptor ligand. This amplified signal overrides the
normal negative regulation of a tumor suppressor, resulting in uncensored cellular growth. Over-expression of EGFR in glioma cell lines has been correlated with tumor invasiveness and inhibition of this receptor in vivo can eliminate this malignant property.73

Secondary malignant astrocytomas are most often associated with a missense mutation or allelic loss of chromosome arm 17p.72,74 At this site is the gene TP53 that encodes the p53 protein; p53 normally functions as a key transcription factor in the regulation of cellular growth and arrest. If a genetic aberration occurs, p53 arrests the damaged cell in the G1 phase of the cell cycle to allow repair mechanisms to commence. If the cell cannot be repaired, p53 induces programmed cell death. Loss of p53 occurs early in secondary malignant astrocytomas and is largely responsible for the accumulation of other genetic errors that ultimately lead to the malignant phenotype. The replacement of wild-type p53 in glioma cell lines induces massive apoptosis in vitro, increases sensitivity to ionizing radiation, and inhibits tumor growth in vivo.75,76

Although there is some genetic overlap with astrocytomas, the malignant transformation of oligodendrogliomas most often involves distinct molecular pathways. Vascular endothelial growth factor (VEGF) is an endothelial cell-specific mitogen that promotes angiogenesis in many solid tumors and over-expression of this gene is the most common aberration in high grade oligodendrogliomas. VEGF antisense gene therapy in glioma cell lines results in fewer blood vessels, more necrosis, and inhibited tumor growth in vivo.77,78 Expression of VEGF and its receptor correlate strongly with oligodendroglioma tumor grade and patient survival.72 Likewise, allelic loss of 1p and 19q is nearly 100% predictive for drug sensitivity and survival in patients with oligodendrogliomas.72 Ongoing research on the genes that promote proliferation, angiogenesis, and invasion of HGGs will continue to provide new targets for tumor suppression in the future.

A second gene therapy strategy involves targeting genes that are directly involved in the induction of apoptosis. Apoptosis, also called programmed cell death or cellular suicide, involves the activation of an intrinsic proteolytic cascade that terminates with the activation of cell death effectors. As discussed above, secondary HGGs often contain mutations in TP53 that prevent the normal activation of programmed cell death. This offers one potential target of gene therapy for gliomas. Many other signals and downstream effectors of apoptosis may also be delivered to tumor cells via gene therapy to oppose the unchecked growth of malignant cells.

Fas ligand and its receptor form a well-studied upstream signal in the apoptotic cascade. Fas is a transmembrane protein of the nerve growth factor/tumor necrosis factor receptor family, binding of the Fas ligand triggers apoptosis. Fas-associated protein with death domain (FADD) binds to the intracellular domain of Fas and is the immediate downstream signal in the cascade. Most malignant astrocytomas express high levels of Fas in contrast to their nonmalignant counterparts.79 Gene therapy with both Fas ligand and FADD effectively inhibit in vitro and in vivo survival of HGGs via induction of apoptosis.80,81

Ultimately, the apoptotic signal induces activation of caspases, a family of cysteine proteases, and the downstream effectors of apoptosis. Caspase 8 is a
well-characterized effector activated by the Fas/Fas ligand signal. Transfer of this gene preferentially induces apoptosis in glioma cell lines when compared to endothelial cells, fibroblasts, and nonmalignant neuronal cells.\textsuperscript{82} It is logical that the transfer of genes that induce tumor suppression or apoptosis may also confer susceptibility to chemotherapy and radiation, which has been demonstrated.\textsuperscript{75,76} Similarly, gene therapeutic techniques may involve the transfer of exogenous genes that sensitize tumor cells to a specific drug or prodrug. The classic example of this approach is delivery of the HSV-1 thymidine kinase (HSV-TK) gene and subsequent therapy with gancyclovir. Unlike the human TK protein, HSV-TK is able to monophosphorylate gancyclovir, a nucleoside analog that is then incorporated into DNA during synthesis and halts cell division. This strategy was first demonstrated to be effective in 1986 by Moolten and has since been reliably reproduced in several model systems.\textsuperscript{83} However, clinical trials utilizing this strategy have been limited by poor and variable gene expression with only anecdotal benefit observed thus far.

Numerous preclinical studies have confirmed that each of these gene therapy strategies shows promise in tumor models \textit{in vitro} and \textit{in vivo}. By transferring genes capable of inducing tumor suppression, apoptosis, or drug susceptibility, researchers have been able to decrease tumor size, aggressiveness, and resistance to chemotherapy or radiation.\textsuperscript{76} The limiting factor in the application of these strategies is the development of vectors and gene promoters that will allow sufficient and specific expression of therapeutic genes in targeted cells. The recent development and rapid improvement in techniques such as serial analysis of gene expression (SAGE) and microarray gene expression analysis now allow the simultaneous determination of differential expression of thousands of potential targets.\textsuperscript{84} Therefore, the identification of novel gene targets is a productive area of research and it is not considered the rate-limiting step for successful gene therapy.

\textbf{9.3.3 VECTORS: VIRAL AND NONVIRAL SYSTEMS}

Once a target gene is identified, a vehicle for transport to the targeted cell population must then be selected. Vectors for use in gene therapy can be viral or nonviral. Any vector for gene therapy of HGGs would ideally:

1. Be stable and relatively easy to produce
2. Be capable of carrying transgenes large enough for desired applications
3. Be able to transf ect target cell efficiently
4. Express a gene of interest in sufficient amounts and for a sufficient time
5. Exert minimal inflammatory effect
6. Demonstrate minimal toxicity to surrounding tissue

None of the vectors employed to date satisfies all these criteria, in fact, most are lacking in several areas. Thus optimizing a vehicle for gene delivery is probably the rate-limiting step for the success of gene therapy as a therapeutic modality.

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9.3.4 RETROVIRUSES

Several viruses have been engineered to be nonpathogenic replication-deficient gene therapy vectors. Most clinical trials to date have used retroviruses due to several attributes of these vectors. Retroviruses are RNA viruses that integrate their genetic information into the genomes of replicating cells. This offers two theoretical advantages when treating brain tumors. First, therapeutic genes should be expressed for the life of a cell. Second, gene expression should be highly specific because tumor cells divide and surrounding cells do not.

However, since only a small portion (approximately 10 to 15%) of glioma cells replicate at any particular time, making transfection with retroviruses highly inefficient. Interestingly though, tumor cells can be affected even without expressing the delivered gene in a phenomena called the “bystander effect” The effect is likely mediated when tumor cells surrounding a transfected cell pick up either the TK gene product or the activated metabolite of the antiviral medication.

Insertion of the retrovirus genome into the host genome carries a distinct disadvantage as well. Whenever a retroviral vector is administered, there is a risk of insertional mutagenesis from random-site insertion that may induce or potentiate neoplastic transformation in a transfected cell. This risk was thought to be very low in humans until a retrovirus gene therapy trial in France was halted when one patient developed leukemia as a result of insertional mutagenesis.

Other important features of retroviruses for gene therapy applications are: (1) retrovirus genomes are small and can only accommodate 9 kb of exogenous information, thus limiting the repertoire of genes they can carry, and (2) the partial immune-privileged nature of the CNS should reduce any immune response to the murine packaging cells necessary for high-titer production of engineered retroviruses.

9.3.5 ADENOVIRUSES

While retroviruses have been the most commonly studied viruses for use in gene therapy applications overall, adenoviruses are the most commonly used DNA viral vectors. Adenoviruses have double-stranded linear DNA genomes and can be engineered to be replication-defective vectors via deletion of early genes that encode transcriptional regulators. Adenoviruses offer some specific advantages over retroviral vectors. The most significant is that they can be produced with relative ease at high titers in cell-free preparations, while retrovirus gene therapy in vivo requires injection of a viral packaging cell line. In addition, their DNA genome makes adenovirus vectors significantly more stable.

As with all vectors, adenoviruses have several properties that confer advantages and disadvantages for use in gene therapy applications. First, the adenovirus genome remains episomal in an infected cell, which means that it does not integrate into the host DNA. While this eliminates the risk of insertional mutagenesis, it appears to result in a shorter duration of transgene expression. Second, adenovirus vectors have the ability to infect both dividing and nondividing cells. This increases the potential targets of adenoviral gene therapy, but makes them less tumor-specific than retroviral
vectors. Third, the adenovirus genome is large, and thus can accommodate the insertion of much more genetic material than retroviruses. However, the larger genome is somewhat more difficult to manipulate. Fourth, adenoviral target cell tropism is controlled by interaction between components of the viral capsid with its receptor. Thus manipulation of the viral capsid may be used to target the vectors to desired cells. However, expression of the adenovirus receptor is absent on many tumor cells, making them resistant to adenovirus infection.

Another important feature of adenoviruses cited in the section on oncolytic viruses is the large immune response generated to the virus. While elimination of adenovirus gene transcription significantly reduces the adaptive immune response, a rapid innate response to the viral particle or capsid remains. This innate immune response is potent enough to result in a substantial loss of adenoviral vectors within 24 hours after injection in vivo. Given the various advantages and disadvantages of each vector system, we have no way to know whether adenoviruses are better suited for gene therapy of gliomas without a direct comparison to retroviruses.

Fortunately, adenoviral vectors and retroviral vectors were compared in a head-to-head trial for gene therapy of brain tumors via delivery of HSV-TK followed by administration of gancyclovir. This Phase I trial with seven patients in each group revealed a significant survival advantage in those treated with adenovirus vectors compared to retrovirus vectors. Patients treated with adenoviruses also had significant increases in side effects, with seizures occurring in two patients, fevers in two, and an increased anti-adenovirus antibody titer in four. The side effects observed in adenovirus-treated patients in this trial underscore the need for improvements in these vectors before more widespread use can be considered.

9.3.6 HERPES SIMPLEX VIRUS

HSV is an enveloped, double-stranded DNA virus that has long been considered a possible agent for delivering genes into mammalian cells. The virus infects virtually all cell types and vertebrate species when tested in vitro, making it potentially valuable as a vector for use in any organ system or animal model. The HSV genome is very large, 152 kb, and as many as 50 kb are available for deletion and replacement with desired transgenes.

Two primary methods have been developed for using HSV as a vector for gene transfer into mammalian cells. The first method (recombinant vectors) consists of HSV particles in which the gene of interest is inserted into a portion of the viral genome. Early studies showed the potential of recombinant HSV vectors to efficiently deliver transgenes into cells of the CNS using marker genes. Because of the large viral genome, these vectors were classically constructed by homologous recombination, an arduous process that limited their use. However, this trend has changed recently and recombinant HSV vectors may still develop into important tools for gene therapy in neurons.

Most work with HSV in gene therapy applications has utilized a second system (defective HSV vectors). Defective HSV vectors are generated by creation
of a DNA plasmid containing the desired transgene along with an HSV origin of DNA replication and packaging sequence. This plasmid is transfected into cells along with a replicating “helper” HSV that then packages the desired transgene into nonreplicating “defective” particles. Several studies demonstrated the potential of defective HSV vectors for gene therapy in the CNS. Most studies using defective HSV vectors for gliomas utilized the TK/gancyclovir mechanism of tumor cell lysis. However the vectors have also been used to deliver other therapeutic genes.

9.3.7 Other Vectors

The shortcomings of retroviral, Ad, and HSV vectors have fueled searches for other gene therapy vectors including viral and nonviral constructs. Other viruses studied include adeno-associated viruses, lentiviruses, and foamy viruses. Nonviral constructs seek to transfer desired transgenes without the associated toxicity of viruses. Encapsulation of plasmid DNA into liposomes is a promising nonviral mechanism for gene therapy that has been applied to brain and other tumors.

Plasmid–liposome complexes have many distinct advantages compared to viral vectors. They (1) transfer genes of essentially unlimited size, (2) cannot recombine to form an infectious agent, (3) protect DNA from the extracellular environment, and (4) evoke weaker inflammatory responses because they lack proteins. However, transfection via plasmids is highly inefficient and is not selective. Gene expression has been found to be relatively transient.

Liposomes can also be used as vectors for delivery of antisense DNA. This is a unique gene therapy approach that uses therapeutic strands of DNA to bind a complementary sequence within a target gene to block synthesis at the transcription level. Antisense oligonucleotides have shown therapeutic benefit (and minimal toxicity) against brain tumors.

9.3.8 Delivery, Neurogenetic Surgery

Delivery is a potential obstacle to the success of gene therapy for HGGs due to the presence of the blood–brain barrier (BBB). Although several systemic approaches are aimed at bypassing or disrupting the BBB, local delivery is a logical and efficient approach for these locally aggressive tumors. If a tumor is accessible to surgery, the surgical cavity can be lined with the vector or vector-producing cells. Alternatively, repeated delivery of a vector may be performed through placement of an Ommaya or Rickham reservoir at the time of surgery. MRI-guided stereotactic injection is a reasonable alternative for surgically inaccessible tumors. Using these methods, gene therapy may be delivered via traditional surgical approaches, prompting some to refer to this mode of delivery as neurogenetic surgery. A more detailed discussion of delivery methods is presented next in the section on convection-enhanced delivery (CED).
9.4 TRANSLATION INTO CLINICAL TRIALS: HUMANS ARE NOT LARGE MICE

The success of in vitro and in vivo preclinical studies led to cautious optimism in regard to the potential clinical utility of gene therapy for HGGs. It has been only 10 years since the first human trial of gene therapy for HGGs. Since then, multiple Phase I and Phase II clinical trials have demonstrated the safety and feasibility of this approach in humans. Unfortunately, the dictum that humans are not large mice holds true for the translation of in vitro and in vivo experimental successes. Despite tumor regression and improved survival in animal models, significant clinical benefit in humans has yet to be achieved.

Only two gene therapy strategies have been tested in clinical trials for HGG, the p53 tumor suppression approach and the HSV-TK/gancyclovir susceptibility approach. A single Phase I trial of p53 gene therapy utilized an adenoviral vector. Delivery was via a two-stage approach, first with stereotactic injection after tumor biopsy followed by tumor resection and direct injection of vector into the remaining tumor bed. This study demonstrated low toxicity and successful transfection of functional p53 gene to tumor cells. Ad vector injection successfully resulted in apoptosis of p53 transfected cells. However, extension of transfection only reached 5 mm from the injection sites. Further studies are needed to improve distribution of this agent prior to Phase II studies designed to determine therapeutic efficacy.

The majority of clinical trials of gene therapy for any tumor utilize transduction of HSV-TK followed by systemic administration of gancyclovir. Phase I and Phase II trials have demonstrated the safety of this approach in brain tumors via both stereotactic and open surgical delivery. These trials have utilized retroviral or adenoviral vectors. As mentioned, in a Phase I trial that directly compared retrovirus and adenovirus delivery, the adenovirus group showed statistically significant improved survival along with dose-limiting inflammatory side effects.

The bystander effect demonstrated in preclinical experiments may also be significant in human clinical trials. A small Phase I trial demonstrated MRI evidence of tumor regression after transfection with HSV-TK and subsequent treatment with gancyclovir, despite transfection of only a small cluster of cells and variable expression of the HSV-TK gene. However, in the only Phase III trial of this approach in brain tumor patients, no significant differences in time to progression or overall survival were observed in gene therapy-treated patients. Major limitations to therapeutic efficacy include poor distribution of the viral vector and poor penetration of gancyclovir across the BBB.

Despite limited clinical success, gene therapy is now a tenable goal and will very possibly become a standard part of multimodal therapy for patients with HGGs in the future. The past decade has provided major conceptual and technological advances in brain tumor biology and molecular genetics; the years to come will provide many new breakthroughs. This vigorous area of basic science research has already been translated into clinical trials (http://clinicaltrials.gov/ct/ gui) and hopefully the best combination of transgene, vector, and delivery method to benefit brain tumor patients will be discovered soon.

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9.5 CONVECTION-ENHANCED DELIVERY OF TARGETED TOXINS AND OTHER AGENTS

9.5.1 INTRODUCTION

Delivery of therapeutic agents to HGGs is a difficult task that has perplexed neurosurgeons and brain tumor researchers for several decades. The effectiveness of some chemotherapeutic agents against gliomas in vitro has been recognized for many years, but the BBB minimizes the amount of drug that penetrates tumors when administered systemically, even with highly lipophilic nitrosureas.124

Toxicity limits how high a systemic dose can be given and prevents satisfactory levels of agents from reaching tumors in the brain. This circumstance led to many attempts to treat brain tumors with intratumoral or local injections of methotrexate or nitrosureas in the 1960s and 1970s, all with minimal beneficial responses.125–129 Despite the lack of therapeutic benefit, these early investigations were encouraging because they found that intratumoral injections of chemotherapeutic agents resulted in lower systemic toxicity.129,130

The revolution in molecular biology techniques and other scientific advances are leading to a dramatic increase in discoveries of potential therapeutic agents for the treatment of cancer. These agents include traditional chemotherapies, molecular therapies, targeted toxins, viruses, liposomal–DNA complexes, viral packaging cells, stem cells, and others.131–133 Although few of the new therapeutic modalities have achieved mainstream use in cancer therapy as yet, it is likely that some will do so soon. To allow brain tumor patients to benefit from these exciting new developments, a method to deliver therapeutic agents to the brain in a safe and effective manner must be developed. It is possible that this stumbling block to progress in the treatment of HGGs will be overcome by promising developments in CED.

9.5.2 CONVECTION-ENHANCED DELIVERY

Traditional means of delivering agents to the brain have involved direct injection into the parenchyma or cerebrospinal fluid. These injections rely on diffusion of the delivered agent to reach brain tissue away from the injected site. Unfortunately, multiple studies demonstrated that diffusion of agents in the brain is extremely limited, particularly with high molecular weight or polar molecules.134–136

Attempts have been made to overcome this limitation with use of multiple intraparenchymal catheters.137 One study involving cisplatin infusion via 68 catheters still did not produce a significant impact on the patient’s prognosis. This suggests that far too many catheters would be required to treat gliomas in this fashion. A more feasible approach is to use fewer catheters and increase the volume of diffusion through each catheter using CED.

CED uses sustained intracerebral infusion to induce a convective interstitial fluid current that has the potential to homogeneously distribute even large molecules great distances within the brain by displacing interstitial fluid.138 In animal models, CED achieved high homogeneous concentrations of various macromolecular therapeutic agents throughout large regions of the brain that were several orders of magnitude greater than those obtainable by systemic delivery.139 The potential benefit of CED
in the treatment of brain tumors in animal models has been demonstrated in several studies.\textsuperscript{139–141}  

A significant limitation to interpreting data from CED experiments comes from the fact that human brains are much larger than those of the animal models routinely used. Although a few studies have been conducted using CED in humans,\textsuperscript{142} no data are available on the actual distribution of agents delivered in the human brain via this method. Data recently submitted for publication demonstrate distribution of at least 10% of the injected concentration of a macromolecule within a nearly spherical radius over 4 cm from the catheter tip throughout the gray and white matter surrounding a tumor resection cavity (D. Bigner, personal communication, 2004).

In addition to this encouraging data on distribution of agents in the human brain using CED, two clinical trials demonstrated the efficacy of CED in treating human brain tumor patients. In a clinical trial by Laske et al., 9 of 15 malignant brain tumor patients had greater than 50% reductions in tumor volume after receiving therapeutic agents via CED.\textsuperscript{142} Although local toxicity was seen at the highest dose administered, no systemic toxicity was observed, suggesting CED is an effective way to deliver therapeutic toxins to the human brain. In a trial by Rand et al., 7 of 9 patients treated with CED had increased tumor necrosis as evidenced by reduced gadolinium enhancement on MRI following therapy.\textsuperscript{143} One patient survived more than 18 months after therapy.

Although these results are encouraging, several limiting factors remain as obstacles to the use of CED in the treatment of HGG patients. First, although a distribution of agent 4 cm from the catheter tip is encouraging, the technique still requires infusion via multiple catheters and careful optimization and planning to deliver therapeutic agent to the region surrounding a tumor or its resection cavity. Second, tumors clearly alter the fluid dynamics in the brain and the effect of this alteration on CED is poorly understood. Despite these limitations, further studies aimed at optimizing catheter design and infusion parameters should identify modifications capable of effectively addressing these issues now that the potential utility of this approach has been established in humans.

### 9.5.3 Targeted Toxins

Although CED could be used to deliver any of a number of therapeutic agents to treat brain tumors, the majority of work to date has utilized targeted toxins. A targeted toxin is attached to a receptor ligand; an immunotoxin consists of a toxin attached to an antibody that recognizes a receptor. In both cases, receptors selected for targeting are over-expressed on tumor cells (for simplicity, this chapter will use the term “targeted toxin” in reference to both moieties). Targeted toxins allow targeted delivery of potent toxins to tumors with relative sparing of normal tissue.\textsuperscript{133} The specificity of these agents is enhanced and systemic toxicity reduced by delivery to an anatomically isolated compartment, such as the intracranial or intrathecal space.\textsuperscript{144}

Bacterial and plant toxins are potent cytotoxic agents that have been exploited in targeted toxin therapy. Such toxins have at least two important advantages over most chemotherapeutic agents: (1) they are far more potent, while most
chemotherapies require >10⁴ molecules to kill a single tumor cell, many toxins require only one,¹³³ and (2) they are active against hypoxic and nongrowing cells, making them potentially effective against tumors that are resistant to chemotherapy and radiation.¹⁴⁵

The powerful potential of targeted toxins derives from a combination of the high potency and toxicity of the toxin with the highly selective binding of a receptor ligand or antibody. Critical to the success of targeted toxin therapy is the identification of a receptor that is ubiquitously highly expressed on the tumor but not on surrounding tissue. This has been accomplished in tumors outside the CNS. Clinical trials using targeted toxin therapy have targeted interleukin-2 receptors in hematologic malignancies¹⁴⁶ and interleukin-13 receptors in squamous cell carcinomas.¹⁴⁷ Other trials have used tumor-specific antibodies to target ovarian, breast, and colon cancers.¹⁴⁸,¹⁴⁹

In order for targeted toxin therapy to be effective against HGGs, a receptor that is commonly over-expressed on the tumors must be identified and targeted. It has been known for several years that HGGs frequently over-express EGFR.¹⁵⁰ Over-expression is often associated with amplification of the EGFR gene. A simultaneous examination of GBM samples for EGFR gene amplification, mRNA, and protein found approximately one-third had gene amplification, all had mRNA, and 85% had detectable EGFR protein¹⁵¹ (McLendon et al., personal communication, 2004). By contrast, EGFR was found in only very low levels in surrounding brain — a circumstance that lends it to targeted toxin treatment with minimal unwanted toxicity.¹⁵²

EGFR has two natural ligands, epidermal growth factor and transforming growth factor alpha (TGF-α). A targeted toxin for the EGFR was designated TP-38. It is a recombinant chimeric protein composed of TGF-α and a genetically engineered form of the pseudomonas exotoxin PE-38. Encouraging results of a Phase I clinical trial examining treatment of patients with recurrent HGGs using CED of TP-38 have recently been submitted for publication.¹⁵³

Other receptors over-expressed on HGGs have been identified. Targeted toxins for interleukin-4 and interleukin-13 receptors showed therapeutic efficacy against HGGs.¹⁵⁴,¹⁵⁵ Further work using sophisticated molecular biology techniques will undoubtedly identify other potential receptors for toxin targeting and enhance the potential of this novel therapy for HGG patients.

9.6 CONCLUSION

The relatively recent revolution in molecular biology techniques has in fact led to many significant discoveries of underlying mechanisms of the development of HGGs, only a few of which were covered here. Even more importantly, a variety of scientific advances led to the development and rapid translation to clinical trials of many novel forms of cancer therapy, broadly increasing the landscape of potential therapies far beyond the traditional modes of surgery, chemotherapy, and radiation.

Although we have not yet discovered the combination of novel therapy and better understanding of underlying tumor mechanisms that will lead to an efficacious new
treatment of HGGs, many promising new therapies are on the horizon. In this environment of rapid new discovery, it remains of utmost importance that neurosurgeons are involved in and informed of the development of these exciting new therapies that may soon allow us to better serve our sickest patients.

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