Potential of gene therapy for brain tumors

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Brain tumors comprise a broad spectrum of biological and clinical entities making it unlikely for any single therapeutic approach to be universally applicable. In particular, malignant glioblastoma multiforme have defied all current therapeutic modalities. Gene therapy offers the potential to augment current neurosurgical, radiation and drug treatments with little increase in morbidity. Many therapeutic transgenes have shown efficacy in experimental models, including generation of toxic compounds, enzymatic activation of pro-drugs, expression of tumor suppressor or apoptotic proteins, inhibition of angiogenesis and enhancement of immune responses to tumor antigens. Vectors have been used as gene delivery vehicles and as cytotoxic agents in their own right by selective replication and lysis of tumor cells, thereby also generating vectors on-site. Brain tumors appear to offer some ‘Achilles’ heels’ in that they are usually contained within the brain and represent a unique dividing cell population there. However, the heterogeneous and invasive characteristics of these tumor cells, as well as sequestration of tumor antigens within a relatively immune privileged location present serious problems for effective therapy. This review will focus on current transgene/vector strategies, including novel therapeutic genes, combinational therapies and new delivery modalities, the latter of which appears to be the rate limiting factor for gene therapy of brain tumors in humans.

INTRODUCTION

Over the decades, brain tumors, in particular glioblastomas multiforme (GBM), have retained their dismal prognosis despite advances in neurosurgical techniques, radiation and drug therapies (1,2). Some of the difficulties encountered include inaccessibility to resective surgery because of anatomical location and single cell invasion of surrounding brain tissue, with tumors usually recurring within a few centimeters of the margins of the resection (3). These migratory tumor cells temporarily exit the cell cycle during migration, making them resistant to therapies that target dividing cells (4). Even within a tumor, most cells are not dividing within a given treatment window. Other complications are damage to normal brain by therapeutic procedures, the relative impermeability of the blood–brain barrier (BBB) and the genetic heterogeneity of tumor cells (5,6).

Malignant gliomas have been a primary target for gene therapy partly because of their dismal prognosis, but also because patients with these tumors are initially able to give informed consent and may agree to experimental procedures for altruistic reasons. Even benign tumors within the nervous system can be severely debilitating and life-threatening, and in such cases partial debulking and inhibition of growth may prove therapeutically effective. Although cures or long-term remission of malignant brain tumors seems unlikely in the near future, extension of meaningful lifespan for months or even years would be a boon. Clinical trials designed to maximize scientific information about gene delivery and potentially toxic effects of the therapy provide a basis of knowledge for future therapeutic strategies. Gene therapy offers the promise of augmenting traditional cancer therapies (drugs, radiation and surgery) as well as bringing into action some novel weapons. Therapeutic genes can, for example, serve to: generate anticancer drugs within the tumor (pro-drug activation) (7), thereby increasing intratumoral drug levels without increasing systemic toxicity; protect sensitive endogenous hematopoietic cells from drug damage by making them drug resistant (8); and allow sustained delivery of secreted fusion proteins which combine a targeting ligand and a toxin/enzyme. Gene-mediated drug activation within the tumor can also be used to sensitize tumors to radiation (9), and radiation in turn can be used to induce expression of transgenes via radiation-activated promoters (10). Neurosurgical procedures are used to introduce cells or vectors into the tumor and to obtain tumor cells for subsequent genotyping or vaccination. Therapeutic genes can act to directly kill or block growth of tumor cells, inhibit angiogenesis, stimulate immune responses to tumor antigens and block tumor cell invasion (Table 1). Vectors themselves can act as selectively toxic agents and be targeted by ligands to receptors that are highly expressed on tumor cells (11,12). Gene delivery to tumors within the brain is a formidable obstacle; even in experimental tumors it is difficult to achieve gene delivery to >5% of the tumor mass. Therefore, transduced cells must be able to exert a therapeutic effect on neighboring
Table 1. Therapeutic genes for tumor therapy

<table>
<thead>
<tr>
<th>Tumor cell killing</th>
<th>Direct cytotoxicity:</th>
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<tbody>
<tr>
<td></td>
<td>Transferrin-toxin fusion</td>
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<tr>
<td></td>
<td>Tetanus toxin</td>
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<td></td>
<td>Diphtheria toxin A</td>
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<td>Pseudomonas exotoxin A</td>
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<th>Indirect or conditional cytotoxicity:</th>
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<tbody>
<tr>
<td>HSV-TK/GCV</td>
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<tr>
<th>E. coli cytosine deaminase/5-fluorocytosine/uracil phosphotransferase</th>
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<tbody>
<tr>
<td>Cytochrome P450/cyclophosphamide/reductase</td>
</tr>
<tr>
<td>Poly(ADP)-ribose synthetase/methatrexate</td>
</tr>
<tr>
<td>Carboxylesterase/CPT-11</td>
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<tr>
<td>Deoxycytidine kinase/arabinoside</td>
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<th>Targeting specific cellular gene</th>
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<tr>
<td>Tumor suppressors:</td>
</tr>
<tr>
<td>p53; p16; p21; PTEN; Rb; p300</td>
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| Apoptosis: |
| Caspases; Bax; Fas ligand |

| Angiogenesis: |
| Endostatin; angiostatin |

<table>
<thead>
<tr>
<th>Antisense VEG; dominant negative VEGF receptors</th>
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<tbody>
<tr>
<td>Antisense EGF; dominant negative EGF receptors</td>
</tr>
<tr>
<td>Antisense basic FGF</td>
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<tr>
<td>Antisense IGF-1</td>
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<th>Immunomodulation</th>
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<tbody>
<tr>
<td>Cytokines:</td>
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<tr>
<td>Interleukines (IL-2; IL-4; IL-6; IL-12; IL-13); TNF-α; GM-CSF; interferon γ</td>
</tr>
<tr>
<td>Inhibition of TGF-β</td>
</tr>
<tr>
<td>Antisense TGF-β; TGF-β soluble receptors; decorin</td>
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<th>Oncolytic viruses</th>
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<tr>
<td>HSV γ34.5-minus</td>
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<tr>
<td>HSV γ34.5-minus, RR-minus</td>
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<tr>
<td>Ad E1B-minus</td>
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<tr>
<td>Ad E1A-minus</td>
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| Non-transduced cells (the ‘bystander effect’). New methods are |
| being explored to achieve delivery to invasive tumor cells over |
| wide swaths of the brain through convection delivery, via the |
| vasculature or cerebrospinal fluid (CSF), or by using migratory |
| vehicle cells (for review see 13). |

Clinical trials of gene therapy for brain tumors to date have |
been focused primarily on Phase I toxicity evaluation. None |
have shown notable efficacy, and many point to the high sensi- |
tivity of normal brain, with possible/probable related conse- |
quences of fever, confusion, hemorrhage, sepsis and paralysis. |
The low response rate observed in these clinical trials, as |
compared with promising preclinical tumor models in rodents, |
is multi-factorial. First, the size of tumors and brain in rodents |
and human is different by several orders of magnitude. Second, |
most experimental tumors have an overall higher percentage of |
dividing cells as compared with human tumors, thus, resulting |
in greater transduction efficiency via retroviral vectors and |
higher sensitivity to drugs and vectors that are selective for |
DNA replication/cell cycling. In addition, rodent gliomas tend |
to grow in the brain as a single mass with infiltrating fronds |
(14), whereas human gliomas send out single invasive cells |
that extend a considerable distance from the main tumor mass |
(15). Furthermore, most rodent gliomas are antigenic even in |
syngeneic animals, whereas human GBMs are associated with |
immune suppression and evasion (16). |

PHARMACOLOGICAL ENHANCEMENT |

Targeting toxin/protein/vector delivery |

Recombinant fusion proteins containing both a ligand binding |
domain for a tumor-enriched receptor and a toxin domain can |
kill tumor cells upon receptor-mediated endocytosis. Examples |
used for brain tumors include a toxin fused to the interleukin |
(IL)-4 ligand (17) or to anti-transferrin (Tf) receptor (R) anti-
| bodies (18). Both the IL-4R and TfR are expressed at high |
levels on human glioma cells and the TfR is also high on the |
luminal surface of brain capillaries (19,20). This targeting |
strategy has been utilized in gene therapy. For example, an |
envelope protein of the retrovirus virion has been fused |
through a protease-sensitive linkage to a polypeptide that |
blocks infection, with high levels of metalloproteinase in the |
vicinity of tumors releasing the peptide and restoring infect-
tivity (21). Ligands or receptor antibodies have also been |
added to the capsid of adenovirus (Ad) virions to enhance |
infec tion of glioma cells, e.g. antibodies to EGFR, which is |
expressed at high levels on GBM (22), a peptide selected for |
blocking the TfR (23), a lysine polypeptide (24) and ligands |
that target heparin sulfate and integrin receptors (25). Bio-
obically active proteins, such as β-galactosidase and viral thymi- |
dine kinase (TK) have been fused to translocating peptides/ |
proteins, such as TAT (26) or VP22 (27,28), to allow their |
movement out of the cell of synthesis into neighboring cells. |

Pro-drug activation |

One powerful use of gene therapy is to augment the toxicity of |
cancer drugs by selectively increasing their concentration |
within the tumor through on-site conversion from a pro-drug. |
This strategy employs pro-drugs, which are non-toxic systemically |
and may cross the BBB more readily than active drugs. This |
approach has also been called ‘suicide’ gene therapy as the |
transduced cells convert a non-toxic pro-drug into a toxic |
molecule, thereby killing themselves. One of the first and most |
widely used pro-drug activation systems was Herpes Simplex |
Virus type-1 (HSV) TK with ganciclovir (GCV) (29). HSV-TK |
phosphorylates the antiviral nucleoside analog, GCV, allowing |
it to be incorporated into replicating DNA leading to cell death |
(30) (Fig. 1). GCV is non-toxic to both non-transduced cells |
and non-dividing cells. Phosphorylated GCV is able to pass |
through gap junctions between adjacent cells and kill neigh-
boring cells (31–36). |

Two other well-characterized pro-drug-activating systems are |
Escherichia coli cytosine deaminase/5-fluorocytosine (CD/5-FC) |
(37) and rat cytochrome P450 2B1/cyclophosphamide (CPA)
CD converts the non-toxic compound 5-FC to 5-fluorouracil (5-FU), a cancer drug. 5-FU inhibits DNA and RNA synthesis and is thereby toxic to both dividing and non-dividing cells (7). Potency is increased by co-expression of uracil phosphotransferase (40). CD/5-FC exerts a strong bystander effect due to membrane diffusibility of 5-FU. The combination of HSV-TK/GCV and CD/5-FC has proven to be very potent for experimental brain tumors and can be combined with other therapies (41,42).

Cytochrome P450 2B1 (CYP2B1) is a mammalian enzyme and thus less antigenic than bacterial proteins, so that transduced cells survive longer and can serve as biological mini-pumps. CYP2B1 is a hepatic-specific enzyme that activates the cancer drug cyclophosphamide (CPA) by conversion to phosphoramide mustard (PM), which is relatively stable and diffusible, but does not readily across the BBB (43). Vector-mediated delivery of CYP2B1 to brain tumors generates the active metabolite on-site thereby increasing intratumoral concentrations. Intratumoral levels of PM can be increased by co-expression of reductase (44) and placement of CPA-containing wafers within the tumor (M. Colvin and E.A. Chiocca, unpublished results). Incorporation of the CYP2B1 gene into replication-conditional HSV vectors allows oncolytic propagation of the virus in tumor cells with concomitant generation of PM (45).

**Tumor suppressors, apoptosis and angiogenesis and other Achilles’ heels**

Tumor cells can be killed by triggering apoptosis. Although this does not have a direct bystander effect, it may increase immune recognition of tumor antigens. Among the various tumor suppressor genes that can trigger apoptosis, p53 is the most commonly used for brain tumor therapy. Loss or mutation of the p53 gene has been shown to promote genomic instability and accelerate the growth rate of cells, and is generally accepted to be an early event in the malignant transformation of up to half of human glioma tumors (46,47). Restoring normal wild-type p53 function can lead to growth arrest (48–50) and enhance apoptotic actions of radiotherapy and chemotherapy (51–53). Combining apoptotic-inducing effects from two different pathways, e.g. p53 and Fas, can override the apoptosis-resistant mechanisms found in some glioma cells (54), e.g. by expression of dominant negative forms of ras (55) or ribozymes against its message (56), as well as delivery of caspases (54,57) and tumor necrosis factor α (TNFα) (58,59).

Primary human gliomas express Fas (60) and hence should be
susceptible to apoptosis via its activation; however, the high toxicity of TRAIL warrants great caution (61).

Brain tumors, like other cancers, require angiogenesis for bulk growth and gene transfer has been used to express anti-angiogenic agents (62). A number of angiogenic factors mediate this neovascularization including angiopoietins, hypoxia inducible factor-1 [which up-regulates vascular endothelial growth factor (VEGF)], basic FGF and platelet factor 4. Levels of VEGF correlated with tumor progression with highest levels found in the most malignant forms (63,64). Strategies to block angiogenesis include, for example, a dominant negative version of the VEGF/flk receptor (65,66), anti-sense to VEGF and an antagonist of the Tie2 receptor (67–69). Angiogenesis can be inhibited via diffusible factors, with the main challenges in gene therapy being continuous production overtime (most vector-mediated gene expression is down-regulated) and arrest rather than elimination of tumors.

Other novel therapeutic genes with promise for brain tumor therapy include the sodium+/iodide– transporter, normally expressed in the thyroid, which allows imaging of gene delivery and radiiodide-mediated toxicity (70,71), a fusogenic protein on the membrane of tumor cells that stimulates cell fusion into a multinucleate, necrotic mass (72), a secretable growth factor that stimulates apoptosis of tumor cells (73), anti-sense against telomerase RNA to block protection of chromosomes ends (74) and connexin to increase passage of toxic molecules between tumor cells (36,75). Attempts to reduce neuroinvasiveness have included modification of the extra-cellular matrix by expression of the tissue inhibitor of mettall-proteinase-2 (TIMP) (76), and anti-sense blockade of β-integrin (77) and fucosyltransferase (78).

**IMMUNE RESPONSE MODIFICATION**

The lack of effective immune responses against glial tumors of the brain is due in part to the immune-privileged status of the brain conferred by the BBB and the lack of conventional lymphatics within the central nervous system (CNS) (79). In addition, successful neoplastic cells typically produce immune-suppressive factors (60,80,81). Tumor antigens are heterogeneous even within the same tumor, and few-to-no identified antigenic markers have been identified that are common across multiple brain tumors, with possible exceptions being mutant EGFR (82) and the IL-13R/feistis antigen (83). Other escape mechanisms include failure to recruit or fully activate dendritic cells and suppression of the T cell-dependent arm of the immune response (84). However, both infiltrating lymphocytes and macrophages are found within high-grade gliomas (85), indicating the potential for lymphocyte homing and presentation of processed tumor antigens. Immunotherapy could be most effective as a ‘mop up’ operation for small tumor foci left behind after other treatments.

Immunotherapy in experimental animals can be mediated by either injection of vectors encoding cytokines or cells producing cytokines into the tumor mass, or by peripheral vaccination with such vectors or cells combined with irradiated tumor cells. A number of cytokines have shown efficacy in experimental brain tumor models including GM-CSF (86), IL-2 (87), IL-12 (88) and IL-4 (89). Future immune alerting schemes will probably include peripheral vaccination with a combination of cells, e.g. autologous dendritic and freshly isolated, viable tumor cells mixed with ‘generic’ cells secreting one or more cytokines. These strategies combine well with chemotherapy (90), oncolytic (inherently antigenic) viruses (91) and radiosurgery (59).

**VECTORS**

**Vector systems**

Given the desperate condition of brain tumor patients, even potentially toxic vectors such as replicating viruses appear warranted. But it should be kept in mind that someone can die faster and incur more brain damage from viral encephalitis than from a brain tumor. Therefore investigators are of two minds with respect to the type of vectors to be employed for brain tumor therapy. On the one hand, non-toxic vectors, such as non-viral vectors, HSV amplicons, gutted Ad and retrovirus (92) seem a cautious choice for sensitive brain tissue. On the other hand, it is critical to expand the range of gene delivery within the brain, and oncolysis by replicating virus vectors and on-site vector generation may be the only effective way to do this.

Non-viral vectors include naked DNA, polycationic polymers and liposomes. These vectors are delivered into the tissue by injection or particle bombardment and typically enter the cytoplasm by endocytosis or transient membrane disruption (reviewed in 93). Transduction efficiency is increased by incorporation of fusion proteins (94), targeting elements (95). DNA transit to the nucleus can be facilitated by high mobility group proteins and nuclear localization signals (96) and viral elements can also be included to prolong DNA stability (97). However, for the treatment of brain tumors, these non-viral vectors are limited by low transfection efficiency and transient expression.

Virus vectors have a high efficiency of gene delivery and multiple therapeutic capabilities (98). Most of the viruses used for gene delivery are common human pathogens with a broad host cell range. They are inherently antigenic—and hence can promote immune responses to tumor antigens—and toxic, through virus proteins or replication. Most humans have or can readily generate antibodies to virus proteins, which provides a level of containment, albeit at the risk of decreasing the efficiency of gene delivery. The commonly used viral vectors for gene delivery into brain tumors include the recombinant HSV, Ad, retrovirus and hybrid vectors derived from them (99,100). Gutless Ad, HSV amplicon and AAV vectors, which like retrovirus vectors express no viral genes, have less potential toxicity, but reduced transduction efficiency.

Recombinant virus vectors are grouped into two types: replication-defective and replication-conditional. Replication-defective Ad vectors have shown limited gene delivery to tumors (101,102) even with a bystander effect (103). In attempts to increase tumor infection by generation of vectors on site, as well as to harness the oncolytic potential of viruses, mutant viruses have been employed that can replicate selectively in tumor cells. Replication-conditional vectors also yield high-level gene expression in tumor cells and can enhance inflammatory cytokines and T cell-mediated immunity (104). Replication-conditional viruses include HSV mutants for dividing cells (105), Ad mutants for p53 mutated cells (106) and reovirus for cells with an activated ras pathway (107).
Tumor selectivity has also been achieved by placement of genes essential to virus replication under promoters that are selectively active in target tumors, e.g. the nestin promoter (108,109) and the myelin basic protein promoter (54).

There are a number of complications envisioned using virus vectors in the brain. Viral antigens may activate latent viruses and cause inflammatory responses (110) or facilitate autoimmunity leading to demyelination (111) and neurodegeneration (112). With replicating vectors, it is very difficult to achieve absolute tumor specificity and low level infection and replication in normal brain could manifest as a ‘smoldering’ infection with neurodegeneration (113). The immune-privilege of the nervous system and immune-compromise of the patient could complicate this problem with some vector inevitably breaching the vasculature and infection proceeding in other tissues. Therefore, if the lifespan of brain tumor patients could be extended by a number of years, these individuals might suffer other consequent debilitating conditions. Given that foreign promoters inserted into virus genomes rarely behave as in their natural genomic setting, and that virus tropism is determined in part by host cell permissiveness at the transcriptional level, engineered, replicating virus vectors may manifest novel tissue tropisms and could potentially be transmitted to other individuals.

Retrovirus

Retrovirus vectors have been the mainstay for most clinical gene therapy protocols and have special appeal for brain tumors given that the classic Moloney Murine Leukemia Virus type can only insert genes into dividing cells, such as tumor and endothelial cells within the neovasculature in the adult brain. Since these vectors tend to have very low titers and are unstable in body fluids, they have been delivered by grafting in vector producer cells (114), injecting virions pseudotyped with vesicular stomatitis virus glycoprotein (VSVG) to stabilize the vector producer cells (114), injecting virions pseudotyped with vesicular stomatitis virus glycoprotein (VSVG) to stabilize the vector producer cells (114), and infecting cells in vitro. Therefore, if the lifespan of brain tumor patients could be extended by a number of years, these individuals might suffer other consequent debilitating conditions. Given that foreign promoters inserted into virus genomes rarely behave as in their natural genomic setting, and that virus tropism is determined in part by host cell permissiveness at the transcriptional level, engineered, replicating virus vectors may manifest novel tissue tropisms and could potentially be transmitted to other individuals.

HSV vectors

HSV is a common human pathogen that naturally establishes life-long, asymptomatic infections of the nervous system with periodic epidermal manifestations, and can infect and express genes in both dividing and non-dividing cells (121). The viral replication takes <10 h, produces thousands of virus progeny and invariably results in cell death. Recombinant virus vectors have a large transgene capacity, up to 50 kb, with up to five transgenes inserted into different loci (122). Replication-deficient vectors typically delete essential, immediate-early genes encoding transcriptional activators, so that viral gene expression is blocked and cytotoxicity is reduced (123). Latent infections of neurons are non-toxic as essentially all viral gene expression is silenced (124). Hence, the possibility exists of expressing therapeutic genes in dividing tumor cells while establishing a benign, latent infection in neurons. Although clinical trials indicate that inoculation of even replication-conditional HSV vectors into human brain can be tolerated, concerns remain about the potential for reactivation of the wild-type virus, which is believed to be latent in the brains of many humans (125), and direct toxicity to neurons or persistent cerebral inflammation due to low-grade viral protein expression or immune responses (126).

Replication-conditional vectors contain mutations in one or several non-essential viral genes for TK, ribonucleotide reductase (RR), UTPase or the neurovirulence factor, γ34.5 (Fig. 2), which can be compensated for by up-regulation of mammalian enzymes in dividing cells. Mutants for γ34.5 (e.g. 1716) have reduced neurovirulence and replicate selectively albeit at a low rate in actively dividing cells (127–129). In a clinical study of CNS glioma, doses of 1716 up to 10^5 p.f.u. were tolerated without apparently related adverse effects (130), but this, like other HSV vectors, does exhibit toxicity in animal models in a dose-dependent manner (131–133). Replication-conditional double mutants, such as G207 (134) or MGH1 (135), which are defective for both γ34.5 and RR, have reduced toxicity, hyper-sensitivity to GCV and temperature sensitivity. In a Phase I clinical trial for malignant glioma, direct intracranial inoculation of G207 at doses up to 3 × 10^6 p.f.u. caused neither acute toxicity, viral shedding or delayed reactivation of latent virus (136). In both the 1716 and G207 trials, the apparent safety of these mutant HSV in brain is remarkable given the immuno-suppressed condition of participants. No consistent decreases in tumor size were noted by imaging, but anecdotal cases of tumor shrinkage or prolonged progression-free intervals were reported.

Adenovirus

Adenovirus typically causes respiratory illness and its genome is not retained in most cells for any extended period. Recombinant Ad vectors have been used extensively in experimental therapies and also consist of replication-defective and conditional forms. Genes (up to 10 kb) can be inserted into regions containing the E1A and/or E1B genes, and E3 and E4 genes (137). Deletion of the E3 region and/or part of E4 increases vector immunogenicity, and hence potential for inflammation (138). The 55 kDa protein from the E1B region inactivates p53 (reviewed in 139). Because p53 function is critical for efficient virus replication, Ad lacking E1B expression can only replicate in cancer cells that lack p53 function (Fig. 3), including human glioma cells (108). Mutation of E1A also promotes selective replication in tumor cells and overexpression of the Ad-encoded death protein enhances toxicity (140). However, Ad vectors have high antigenicity and have been associated with toxic, inflammatory responses in the brain (110,141). Clinical trials for brain tumors using replication-defective Ad vectors vector with HSV-TK/GCV showed tolerance up to 2 × 10^10 viral particles, but no confirmed benefit (103).
The rate limiting step to gene therapy for brain tumors lies in achieving comprehensive gene delivery (13). This results from limited and risky access to the brain, the highly invasive nature of these tumor cells and difficulties of access across the BBB and BTB. A number of innovative approaches have been evaluated which appear to be able to extend the range of delivery.

Direct, stereotactic injection is the most common route of delivery, with the volume and number of injections being limited by inherent toxicity of fluids and the potential for hemorrhage. The number of vectors, delivery period and range of gene delivery can be increased by slow and convection-enhanced delivery (142), incorporation of stable virus particles into biodegradable microspheres (143) and pre-exposure to proteases to degrade extracellular matrix proteins (144). Still, in most schemes the vector only diffuses a few millimeters from the injection site (145,146).

Vectors can be ‘propelled’ away from the injection site using replication-conditional vectors, which can ‘leap-frog’ from one tumor cell to the next, generating vector progeny in their wake. Migratory cells, such as glioma (147), endothelial (148) and neuroprogenitor cells (149), can be used to carry replication-conditional vectors in an arrested, but activatable state (150) or serve as producers for retrovirus vectors, and not kill the ‘messenger’ cell. The use of neuroprogenitor cells, in particular, appears to offer a means of accessing invasive tumor cells (151). These cells (also referred to as neural stem cells or neuroprecursor cells) are characterized by their ability to self-renew and their potential to differentiate into neurons and glia (152,153). They are present in both the developing and adult CNS and respond to neuronal injury by migration to damaged regions. Neuroprogenitor cells have been used to deliver IL-4 to experimental gliomas (154), but results were confounded by the use of C6 glioma cells in a non-syngeneic host (155). Neuroprogenitor cells armed with the CD gene, combined with 5-FC treatment, have also shown therapeutic efficacy in an experimental glioma (149).

Other routes of access to the brain include the CSF and vasculature. Intraventricular injection of Ad and HSV vectors yields extensive labeling along the ependyma and meninges with some penetration into the cortical layers (133,141). However, in both cases toxicity was observed due to inflammation and immune responses, as observed with retrovirus producer cells (146,156). The ventricles appear to be an especially sensitive compartment of the brain where less toxic vectors may need to be applied. The brain vasculature is very extensive and, although the BBB restricts entry of drugs and vectors, the BTB is more permeable (120,157). The BTB has proven leaky to virus vectors, including Ad (158) and HSV (159), and selective entry into tumor versus normal brain can be increased using pharmacologic agents (160). This allows selective delivery to regions of tumor neovascularization, typical of the expanding, invasive margin of the tumor and new foci of tumor formation, both of which are critical areas for therapeutic intervention.

CONCLUSION

Brain tumors present many unique challenges and opportunities for innovative therapies. Foremost is the high sensitivity of the brain to damage that can compromise functional capacity, and, as with current treatment modalities, the morbidity of the treatment must always be weighed against possible therapeutic gains. The hope of gene therapy for brain tumors lies in the potential to extend functional lifespan with little additional,
Gene therapy does not provide ‘magic bullets’, but rather offers a ‘Pandora’s box’ of possible counteragents to some of the difficulties in treating brain tumors. Some potential solutions have been identified, including use of on-site vector generation and migratory cells that home to tumors, pharmacologic means of selectively opening the BTB, and an array of therapeutic genes, many of which produce ‘bystander’ and synergistic effects.

There are a wide range of adult and pediatric tumor types, including astrocytomas, oligodendrogliomas, meningiomas, ependymomas, neuroblastomas, medulloblastomas and glioblastomas. These have been classified on the basis of pathological and antigenic profiles and have varying prognoses. The advent of molecular genetic technologies will allow a thorough genotypic classification based on mutant genes and the pattern of gene expression (161,162). This genetic information can be used to tailor ‘genetic therapy’. For example, tumors can be typed with respect to p53 status and downstream events to see whether they will be susceptible to p53-based therapy. Genetic changes associated with loss of genes on chromosome 1p and 19q can predict a good response to chemotherapy (163). The expression of the trkC receptor by medulloblastomas may herald a therapeutic response to NT3 (73,164). The next step is to hone vectors so that they function as ‘smart bombs’ that are directed to the specific genotypic and phenotypic properties and progression status of particular tumors. Recent advances in imaging of tumor geography, molecular genotyping of tumor cells and vectors that infect tumor cells selectively and have reduced toxicity to normal cells, will help tremendously in directing these efforts.

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REFERENCES


