 Human Molecular Genetics, 2001, Vol. 10, No. 7 777–787

Potential of gene therapy for brain tumors

Paula Y.P. Lam[1,2] and Xandra O. Breakefield[1,+]

[1]Molecular Neurogenetics Unit, Massachusetts General Hospital, and Department of Neurology and Neuroscience Program, Harvard Medical School, Boston, MA 02114, USA and [2]National Cancer Centre, Division of Molecular and Cellular Research, National Cancer Centre, 169610 Singapore

Received 12 January 2001 ; Revised and Accepted 26 January 2001

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 hemapoietic 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

[+]To whom correspondence should be addressed at: Massachusetts General Hospital East, Deptartment of Molecular Neurogenetics, 13th Street, Building 149, Charlestown, MA 02129, USA. Tel: +1 617 726 5728; Fax: +1 617 724 1537; Email: breakefield@helix.mgh.harvard.edu

Table 1. Therapeutic genes for tumor therapy

Tumor cell killing
Direct cytotoxicity:
Transferrin–toxin fusion
Tetanus toxin
Diphtheria toxin A
Pseudomonas exotoxin A
Indirect or conditional cytotoxicity:
HSV-TK/GCV
E.coli cytosine deaminase/5-fluorocytosine/uracil phosphotransferase
Cytochrome P450/cyclophosphamide/reductase
Folylpolyglutamyl synthethase/methatrexate
Carboxylesterase/CPT-11
Deoxycytidine kinase/arabinoside
Targeting specific cellular gene
Tumor suppressors:
p53; p16; p21; PTEN; Rb; p300
Apoptosis:
Caspases; Bax; Fas ligand
Angiogenesis:
Endostatin; angiostatin
Antisense VEG; dominant negative VEGF receptors
Antisense EGF; dominant negative EGF receptors
Antisense basic FGF
Antisense IGF-1
Immunomodulation
Cytokines:
Interleukines (IL-2; IL-4; IL-6; IL-12, IL-13); TNF-α; GM-CSF; interferon γ
Inhibition of TGF-β
Antisense TGF-β; TGF-β soluble receptors; decorin
Oncolytic viruses
HSV γ34.5-minus
HSV γ34.5-minus, RR-minus
Ad E1B-minus
Ad E1A-minus

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 sensitivity of normal brain, with possible/probable related consequences 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) antibodies (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 infectivity (21). Ligands or receptor antibodies have also been added to the capsid of adenovirus (Ad) virions to enhance infection of glioma cells, e.g. antibodies to EGFR, which is expressed at high levels on GBM (22), a peptide selected for binding to the TfR (23), a lysine polypeptide (24) and ligands that target heparin sulfate and integrin receptors (25). Biologically active proteins, such as β-galactosidase and viral thymidine 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 neighboring 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)

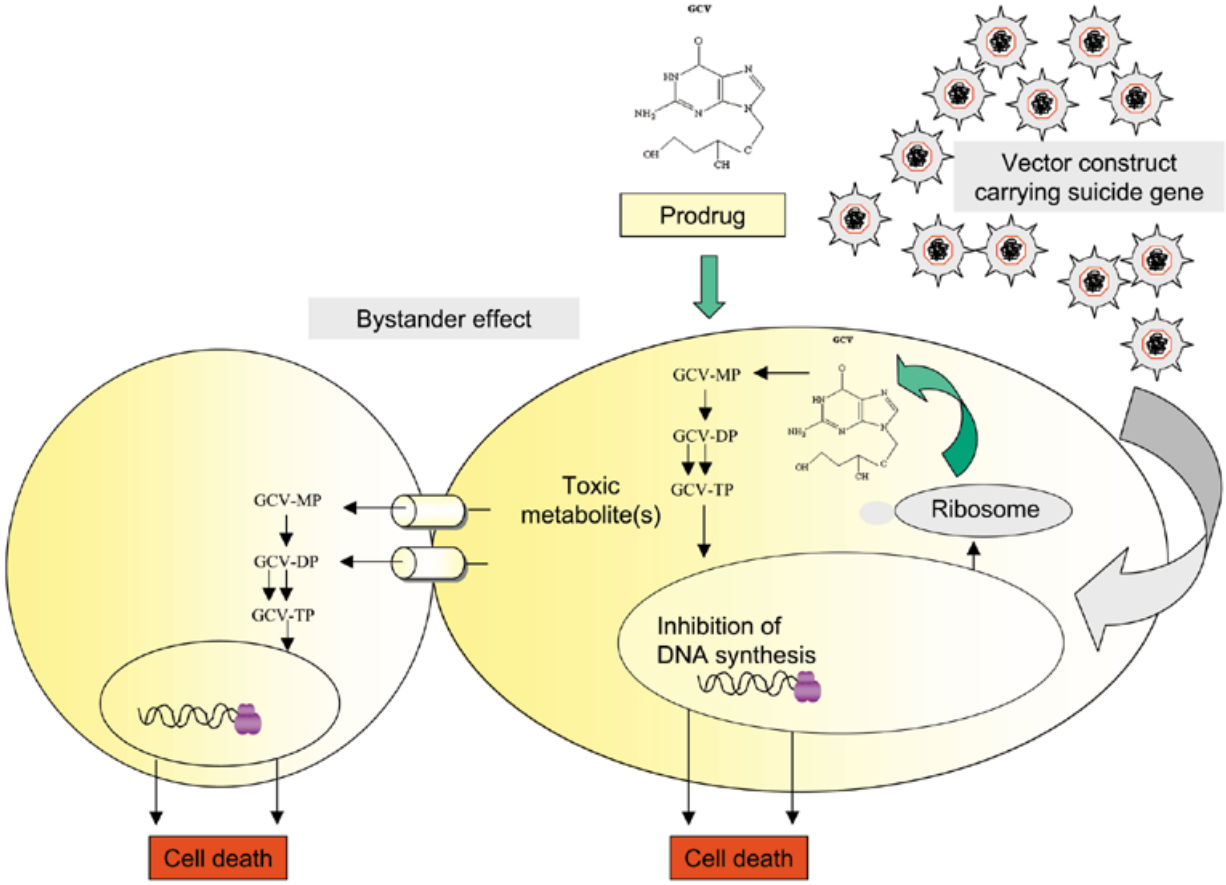

Figure 1. Mode of the Herpes Simplex Virus TK/GCV paradigm. Cells expressing HSV-TK phosphorylate GCV efficiently to di- and triphosphate metabolites. GCV-triphosphate (GCV-TP) is the active form of the drug. Incorporation into elongating DNA during cell proliferation results in premature chain termination and eventual cell death. Phosphorylated GCV is capable of passing through cellular gap junctions to confer cytotoxic effects on non-transduced, neighboring cells.

(38,39). 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 minipumps. 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 radioiodide-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 stimulate apoptosis of tumor cells (73), anti-sense against telomerase RNA to block protection of chromosome ends (74) and connexin to increase passage of toxic molecules between tumor cells (36,75). Attempts to reduce neuroinvasiveness have included modification of the extracellular matrix by expression of the tissue inhibitor of metallproteinase-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 heterogeneic 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/testis 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. Virus 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 compound 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 virions (115) or packaged in human cells (116), or by converting tumor cells to producer cells (117,118). Primary safety concerns with retrovirus vectors include the possible presence of replication-competent, recombinant (RCR) virus, which can co-infect cells and yield continuing production of RCR and retrovirus vectors *in vivo*. This presents a risk if the vector contains a gene that has toxic or debilitating effects on normal cells. Also, extended retrovirus production *in vivo* has been associated in non-human primates with genomic integration events leading to leukemia (119). Still, the wide use of these vectors in the human population with extensive monitoring has inspired confidence that they are relatively safe. Unfortunately the first large-scale, gene therapy Phase III trial for brain tumors in which producer cells generating retrovirus vectors bearing HSV-tk were implanted, followed by GCV treatment, did not have any treatment benefit (120).

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 virus 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, hypersensitivity to GCV and temperature sensitivity. In a Phase I clinical trial for malignant glioma, direct intracranial inoculation of G207 at doses up to 3×10^9 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 immunosuppressed 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).

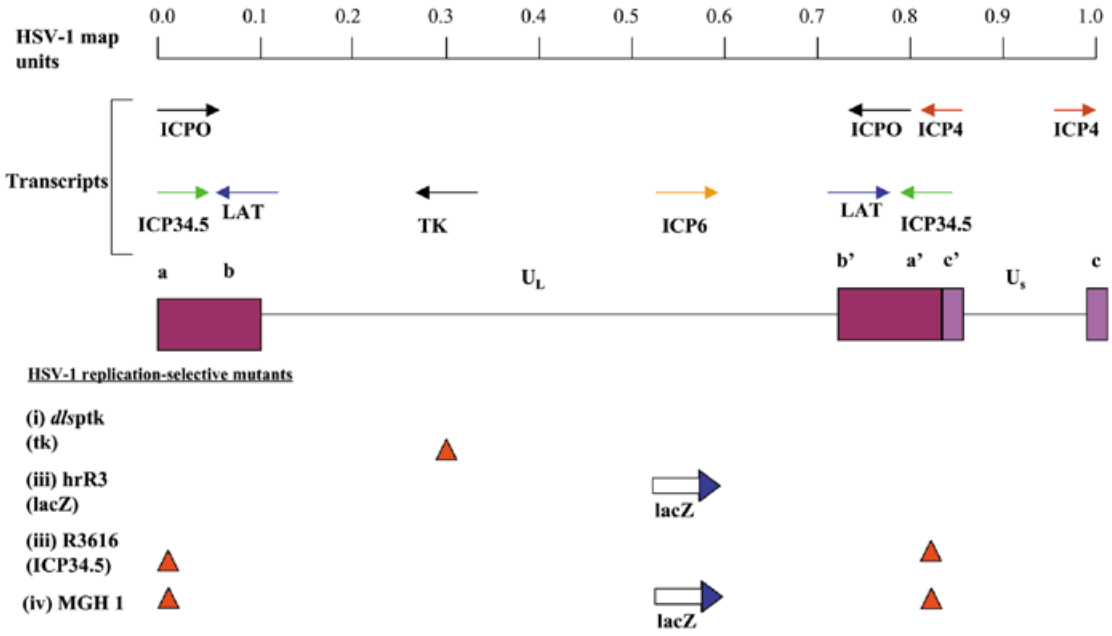

Figure 2. HSV recombinant virus vectors. Structure of 150 kb HSV genome. The boxes represent inverted repeat sequences flanking the long (U_L) and short (U_S) unique sequences of HSV DNA (thin lines). Some transcripts are indicated as arrows in the direction of transcription. (i) Mutant dlsptk contains a deletion in the *tk* gene; (ii) hrR3 contains a lacZ marker insertion in the RR gene; (iii) R3616 contains a 1 kb deletion in both copies of the $\gamma_1$34.5 gene, and (iv) G207 and MGH-1 have a deletion/lacZ insertion in the RR locus and mutation in $\gamma_1$34.5.

DELIVERY MODALITIES

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,

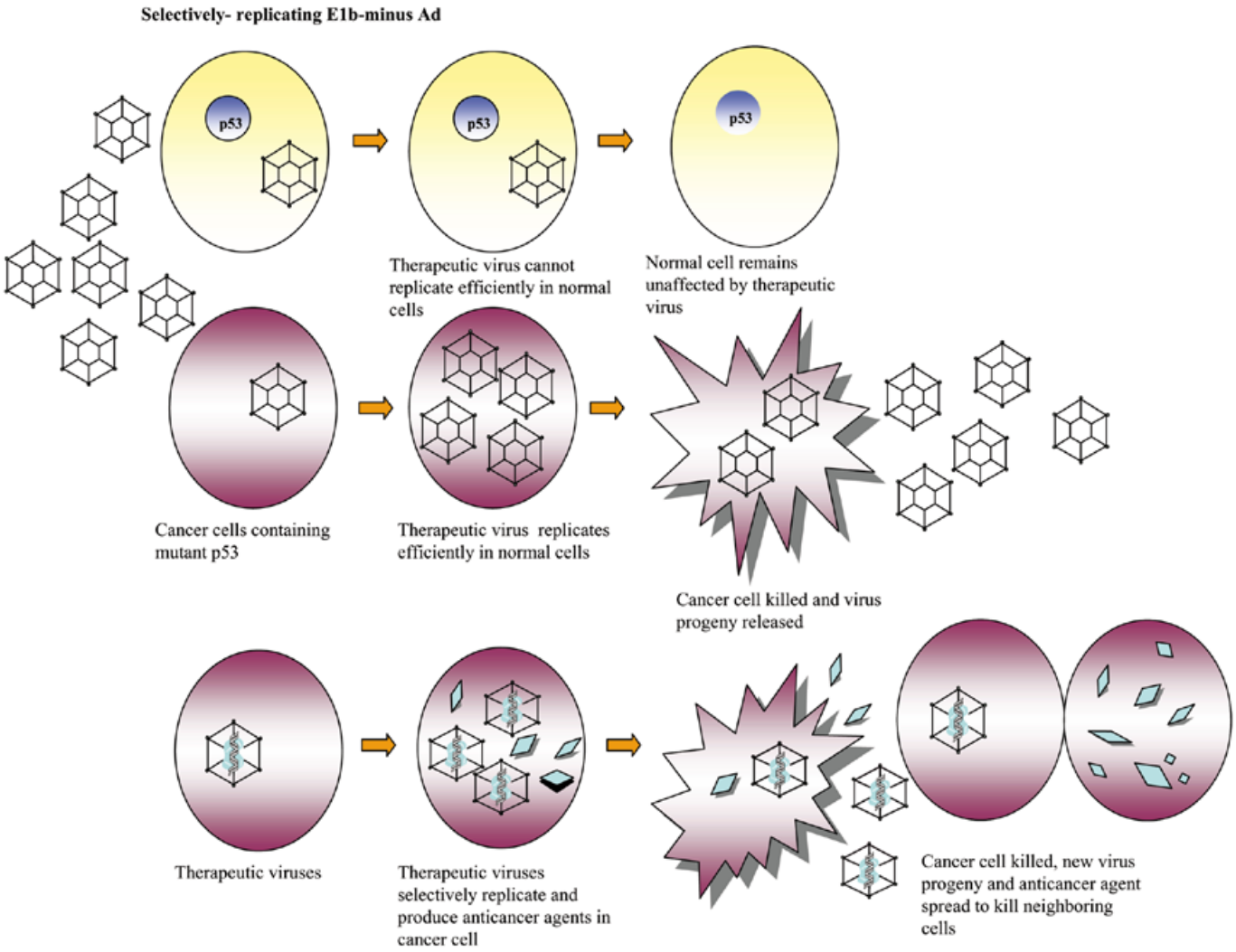

Figure 3. Modes of action of conditional oncolytic Ad vectors. Adenoviruses lacking expression of E1B 55 kDa replicate selectively in cancer cells that lack p53 function (middle panel), while normal cells are resistant to virus replication (upper panel). Oncolysis can be improved further by incorporating therapeutic genes, e.g. pro-drug-converting enzymes, with an added bystander effect to kill surrounding untransduced tumor cells (lower panel).

and possibly reduced, morbidity as compared with current treatments. 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.

ACKNOWLEDGEMENTS

We thank Ms Suzanne McDavitt for skilled preparation of this manuscript. Funding has been provided by National Cancer Centre of Singapore Pte Ltd (P.L.) and NINDS NS24279 (X.O.B.) and NIMH NCI CA69246 (X.O.B.).

REFERENCES

1. Forsyth, P.A. and Roa, W.H. (1999) Primary central nervous system tumors in adults. *Curr. Treat. Options Neurol.*, **1**, 377–394.
2. Black, P.M. and Loeffler, J. (1997) *Cancer of the Nervous System*. Blackwell, Oxford, UK
3. Damek, D.M. and Hochberg, F.H. (1997) Clinical aspects of brain tumor. *Curr. Opin. Neurol.*, **10**, 452–458.
4. Berens, M.E. and Giese, A. (1999) '...those left behind.' Biology and oncology of invasive glioma cells. *Neoplasia*, **1**, 208–219.
5. Ng, H.-K. and Lam, P. (1998) The molecular genetics of CNS tumors. *Pathology*, **30**, 196–202.
6. von Deimling, A., Louis, D.N. and Wiestler, O.D. (1995) Molecular pathways in the formation of gliomas. *Glia*, **15**, 328–338.
7. Aghi, M., Hochberg, F. and Breakefield, X.O. (2000) Prodrug-activating enzymes in cancer gene therapy. *J. Gene Med.*, **2**, 148–164.
8. Licht, T., Goldenberg, S.K., Vieira, W.D., Gotesman, M.M. and Pastan, I. (2000) Drug selection of MDR1-transduced hematopoietic cells *ex vivo* increases transgene expression and chemoresistance in reconstituted bone marrow in mice. *Gene Ther.*, **7**, 348–358.
9. Hanna, N.N., Hallahan, D.E., Wayne, J.D. and Weischselbaum, R.R. (1996) Modification of the radiation response by the administration of exogenous genes. *Semin. Radiat. Oncol.*, **6**, 321–328.
10. Stackhouse, M.A. and Buchsbaum, D.J. (2000) Radiation to control gene expression. *Gene Ther.*, **7**, 1085–1086.
11. Wickman, T.J. (2000) Targeting adenovirus. *Gene Ther.*, **7**, 110–114.
12. Alemany, R., Gomex-Manzano, C., Balague, C., Yung, W.K., Curiel, D.T., Kyritsis, A.P. and Fueyo, J. (1999) Gene therapy for gliomas: molecular targets, adenoviral vectors and oncolytic adenoviruses. *Exp. Cell Res.*, **252**, 1–12.
13. Hampl, J., Brown, A., Rainov, N. and Breakefield, X.O. (2001) Methods for gene delivery to neural tissue. In China, H.R. and Moldin, S.O. (eds), *Methods in Neurogenetics.* CRC Press, Boca Raton, FL, in press.
14. Scharf, J.M., Boviatsis, E.J., Fleet, C., Breakefield, X.O. and Chiocca, E.A. (1994) Genetically modified rat 9L gliosarcoma cells facilitate detection of infiltrating tumor cells in a rat model of brain neoplasms. *Transgenics*, **1**, 219–224.
15. Dalrymple, S.J., Parkisi, J.E., Roche, P.C., Ziesmer, S.C., Scheithauer, B.W. and Kelly, P.J. (1994) Changes in proliferating cell nucleus antigen expression in glioblastoma multiforme cells along a stereotactic biopsy trajectory. *Neurosurgery*, **35**, 1036–1044.
16. Nitta, T., Hishii, M., Sato, K. and Okumura, K. (1994) Selective expression of interleukin-10 gene within glioblastoma multiforme. *Brain Res.*, **649**, 122–128.
17. Puri, R.K. (1999) Development of a recombinant interleukin-4-Pseudomonas exotoxin for therapy of glioblastoma. *Toxicol. Pathol.*, **27**, 53–57.
18. Laske, D.W., Ileroil, O., Akbasak, A., Youle, R.J. and Oldfield, E.H. (1994) Efficacy of direct intratumoral therapy with targeted protein toxins for solid human gliomas in nude mice. *J. Neurosurg.*, **80**, 520–526.
19. Recht, L., Torres, C.O., Smith, T.W., Raso, V. and Griffin, T.W. (1990) Transferrin receptor in normal and neoplastic brain tissue: implications for brain-tumor immunotherapy. *J. Neurosurg.*, **72**, 941–945.
20. Jefferies, W.A., Brandon, M.R., Hunt, S.V., Williams, A.F., Gatter, K.C. and Mason, D.Y. (1984) Transferrin receptor on endothelium of brain capillaries. *Nature*, **312**, 162–163.
21. Peng, K.W., Vile, R., Cosset, F.L., Russell, S. (1999) Selective transduction of protease-rich tumors by matrix-metalloproteinase-targeted retroviral vectors. *Gene Ther.*, 6, 1552–1557.
22. Dmitriev, I., Kashentseva, E., Rogers, B.E., Krasnykh, V. and Curiel, D.T. (2000) Ectodomain of coxsackievirus and adenovirus receptor genetically fused to epidermal growth factor mediates adenovirus targeting to epidermal growth factor receptor-positive cells. *J. Virol.*, **74**, 6875–6884.
23. Xia, H. anderson, B., Mao, Q. and Davidson, B.L. (2000) Recombinant human adenovirus: targeting to the human transferrin receptor improves gene transfer to brain microcapillary endothelium. *J. Virol.*, **74**, 11359–11366.
24. Yoshida, Y., Sadata, A., Zhang, W., Saito, K., Shinoura, N. and Hamada, H. (1998) Generation of fiber-mutant recombinant adenoviruses for gene therapy of malignant glioma. *Hum. Gene Ther.*, **9**, 2503–2515.
25. Staba, M.J., Wickman, T.J., Kovesdi, I. and Hallahan, D.E. (2000) Modifications of the fiber in adenovirus vectors increase tropism for malignant glioma models. *Cancer Gene Ther.*, **7**, 13–19.
26. Schwarze, S.R., Ho, A., Vocero-Akbani, A. and Dowdy, S.F. (1999) *In vivo* protein transduction: delivery of a biologically active protein into the mouse. *Science*, **285**, 1569–1572.
27. Brewis, N., Phelan, A., Webb, J., Drew, J., Elliott, G. and O'Hare, P. (2000) Evaluation of VP22 spread in tissue culture. *J. Virol.*, **74**, 1051–1056.
28. Dilber, M.S., Phelan, A., Aints, A., Mohamed, A.J., Elliott, G., Smith, C.I. and O'Hare, P. (1999) Intercellular delivery of thymidine kinase prodrug activating enzyme by the herpes simplex virus protein, VP22. *Gene Ther.*, **6**, 12–21.
29. Moolten, F.L. (1986) Tumor chemosensitivity conferred by inserted herpes thymidine kinase genes: paradigm for a prospective cancer control strategy. *Cancer Res.*, **46**, 5276–5281.
30. Ilsley, D.D., Lee, S.H., Miller, W.H. and Kuchta, R.D. (1995) Acyclic guanosine analogs inhibit DNA polymerases alpha, delta and epsilon with very different potencies and have unique mechanisms of action. *Biochemistry*, **34**, 2504–2510.
31. Moolten, F.L. and Wells, J.M. (1990) Curability of tumors bearing herpes thymidine kinase genes transferred by retrovirus vectors. *J. Natl Cancer Inst.*, **82**, 297–300.
32. Takamiya, Y., Short, M.P., Moolten, F.L., Fleet, C., Mineta, T., Breakefield, X.O. and Martuza, R.L. (1993) An experimental model of retrovirus gene therapy for malignant brain tumors. *J. Neurosurg.*, **79**, 104–110.
33. Culver, K.W., Ram, Z., Walbridge, S., Hirouki, K., Oldfield, E.H. and Blaese, R.M. (1992) *In vivo* gene transfer with retroviral producer cells for treatment of experimental brain tumors. *Science*, **256**, 1550–1552.
34. Freeman, S.M., Abboud, C., Whartenby, K., Packman, C., Koeplin, D., Moolten, F. and Abraham, G. (1993) The 'bystander effect': Tumor regression when a fraction of the tumor mass is genetically modified. *Cancer Res.*, **53**, 5274–5283.
35. Elshami, A.A., Saavedra, A., Zhang, H., Kucharczuk, J.C., Spray, D.C., Fishman, G.I., Amin, K.M., Kaiser, L.R. and Albelda, S.M. (1996) Gap junctions play a role in the 'bystander effect' of the herpes simplex virus thymidine kinase/ganciclovir system *in vitro*. *Gene Ther.*, **3**, 85–92.
36. Dilber, M.S., Abedi, M.R., Christensson, B., Bjorkstrand, B., Kidder, G.M., Naus, C.C., Gahrton, G. and Smith C.I. (1997) Gap junctions promote the bystander effect of herpes simplex virus thymidine kinase *in vivo*. *Cancer Res.*, **57**, 1523–1528.
37. Mullen, C.A., Kilstrup, M. and Blasé, R.M. (1992) Transfer of the bacterial gene for cytosine deaminase to mammalian cells confers lethal sensitivity to 5-fluorocytosine: a negative selection system. *Proc. Natl Acad. Sci. USA*, **89**, 33–37.
38. Clarke, L. and Waxman, D.J. (1989) Oxidative metabolism of cyclophosphamide: identification of the hepatic monooxygenase catalysts of drug activation. *Cancer Res.*, **49**, 2344–2350.
39. Wei, M.X., Tamiya, T., Chase, M., Boviatsis, E.J., Chang, T.K.H., Kowall, N.W., Hochberg, F.H., Waxman, D.J., Breakefield, X.O. and Chiocca, E.A. (1994) Experimental tumor therapy in mice using the cyclophosphamide-activating cytochrome P450 2B1 gene. *Hum. Gene Ther.*, **5**, 969–978.
40. Adachi, Y., Tamiya, T., Ichikawa, T., Terada, K., Ono, Y., Matsumoto, K., Furuta, T., Hamada, H. and Ohmoto, T. (2000) Experimental gene therapy for brain tumors using adenovirus-mediated transfer of cytosine deaminase gene and uracil phosphoribosyltransferase gene with 5-fluorocytosine. *Hum. Gene Ther.*, **11**, 77–89.
41. Aghi, M., Kramm, C.M., Chou, T.C., Breakefield, X.O. and Chiocca, E.A. (1998) Synergistic anticancer effects of ganciclovir/thymidine kinase and 5-fluorocytosine/cytosine deaminase gene therapies. *J. Natl Cancer Inst.*, **90**, 370–380.
42. Freytag, S.O., Rogulski, K.R., Paielli, D.L., Gilbert, J.D. and Kim, J.H. (1998) A novel three-pronged approach to kill cancer cells selectively: concomitant viral, double suicide gene and radiotherapy. *Hum. Gene Ther.*, **9**, 1323–1333.
43. Genka, S., Deutsch, J., Stahle, P.L., Shetty, U.H., John, V., Robinson, C., Rapoport, S.I. and Greig N.H. (1990) Brain and plasma pharmacokinetics and anticancer activities of cyclophosphamide and phosphoramide mustard in the rat. *Cancer Chemother. Pharmacol.*, **27**, 1–7.
44. Chen, L., Yu, L.J. and Waxman, D.J. (1997) Potentiation of cytochrome P450/cyclophosphamide-based cancer gene therapy by coexpression of the P450 reductase gene. *Cancer Res.*, **57**, 4830–4837.
45. Aghi, M., Chao Chou, T., Suling, K., Breakefield, X.O. and Chiocca, E.A. (1999) Multimodal cancer treatment mediated by a replicating oncolytic virus that delivers the oxazaphosphorine/rat cytochrome P450 2B1 and ganciclovir/herpes simplex virus thymidine kinase gene therapies. *Cancer Res.*, **59**, 3861–3865.

46. Yahanda, A.M., Bruner, J.M., Donehower, L.A. and Morrison, R.S. (1995) Astrocytes derived from p53-deficient mice provide a multistep in vitro model for development of malignant gliomas. *Mol. Cell. Biol.*, **15**, 4249–4259.
47. Albertoni, M., Daub, D.M., Arden, K.C., Viars, C.S., Powell, C. and Van Meir, E.G. (1998) Genetic instability leads to loss of both p53 alleles in a human glioblastoma. *Oncogene*, **16**, 321–326.
48. Rosenfeld, M.R., Meneses, P., Dalmau, J., Drobnjak, M., Cordon-Cardo, C. and Kaplitt, M.G. (1995) Gene transfer of wild-type p53 results in restoration of tumor-suppressor function in a medulloblastoma cell line. *Neurology*, **45**, 1533–1539.
49. Gomez-Manzano, C., Fueyo, J., Kyritsis, A.P., Steck, P.A., Roth, J.A., McDonnell, T.J., Steck, K.D., Levin, V.A. and Yung, W.K. (1996) Adenovirus-mediated transfer of the p53 gene produces rapid and generalized death of human glioma cells via apoptosis. *Cancer Res.*, **56**, 694–699.
50. Cirielli, C., Inyaku, K., Capogrossi, M.C., Yuan, X. and Williams, J.A. (1999) Adenovirus-mediated wild-type p53 expression induces apoptosis and suppresses tumorigenesis of experimental intracranial human malignant glioma. *J. Neurooncol.*, **43**, 99–108.
51. Broaddus, W.C., Liu, Y., Steele, L.L., Gillies, G.T., Lin, P.S., Loudon, W.G., Valerie, K., Schmidt-Ullrich, R.K. and Fillmore, H.L. (1999) Enhanced radiosensitivity of malignant glioma cells after adenoviral p53 transduction. *J. Neurosurg.*, **91**, 997–1004.
52. Biroccio, A., Bufalo, D.D., Ricca, A., D'Angelo, C., D'Orazi, G., Sacchi, A., Soddu, S. and Zupi, G. (1999) Increase of BCNU sensitivity by wt-p53 gene therapy in glioblastoma lines depends on the administration schedule. *Gene Ther.*, **6**, 1064–1072.
53. Trepel, M., Groscurth, P., Malipiero, U., Gulbins, E., Dichgans, J. and Weller, M. (1998) Chemosensitivity of human malignant glioma: modulation by p53 gene transfer. *J. Neurooncol.*, **39**, 19–32.
54. Shinoura, N., Saito, K., Yoshida, Y., Hashimoto, M., Asai, A., Kirino, T. and Hamada, H. (2000) Adenovirus-mediated transfer of bax with caspase-8 controlled by myelin basic protein promoter exerts an enhanced cytotoxic effect in gliomas. *Cancer Gene Ther.*, **7**, 739–748.
55. Shu, J., Lee, J.H., Harwalkar, J.A., Oh-Siskovic, S., Stacey, D.W. and Golubic, M. (1999) Adenovirus-mediated gene transfer of dominant negative Ha-Ras inhibits proliferation of primary meningioma cells. *Neurosurgery*, **44**, 579–587.
56. Zhang, Y.A., Nemunaitis, J. and Tong, A.W. (2000) Generation of a ribozyme-adenoviral vector against K-ras mutant human lung cancer cells. *Mol. Biotechnol.*, **15**, 39–49.
57. Jacobson, M.D., Brown, M., Bertin, J., Chiang, L., Horsburgh, B., Bakowska, J., Breakefield, X.O. and DiStefano, P. (2000) Herpes simplex virus amplications as tools to analyze pro-apoptotic gene function in primary neurons. *Soc. Neurosci. Abstract.*, **227**.17
58. Walczak, H., Miller, R.E., Ariail, K., Gliniak, B., Griffith, T.S., Kubin, M., Chin, W., Jones, J., Woodward, A., Le, T. *et al.* (1999) Tumoricidal activity of tumor necrosis factor-related apoptosis-inducing ligand *in vivo*. *Nature Med.*, **5**, 157–163.
59. Niranjan, A., Moriuchi, S., Lunsford, L.D., Kondziolka, D., Flickinger, J.C., Fellows, W., Rajendiran, S., Tamura, M., Cohen, J.B. and Glorioso, J.C. (2000) Effective treatment of experimental glioblastoma by HSV vector-mediated TNF alpha and HSV-tk gene transfer in combination with radiosurgery and ganciclovir administration. *Mol. Ther.*, **2**, 114–120.
60. Parney, I.F., Farr-Jones, M.A., Chang, L.J. and Petruk, K.C. (2000) Human glioma immunobiology *in vitro*: implications for immunogene therapy. *Neurosurgery*, **46**, 1169–1177.
61. Nagata, S. (2000) Steering anti-cancer drugs away from the TRAIL. *Nat. Med.*, **6**, 502–503.
62. Feldman, A.L. and Libutti, S.K. (2000) Progress in antiangiogenic gene therapy of cancer. *Cancer*, **89**, 1181–1194.
63. Ikeda, E., Achen, M.G., Breier, G. and Risau, W. (1995) Hypoxia-induced transcriptional activation and increased mRNA stability of vascular endothelial growth factor in C6 glioma cells. *J. Biol. Chem.*, **270**, 19761–19766.
64. Oehring, R.D., Miletic, M., Valter, M.M., Pietsch, T., Neumann, J., Fimmers, R. and Schlegel, U. (1999) Vascular endothelial growth factor (VEGF) in astrocytic gliomas – a prognostic factor? *J. Neurooncol.*, **45**, 117–125.
65. Milauer, B., Shawyer, L.K., Plate, K.H., Risau, W., Ullrich, A. (1994) Glioblastoma growth inhibited *in vivo* by a dominant-negative Flk-1 mutant. *Nature*, **367**, 576–578.
66. Machein, M.R., Risau, W. and Plate, K.H. (1999) Antiangiogenic gene therapy in a rat glioma model using a dominant-negative vascular endothelial growth factor receptor 2. *Hum. Gene Ther.*, **10**, 1117–1128.
67. Saleh, M., Stacker, S.A. and Wilks, A.F. (1996) Inhibition of growth of C6 glioma cells i*n vivo* by expression of antisense vascular endothelial growth factor sequence. *Cancer Res.*, **56**, 393–401.
68. Sasaki, M., Wizigmann-Voos, S., Risau, W. and Plate, K.H. (1999) Retrovirus producer cells encoding antisense VEGF prolong survival of rats with intracranial GS9L gliomas. *Int. J. Dev. Neurosci.*, **17**, 579–591.
69. Maisonpierre, P.C., Suri, C., Jones, P.F., Bartunkova, S., Wiegand, S.J., Radziejewski, C., Compton, D., McClain, J., Aldrich, T.H., Papadopoulos, N. *et al.* (1997) Angiopoietin-2, a natural antagonist for Tie2 that disrupts *in vivo* angiogenesis. *Science*, **277**, 55–60.
70. Cho, J.Y., Xing, S., Liu, X., Buckwalter, T.L., Hwa, L., Sferra, T.J., Chiu, I.M. and Jhiang, S.M. (2000) Expression and activity of human Na+/I- symporter in human glioma cells by adenovirus-mediated gene delivery. *Gene Ther.*, **7**, 740–749.
71. Boland, A., Ricard, M., Opolon, P., Bidart, J.M., Yeh, P., Filetti, S., Schlumberger, M. and Perricaudet, M. (2000) Adenovirus-mediated transfer of the thyroid sodium/iodide symporter gene into tumors for targeted radiotherapy. *Cancer Res.*, **60**, 3483–3492.
72. Diaz, R.M., Bateman, A., Emiliusen, L., Fielding, A., Trono, D., Russell, S.J. and Vile, R.G. (2000) A lentiviral vector expression a fusogenic glycoprotein for cancer gene therapy. *Gene Ther.*, **7**, 1656–1663.
73. Kim, J.Y., Sutton, M.E., Lu, D.J., Cho, T.A., Goumnerova, L.C., Goritchenko, L., Kaufman, J.R., Lam, K.K., Billet, A.L., Tarbell, N.J. *et al.* (1999) Activation of neurotrophin-3 receptor TrkC induces apoptosis in medulloblastomas. *Cancer Res.*, **59**, 711–719.
74. Kondo, S., Kondo, Y., Li, G., Silverman, R.H. and Cowell, J.K. (1998) Targeted therapy of human malignant glioma in a mouse model by 2–5A antisense directed against telomerase RNA. *Oncogene*, **16**, 3323–3330.
75. Marconi, P., Tamura, M., Moriuchi, S., Krisky, D.M., Niranjan, A., Goins, W.F., Cohen, J.B. and Glorioso, J.C. (2000) Connexin 43-enhanced suicide gene therapy using herpes viral vectors. *Mol. Ther.*, **1**, 71–81.
76. Hoshi, M., Harada, A., Kawase, T., Uyemura, K. and Yazaki, T. (2000) Antitumoral effects of defective herpes simplex virus-mediated transfer of tissue inhibitor of metalloproteinases-2 gene in malignant glioma U87 in vitro: consequences for anti-cancer gene therapy. *Cancer Gene Ther.*, **7**, 799–805.
77. Paulus, W., Baur, I., Beutler, A.S. and Reeves, S.A. (1996) Diffuse brain invasion of glioma cells requires beta 1 integrins. *Lab. Invest.* **75**, 819–826.
78. Weston, B.W., Hiller, K.M., Mayben, J.P., Manousos, G.A., Bendt, K.M., Liu, R. and Cusack, J.C.J. (1999) Expression of human alpha(1, 3)fucosyltransferase antisense sequences inhibits selectin-mediated adhesion and liver metastasis of colon carcinoma cells. *Cancer Res.*, **59**, 2127–2135.
79. Miller, D.W. (1999) Immunobiology of the blood-brain barrier. *J. Neurovirol.*, **5.**, 570–578.
80. Weller, M. and Fontana, A. (1995) The failure of current immunotherapy for malignant glioma. Tumor-derived TGF-beta, T-c apoptosis and the immune privilege of the brain. *Brain Res. Rev.*, **21**, 128–151.
81. Saas, P., Walker, P.R., Hahne, M., Quiquerez, A.L., Schnuriger, V., Perrin, G., French, L., Van Meir, E.G., de Tribolet, N., Tschopp, J. *et al.* (1997) Fas ligand expression by astrocytoma *in vivo*: maintaining immune privilege in the brain? *J. Clin. Invest.*, **99**, 1173–1178.
82. Campa, M.J., Kuan, C.T., O'Connor-McCourt, M.D., Bigner, D.D. and Patz, E.F.J. (2000) Design of a novel small peptide targeted against a tumor-specific receptor. *Biochem. Biophys. Res. Commun.*, **275**, 631–636.
83. Debinski, W. and Gibo, D.M. (2000) Molecular expression analysis of restrictive receptor for interleukin 13, a brain tumor-associated cancer/testis antigen. *Mol. Med.*, **6**, 440–449.
84. Kuppner, M.C., Hamou, M.F., Sawamura, Y., Bodmer, S. and de Tribolet, N. (1989) Inhibition of lymphocyte function by glioblastoma-derived transforming growth factor beta 2. *J. Neurosurg.*, **71**, 211–217.
85. Hitchcock, E.R. and Morris, C.S. (1988) Mononuclear cell infiltration in central portions of human astrocytomas. *J. Neurosurg.*, **68**, 432–437.
86. Herrlinger, U., Jacobs, A., Quinones, A., Woiciechowsky, C., Sena-Esteves, M., Rainov, N.G., Fraefel, C. and Breakefield, X.O. (2000) Helper virus-free Herpes simplex virus type 1 amplicon vectors for granulocyte-macrophage colony-stimulating factor-enhanced vaccination therapy for experimental glioma. *Hum. Gene Ther.*, **11**, 1429–1438.
87. Glick, R.P., Lichtor, T., de Zoeten, E., Deshmukh, P. and Cohen, E.P. (1999) Prolongation of survival of mice with glioma treated with semi-allogeneic fibroblasts secreting interleukin-2. *Neurosurgery*, **45**, 867–874.
88. DiMeco, R., Rhines, L.D., Hanes, J., Tyler, B.M., Brat, D., Torchiana, E., Guarnieri, M., Colombo, M.P., Pardoll, D.M., Finocchiaro, G. *et al.*

(2000) Paracrine delivery of IL-12 against intracranial 9L gliosarcoma in rats. *J. Neurosurg.*, **92**, 419–427.
89. Sampson, J.H., Ashley, D.M., Archer, G.E., Fuchs, H.E., Dranoff, G., Hale, L.P. and Bigner, D.D. (1997) Characterization of a spontaneous murine astrocytomas and abrogation of its tumorigenicity by cytokine secretion. *Neurosurgery*, **41**, 1365–1372.
90. Sampath, P., Hanes, J., DiMeco, F., Tyler, B.M., Brat, D., Pardoll, D.M. and Brem, H. (1999) Paracrine immunotherapy with interleukin-2 and local chemotherapy is synergistic in the treatment of experimental brain tumors. *Cancer Res.*, **59**, 2107–2114.
91. Parker, J.N., Gillespie, G.Y., Love, C.E., Randall, S., Whitley, R.J. and Markert, J.M. (2000) From the cover: engineered herpes simplex virus expression IL-12 in the treatment of experimental murine brain tumors. *Proc. Natl Acad. Sci. USA*, **97**, 2208–2213.
92. Costantini, L.C., Bakowska, J.C., Breakefield, X.O. and Isacson, O. (2000) Gene therapy in the CNS. *Gene Ther.*, **7**, 93–109.
93. Cristiano, R.J., Xu, B., Nguyen, D., Schumacher, G., Kataoka, M., Spitz, F.R. and Roth, J.A. (1998) Viral and nonviral gene delivery vectors for cancer gene therapy. *Cancer Detect. Prev.*, **22**, 445–454.
94. Saeki, Y., Matsumoto, N., Nakano, Y., Mori, M., Awai, K. and Kaneda, Y. (1997) Development and characterization of cationic liposomes conjugated with HSJ (Sendai virus): reciprocal effect of cationic lipid for *in vitro* and *in vivo* gene transfer. *Hum. Gene Ther.*, **8**, 2133–2141.
95. Shi, N. and Pardridge, W.M. (2000) Noninvasive gene targeting to the brain. *Proc. Natl Acad. Sci. USA*, **97**, 7567–7572.
96. Aronsohn, A.I. and Hughes, J.A. (1998) Nuclear localization signal peptides enhance cationic liposome-mediated gene therapy. *J. Drug Target.*, **5**, 163–169.
97. Kaneda, Y., Saeki, Y. and Morishita, R. (1999) Gene therapy using HVJ-liposomes: the best of both worlds? *Mol. Med. Today*, **5**, 298–303.
98. Pilaro, A.M. and Serabian, M.A. (1999) Preclinical development strategies for novel gene therapeutic products. *Toxicol. Pathol.*, **27**, 4–7.
99. Lam, P. and Breakefield, X.O. (2000) Hybrid vector designs to control the delivery, fate and expression of transgenes. *J. Gene Med.*, **2**, 395–408.
100. Kay, M.A., Glorioso, J.C. and Naldini, L. (2001) Viral vectors for gene therapy: the art of turning infectious agents into vehicles of therapeutics. *Nat. Med.*, **7**, 33–127.
101. Verma, I.M. and Somia, N. (1997) Gene therapy – promises, problems and prospects. *Nature*, **389**, 239–242.
102. Gupta, N. (2000) Current status of viral gene therapy for brain tumors. *Expert. Opin. Invest. Drugs*, **9**, 713–726.
103. Trask, T.W., Trask, R.P., Akuilar-Cordova, E., Shine, H.D., Wyde, P.R., Goodman, J.C., Hamilton, W.J., Rojas-Martinez, A., Chen, S.H., Woo, S.L. *et al.* (2000) Phase I study of adenoviral delivery of the HSV-tk gene and ganciclovir administration in patients with current malignant brain tumors. *Mol. Ther.*, **1**, 195–203.
104. Toda, M., Rabkin, S.D., Kojima, H. and Martuza, R.L. (1999) Herpes simplex virus as an *in situ* cancer vaccine for the induction of specific anti-tumor immunity. *Hum. Gene Ther.*, **10**, 385–393.
105. Martuza, R.L., Malick, A., Markert, J.M., Ruffner, K.L. and Coen, D.M. (1991) Experimental therapy of human glioma by means of a genetically engineered virus mutant. *Science*, **252**, 854–856.
106. Bischoff, J.R., Kim, D.H., Williams, A., Heise, C., Horn, S., Muna, M., Ng, L., Nye, J.A., Sampson-Johannes, A., Fattaey, A. *et al.* (1996) An adenovirus mutant that replicates selectively in p53-deficient human tumor cells. *Science*, **274**, 373–376.
107. Coffey, M.C., Strong, J.E., Forsyth, P.A. and Lee, P.W. (1998) Retrovirus therapy of tumors with activated Ras pathway. *Science*, **282**, 1332–1334.
108. Heise, C., Sampson-Johannes, A., Williams, A., McCormick, F., Von Hoff, D.D. and Kirn, D.H. (1997) ONYX-015, an E1B gene-attenuated adenovirus, causes tumor-specific cytolysis and antitumoral efficacy that can be augmented by standard chemotherapeutic agents. *Nat. Med.*, **3**, 639–645.
109. Kurihara, H., Zama, A., Tamura, M., Takeda, J., Sasaki, T. and Takeuchi, T. (2000) Glioma/glioblastoma-specific adenoviral gene expression using the nestin gene regulator. *Gene Ther.*, **7**, 686–693.
110. Dewey, R.A., Morrissey, C.M., Cowsill, C.M., Stone, D., Bolognani, F., Dodd, N.J.F., Southgate, T.D., Klatzmann, D., Lassmann, H., Castro, M.G. *et al.* (1999) Chronic brain inflammation and persistent HSV1-TK expression in survivors of syngeneic glioma treated by adenovirus-mediated gene therapy: implications for clinical trials. *Nat. Med.*, **5**, 1256–1263.
111. Stohlman, S.A. and Hinton, D.R. (2001) Viral induced demyelination. *Brain Pathol.*, **11**, 92–106.
112. Albert, M.L., Darnell, J.C., Bender, A., Francisco, L.M., Bhardwaj, N. and Darnell, R.B. (1998) Tumor-specific killer cells in paraneoplastic cerebellar degeneration. *Nat. Med.*, **4**, 1321–1324.
113. Huang, Q., Vonsattel, J-P., Schaffer, P.A., Martuza, R.L., Breakefield, X.O. and DiFiglia, M. (1992) Introduction of a foreign gene (*lacZ* of *E. coli*) into rat neostriatal neurons using herpes simplex virus mutants: a light and electron microscopic study. *Exp. Neurol.*, **115**, 303–316.
114. Short, M.P., Choi, B., Lee, J., Malick, A., Breakefield, X.O. and Martuza, R.L. (1990) Gene delivery to glioma cells in rat brain by grafting of a retrovirus packaging cell line. *J. Neurosci. Res.*, **27**, 427–439.
115. Abe, A., Chen, S.T., Miyanohara, A., Friedmann, T. (1998) *In vitro* cell-free conversion of noninfectious Moloney retrovirus particles to an infectious form by the addition of the vesicular stomatitis virus surrogate envelope G protein. *J. Virol.*, **72**, 6356–6361.
116. Takeuchi, Y., Porter, C.D., Strahan, K.M., Preece, A.F., Gustafsson, K., Cosset, F.L., Weiss, R.A. and Collins, M.K. (1996) Sensitization of cells and retroviruses to human serum by (alpha 1–3) galactosyltransferase. *Nature*, **379**, 85–88.
117. Ramsey, W.J., Caplen, N.J., Li, Q., Higginbotham, J.N., Shah, M. and Blaese, R.M. (1998) Adenovirus vectors as transcomplementing templates for the production of replication defective retroviral vectors. *Biochem. Biophys. Res. Commun.*, **246**, 912–919.
118. Sena-Esteves, M., Saeki, Y., Camp, S., Chiocca, E.A. and Breakefield, X.O. (1999) Single-step conversion of cells to retrovirus vector producers with herpes simplex virus-Epstein Barr virus hybrid amplicons. *J. Virol.*, **73**, 10426–10439.
119. Vanin, E.F., Kaloss, M., Broscius, C. and Nienhuis, A.W. (1994) Characterization of replication-competent retroviruses from nonhuman primates with virus-induced T-cell lymphomas and observations regarding the mechanism of oncogenesis. *J. Virol.*, **68**, 4241–4250.
120. Rainov, N.G. (2000) 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.*, **11**, 2389–2401.
121. Latchman, D.S. (1999) Herpes virus vectors for gene therapy in the nervous system. *Biochem. Soc. Trans*, **27**, 847–851.
122. Glorioso, J.C., Bender, M.A., Goins, W.F., Fink, D.J. and DeLuca, N. (1995) Herpes simplex virus as a gene-delivery vector for the central nervous system. *Viral Vectors*. Academic Press, New York, NY, pp 1–23.
123. Howard, M.K., Coffin, R.S., Maclean, A.R., Brown, S.M., Bailey, D. Anderson, P.N., Burnstock , G. and Latchman, D.S. (1997) Gene delivery to rat enteric neurons using herpes simplex virus-based vectors. *J. Mol. Neurosci.*, **9**, 65–74.
124. Millhouse, S. and Wigdahl, B. (2000) Molecular circuitry regulating herpes simplex virus type 1 latency in neurons. *J. Neurovirol.*, **6**, 6–24.
125. Sanders, V.J., Felisan, S., Waddell, A. and Tourtellotte, W.W. (1996) Detection of herpesviridae in postmortem multiple sclerosis brain tissue and controls by polymerase chain reaction. *J. Neurovirol.*, **2**, 249–258.
126. Lellouch-Tubiana, A., Fohlen, M., Robain, O. and Rozenberg, F. (2000) Immunocytochemical characterization of long-term persistent immune activation in human brain after herpes simplex encephalitis. *Neuropathol. Appl. Neurobiol.*, **26**, 285–294.
127. Brown, S.M., MacLean, A.R., Aitken, J.D. and Harland, J. (1994) ICP34.5 influences herpes simplex virus type 1 maturation and egress from infected cells *in vitro*. *J. Gen. Virol.*, **75**, 3679–3686.
128. Kucharczuk, J.C., Randazzo, B., Chang, M.Y., Amin, K.M., Elshami, A.A., Sterman, D.H., Rizk, N.P., Molnar-Kimber, K.L., Brown, S.M., MacLean, A.R. *et al.* (1997) Use of a 'replication-restricted' herpes virus to treat experimental human malignant mesothelioma. *Cancer Res.*, **57**, 466–471.
129. Tan, S-L. and Katze, M.G. (2000) HSV.com: Maneuvering the internetworks of viral neuropathogenesis and evasion of the host defense. *Proc. Natl Acad. Sci. USA*, **97**, 5684–5686.
130. Rampling, R., Cruickshank, G., Papanastassiou, V., Nicoll, J., Hadley, D., Brennan, D., Petty, R., MacLean, A., Harland, J., McKie, E. *et al.* (2000) Toxicity evaluation of replication-competent herpes simplex virus (ICP 34.5 null mutant 1716) in patients with recurrent malignant glioma. *Gene Ther.*, **7**, 859–866.
131. Lasner, T.M., Tal-Singer, R., Kesari, S., Lee, V.M., Trojanowski, J.Q. and Fraser, N.W. (1998) Toxicity and neuronal infection of a HSV-1 ICP34.5 mutant in nude mice. *J. Neurovirol.*, **4**, 100–105.
132. McKie, E.A., MacLean, A.R., Lewis, A.D., Cruickshank, G., Rampling, R., Barnett, S.C., Kennedy, P.G. and Brown, S.M. (1996) Selective in vitro replication of herpes simplex virus type 1 (HSV-1) ICP34.5 null

mutants in primary human CNS tumors – evaluation of a potentially effective clinical therapy. *Br. J. Cancer*, **74**, 745–752.
133. Kramm, C.M., Rainov, N.G., Sena-Esteves, M., Chase, M., Pechan, P.A., Chiocca, E.A. and Breakefield, X.O. (1996) Herpes vector-mediated delivery of thymidine kinase gene to disseminated CNS tumors. *Hum. Gene Ther.*, **7**, 291–300.
134. Mineta, T., Rabkin, S.D., Yazaki, T., Hunter, W.D. and Martuza, R.L. (1995) Attenuated multi-mutated herpes simplex virus-1 for the treatment of malignant gliomas. *Nature Med.*, **1**, 938–943.
135. Kramm, C.M., Chase, M., Herrlinger, U., Jacobs, A., Pechan, P., Rainov, N.G., Sena-Esteves, M., Aghi, M., Barnett, F.H., Chiocca, E.A. and Breakefield, X.O. (1997) Therapeutic efficiency and safety of a second-generation replication-conditional HSV1 vector for brain tumor gene therapy. *Hum. Gene Ther.*, **8**, 2057–2068.
136. Markert, J.M., Medlock, M.D., Rabkin, S.D., Gillespie, G.Y., Todo, T., Hunter, W.D., Plamer, C.A., Feigenbaum, F., Tornatore, C., Tufaro, F. *et al.* (2000) Conditionally replicating herpes simplex virus mutant, G207 for the treatment of malignant glioma: results of a phase I trial. *Gene Ther.*, **7**, 867–874.
137. Benihoud, K., Yeh, P. and Perricaudet, M. (1999) Adenovirus vectors for gene delivery. *Curr. Opin. Biotechnol.*, **10**, 440–447.
138. Kaplan, J.M., Armentano, D., Scaria, A., Woodworth, L.A., Panninton, S.E., Wadsworth, S.C., Smith, A.E. and Gregory, R.J. (1999) Novel role for E4 region genes in protection of adenovirus vectors from lysis by cytotoxic T lymphocytes. *J. Virol.*, **73**, 4489–4492.
139. Boulanger, P.A. and Blair, G.E. (1991) Expression and interactions of human adenovirus oncoproteins. *Biochem. J.*, **15**, 281–299.
140. Doronin, K., Toth, K., Kuppuswamy, M., Ward, P., Tollefson, A.E. and Wold, W.S. (2000) Tumor-specific, replication-competent adenovirus vectors overexpression the adenovirus death protein. *J. Virol.*, **74**, 6147–6155.
141. Driesse, M.J., Esandi, M.C., Kros, J.M., Avezaat, C.J.J., Vecht, C.J., Zurcher, C., van der Velde, I., Valerio, D., Bout, A., Sillevis Smitt, P.A.E. (2000) Intra-CSF administered recombinant adenovirus causes an immune response-mediated toxicity. *Gene Ther.*, **7**, 1401–1409.
142. Bankiewicz, K.S., Eberling, J.L., Kohutnicka, M., Jagust, W., Pivirotto, P., Bringas, J., Cunningham, J., Budinger, T.F. and Harvey-White, J. (2000) Convection-enhanced delivery of AAV vector in parkinsonian monkeys; *in vivo* detection of gene expression and restoration of dopaminergic function using pro-drug approach. *Exp. Neurol.*, **164**, 2–14.
143. Davidson, B.L., Hilfinger, J.M. and Beer, S.J. (1997) Extended release of adenovirus from polymer microspheres: potential use in gene therapy for brain tumors. *Adv. Drug Deliv. Rev.*, **27**, 59–66.
144. Kuriyama, N., Kuriyama, H., Julin, C.M., Lamborn, K. and Israel, M.A. (2000) Pretreatment with protease is a useful experimental strategy for enhancing adenovirus-mediated cancer gene therapy. *Hum. Gene Ther.*, **11**, 2219–2230.
145. Boviatsis, E.J., Scharf, J.M., Chase, M., Harrington, K., Kowall, N.W., Breakefield, X.O. and Chiocca, E.A. (1994) Antitumor activity and reporter gene transfer into rat brain tumors inoculated with herpes simplex virus vectors defective in thymidine kinase or ribonucleotide reductase. *Gene Ther.*, **1**, 323–331.
146. Ram, Z., Culver, K.W., Oshiro, E.M., Viola, J.J., DeVroom, H.L., Otto, E., Long, Z., Chiang, Y., McCarrity, G.J., Muul, L.M. *et al.* (1997) Therapy of malignant brain tumors by intratumoral implantation of retroviral vector-producing cells. *Nat. Med.*, **3**, 1354–1361.
147. Tamura, M., Ikenaka, K., Tamura, K., Yoshimatsu, T., Miyao, Y., Kishima, H., Mabuchi, E. and Shimizu, K. (1998) Transduction of glioma cells using a high-titer retroviral vector system and their subsequent migration in brain tumors. *Gene Ther.*, **5**, 1698–1704.
148. Lal, B., Indurti, R.R., Couraud, P.O., Goldstein, G.W. and Laterra, J. (1994) Endothelial cell implantation and survival within experimental gliomas. *Proc. Natl Acad. Sci. USA*, **91**, 9695–9699.
149. Aboody, K.S., Brown, A., Rainov, N.G., Bower, K.A., Liu, S., Yang, W., Small, J.E., Herrlinger, U., Ourednik, V., Black, P.M., Breakefield, X.O., Snyder, E.Y. (2000) Neural stem cells display extensive tropism for pathology in adult brain: evidence from intracranial gliomas. *Proc. Natl Acad. Sci. USA*, **97**, 12846–12851.
150. Herrlinger, U., Woiciechowski, C., Sena-Esteves, M., Aboody, K.S., Jacobs, A.H., Rainov, N.G., Snyder, E.Y. and Breakefield, X.O. (2000b) Neural precursor cells for delivery of replication-conditional HSV-1 vectors to intracerebral gliomas. *Mol. Ther.*, **1**, 347–357.
151. Noble, M. (2000) Can neural stem cells be used to track down and destroy migratory brain tumor cells while also providing a means of repairing tumor-associated damage? *Proc. Natl Acad. Sci. USA*, **97**, 12393–12395.
152. Gage, F.H. (2000) Mammalian neural stem cells. *Science*, **287**, 1433–1438.
153. Panchision, D., Hazel, T. and McKay, R. (1998) Plasticity and stem cells in the vertebrate nervous system. *Curr. Opin. Cell Biol.*, **10**, 727–733.
154. Benedetti, S., Pirola, B., Pollo, B., Magrassi, L., Bruzzone, M.G., Rigamonti, D., Galli, R., Selleri, S., Di Meco, F., De Fraja, C. *et al.* (2000) Gene therapy of experimental brain tumors using neural progenitor cells. *Nat. Med.*, **6**, 447–450.
155. Beutler, A.S., Banck, M.S., Wedekind, D. and Hedrich, H.J. (1999) Tumor gene therapy made easy: allogeneic major histocompatibility complex in the C6 rat glioma model. *Hum. Gene Ther.*, **10**, 95–101.
156. Oshiro, E.M., Viola, J.J., Oldfield, E.H., Walbridge, S., Bacher, J., Frank, J.A., Blaese, R.M. and Ram, Z. (1995) Toxicity studies and distribution dynamics of retroviral vectors following intrathecal administration of retroviral vector-produced cells. *Cancer Gene Ther.*, **2**, 87–95.
157. Carmeliet, P. and Jain, R.K. (2000) Angiogenesis in cancer and other diseases. *Nature*, **407**, 249–257.
158. Chauvet, A.E., Kesava, P.P., Goh, C.S. and Badie, B. (1998) Selective intra-arterial gene delivery into a canine meningioma. *J. Neurosurg.*, **88**, 870–873.
159. Nilaver, G., Muldoon, U., Kroll, R.A., Pagel, M.A., Breakefield, X.O., Davidson, B.L. and Neuwelt, E.A. (1995) Delivery of herpes virus and adenovirus to nude rat intracerebral tumors following osmotic blood-brain barrier disruption. *Proc. Natl Acad. Sci. USA*, **92**, 9829–9833.
160. Rainov, N.G., Zimmer, C., Chase, M., Kramm, C., Chiocca, E.A., Weissleder, R. and Breakefield, X.O. (1995) Selective uptake of viral and monocrystalline particles delivered intra-arterially to experimental brain neoplasms. *Hum. Gene Ther.*, **6**, 1543–1552.
161. Kroes, R.A., Jastrow, A., McLone, M.G., Yamamoto, H., Colley, P., Kersey, D.S., Yong, V.W., Mkrdichian, E., Cerulo, L., Leestma, J. *et al.* (2000) The identification of novel therapeutic targets for the treatment of malignant brain tumors. *Cancer Lett.*, **156**, 191–198.
162. Caskey, L.S., Fuller, G.N., Bruner, J.M., Yung, W.K., Sawaya, R.E., Holland, E.C. and Zhang, W. (2000) Toward a molecular classification of the gliomas: histopathology, molecular genetics and expression profiling. *Histol. Histopathol.*, **15**, 971–981.
163. Bauman, G.S., Ino, Y., Ueki, K., Zlatescu, M.C., Fisher, B.J., Macdonald, D.R., Stitt, L., Louis, D.N. and Cairncross, J.G. (2000) Allelic loss of chromosome 1p and radiotherapy plus chemotherapy in patients with oligodendrogliomas. *Int. J. Radiat. Oncol. Biol. Phys.*, **48**, 825–830.
164. Grotzer, M.A., Eggert, A., Zuzak, T.J., Janss, A.J., Marwaha, S., Wiewrodt, B.R., Ikegaki, N., Brodeur, G.M. and Phillips, P.C. (2000) Resistance to TRAIL-induced apoptosis in primitive neuroectodermal brain tumor cells correlates with a loss of caspase-8 expression. *Oncogene*, **19**, 4604–4610.