Oncolytic adenoviral therapy for glioblastoma multiforme

ADAM M. SONABEND, M.D., ILYA V. ULASOV, PH.D., YU HAN, B.A.,
AND MACIEJ S. LESNIAK, M.D.
Division of Neurosurgery, The University of Chicago, Illinois

✓ Adenoviruses historically have been one of the main vectors used in human gene therapy. To date, the majority of brain tumor trials of these vectors have used replication-defective viruses. The relative lack of success obtained with replication-defective vectors has prompted a search for new and improved therapies. In this context, oncolytic (conditionally replicative) adenoviruses, which selectively bind and replicate only in tumor cells, have gained increasing importance. These adenoviruses, once they are rendered conditionally replicative by transductional and transcriptional modifications, offer significant promise for patients with malignant glioma. In this review, the authors discuss the genetic approaches to adenoviral modification and their applications in the field of neurooncology.

KEY WORDS • brain neoplasm • glioma • gene therapy • adenovirus • conditionally replicative adenovirus

MALIGNANT brain tumors remain refractory to current medical and/or surgical therapy. The characteristic resistance to treatment shown by high-grade gliomas resides in their biological behavior and their location within the CNS. As is true with most cancers, gliomas are subject to constant genotypic and phenotypic alterations that can generate treatment-resistant clones. These cell populations are selected once a therapy is administered. In addition, high-grade gliomas cannot be effectively targeted by systemic chemotherapy because the blood-brain barrier limits the penetration of most agents to brain tumors. With respect to surgical treatment, the complete resection of high-grade gliomas remains a virtually impossible task because the nature of these tumors is to infiltrate diffusely within surrounding brain parenchyma.

To increase the length of survival time of patients with malignant brain tumors, novel therapeutic alternatives are currently being explored. Some of these experimental treatment strategies are based on advances in immunotherapy, stem cell therapy, local chemotherapy, and radiotherapy.

In addition, gene therapy is becoming a promising alternative. In essence, gene therapy consists of the delivery of a gene of interest to tumor cell populations to control and, when possible, kill the growing tumor. Viruses are prominent vehicles for gene therapy, and some adenoviral vectors exhibit oncolytic properties. To this end, a variety of viral vectors have been developed, with oncolytic viruses emerging as an innovative therapeutic tool for these tumors.

To be effective, a virus used for oncolytic therapy must have several features. The desired properties of these vectors include selectivity for the tumor target, minimal brain and systemic toxicities, and the capacity to penetrate and diffuse throughout the brain to reach all neoplastic foci residing beyond the resection border of the tumor. In addition, the viral vector needs to remain active despite evoking an immune response. The goal of developing an ideal vehicle for treatment of malignant brain tumors remains to be achieved. A wide variety of viral vectors have been developed and tested in the setting of gene therapy for malignant gliomas. These are based on different kinds of viruses, such as herpes simplex virus, retrovirus, measles virus, reovirus, and adenovirus. Some have shown promising results when tested in animal models of intracranial gliomas, but to date, clinical trials performed in humans have not shown a significant increase in survival. A more detailed description involving these vectors is beyond the scope of this review; we present here only the advances in the field of adenoviral vectors because these viruses offer promising alternatives to help achieve the goal of developing an effective oncolytic therapy for gliomas.

Biology of the Adenovirus

Adenoviruses are well suited for therapeutic gene delivery tasks. In general, they can be easily manipulated in vitro, have the ability to grow to high titers, and are capable of carrying significant transgenes of interest. An adenoviral particle is composed of an icosahedral capsid surrounding an inner nucleoprotein core. The capsid facets consist of hexon proteins, and the vertices consist of a penton base that joins the fiber protein. An adenoviral genome is presented by a double-stranded DNA molecule approximately 36 kb long and includes different regions that overlap.
An adenoviral replication cycle consists of a series of sequential and critical steps that result in the formation of new viral particles. A critical step in this process is the binding of the virus to the cell via CAR, which is expressed on the host cell membrane. After this initial binding event, the adenoviral penton base recognizes αv integrins and triggers viral internalization through clathrin-dependent endosomes. Once in the endosomes, adenoviral particles are able to escape to the cytoplasm, where they are transported toward the nuclear membrane, and free viral DNA is allowed entry to the nucleus.

Following viral DNA entry to the nucleus, genomic transcriptional regions composed of early (E1A), immediate early (E1B, E2, E3, and E4), intermediate (IX and IVA2), and late genes are expressed in an orderly fashion. These genes are confined to different transcription units that run in a bidirectional manner. Viral mRNA is transported to the cytoplasm, enzymes are synthesized, DNA is replicated, and finally, structural proteins assemble capsids containing nucleoproteins. New viral particles are then released from the host cell.

**Oncolytic Vectors**

In general, viral vectors for cancer therapy can be divided into those that are replication-defective and replication-competent, based on their reproductive capacities. Although both types of vectors can target genes into neoplastic cells, only the latter show oncolytic potential. Conditionally replicative adenovirus is a naturally selected or genetically engineered adenovirus that preferentially replicates in and kills tumor cells. Ideally, this virus is unable to replicate in normal cells.

Initially, adenoviral vectors for cancer gene therapy were manipulated to render them replication-defective. This characteristic was desired to avoid promiscuous transgene expression in healthy tissue. However, such vectors were incapable of expressing transgenes into enough cells to achieve a significant clinical improvement. Disillusionment came when the vectors were tested during in vivo and human studies. For example, a Phase I clinical trial of p53 gene therapy was performed using a replication-defective adenovirus vector with wild-type p53 (Ad-p53, INGN 201) against malignant brain tumors. The vector was injected intratumorally through a catheter that was implanted using stereotactic guidance. Specimens from treated tumors were obtained and analyzed afterward. In all patients, exogenous p53 protein was detected within the nuclei of astrocytic tumor cells, and transgene expression induced apoptosis of targeted cells. However, with the use of this replication-defective vector, transgene expression was limited to within 5 mm of the injection site.

The genomic deletions of replication-defective viruses result in replication impairments that vary depending on the kind of cells these viruses infect. This principle was illustrated with E1A-deleted (H5dl 312) or E1A plus E1B-deleted (H5dl 434) adenoviruses. These vectors are able to replicate, albeit with delayed kinetics compared with wild-type adenoviruses, in cell cultures from normal brain and low-grade tumors such as meningioma and astrocytoma Grade I or II. Nevertheless, these vectors are unable to replicate or do so poorly and considerably more slowly in cells derived from higher-grade brain tumors such as anaplastic astrocytoma, GBM, or gliosarcoma.

With respect to the cytopathic effect linked to viral infection, replication-defective adenoviruses trigger a rather slow rate of damage in low-grade tumor cells or normal brain cells, and have no effect on high-grade tumor cells. Compared with a 5-day average for wild-type adenovirus infection, advanced cytopathological features were noted only 4 weeks after H5dl 312 or H5dl 434 infection of meningioma, astrocytoma, and normal brain cells. Cytopathological features were not observed after H5dl 312 or H5dl 434 infection of glioblastoma, anaplastic astrocytoma, and gliosarcoma cells. The insignificant cellular damage and replication capacity of these vectors correlate with their incapability to induce viral DNA synthesis on host cells.

Low infectivity and poor therapeutic gene transduction have resulted in minimal clinical efficacy, thereby dampening enthusiasm for replication-defective vectors. Recently, however, oncolytic adenoviral vectors have emerged as agents that might overcome these challenges. The replication of CRAd in cancer cells seems to be the key feature needed for a successful oncolytic therapy. In this scenario, the culmination of every viral reproductive cycle leads to cell destruction and the release of new viral particles. These progenies are then able to infect neighboring neoplastic cells, further enhancing the killing effect.

**Strategies Involving Adenoviral Targeting**

The specificity of CRAAd’s replication and transgene expression in tumor cells is necessary to limit toxicity to surrounding healthy brain tissue. The tropism of CRAds can be improved using strategies that involve the manipulation of adenoviral replication events. Some of the strategies used are as follows: 1) deletion of viral genomic regions that are not needed for replication in cancer cells with specific pathway alterations; 2) facilitation of viral transduction in neoplastic cells; and 3) transcriptional targeting of viral genes or transgenes using tumor-specific promoters.

**Deletions of Viral Genome**

Various vectors have been targeted toward cancer cells by deleting the genes responsible for bypassing those cells’ antiviral proteins. Without these genes, the designed vectors will only be able to replicate within cancer cells with disrupted antiviral mechanisms. The deletion of viral genes to enhance specificity for the eradication of neoplastic cells is a principle well exemplified by the actions of two oncolytic adenoviruses, ONYX 015 and Ad5-Delta24 (Table 1). The CRAd vector dl 1520, also called ONYX 015, has a deletion in the viral genomic region coding E1B 55kd. This deletion effectively limits the replication of the virus to neoplastic cells that have a defective p53 pathway. Recently, it has been suggested that E1B 55kd-deleted ONYX 015 replicates in neoplastic cells due to aberrations in the nuclear mRNA export of cancer cells rather than by p53 alteration.

A Phase I clinical trial to examine the effects of injection of ONYX 015 into peritumoral regions of recurrent malignant gliomas was recently completed and published. In that study, ONYX 015 was injected into the walls of tumor
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### TABLE 1

<table>
<thead>
<tr>
<th>Adenoviral Vector</th>
<th>Modification of Adenovirus</th>
<th>Target on Glioma</th>
<th>Examples of Oncolytic Activity Against Glioma</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ad5-Delta24</td>
<td>deletion of 24-bp on E1 viral genomic region; this deletion impairs vector’s capacity to interfere w/ Rb protein</td>
<td>deficient Rb pathway</td>
<td>in vitro: lysis of most human glioma cells was observed w/10–14 days after infection w/ Delta24 at 10 pfu/cell</td>
<td>34</td>
</tr>
<tr>
<td>ONYX 015 (dl1520)</td>
<td>deletion on viral genomic region coding E1B 55K; this deletion restricts replication of this CRAd to cells w/ defective p53 pathway</td>
<td>defective p53 pathway</td>
<td>human study: Phase I clinical trial proved that injection of ONYX 015 into brain surrounding a resected malignant glioma is relatively safe in humans</td>
<td>11,16,44, 46,51</td>
</tr>
</tbody>
</table>

* All tables are slightly modified from the versions that appeared in “Conditionally replicative adenoviral vectors for malignant glioma,” Sonabend AM, Ulasov IV, Lesniak MS. 2006. Copyright John Wiley & Sons Limited. Reproduced with permission.

Resection cavities. This trial proved that the injection of up to $10^{10}$ plaque-forming units of ONYX 015 into brain tissue surrounding a resected malignant glioma is safe in humans. Another adenoviral vector, Ad5-Delta24, is an interesting alternative example that illustrates the same principle. It carries a 24-bp deletion in the E1 viral genomic region that impairs the capacity of the virus to interfere with Rb, which is a tumor suppressor protein with a pathway that is commonly altered in gliomas. A hypophosphorylated form of Rb controls the transition from the G1 to the S cell phase by binding to E2F transcription factors. Then, Ad5-Delta24 replicates in and destroys cancer cells that have a deficient Rb pathway. An advantage of Delta24 is that its oncolytic properties are independent of the host cell’s p53 status.

The vector Ad5-Delta24 has been tested in vivo in human glioma xenografts in nude mice. In that model, a single dose of this vector induced 66.3% inhibition of tumor growth and multiple injections induced 83.8% inhibition. On the other hand, normal fibroblast or cancer cells with restored Rb activity were resistant to this virus. Interestingly, it recently has been described that the shift to the S phase caused by Ad5-Delta24 upregulates the expression of topoisoerase I. This enzyme is responsible for the relaxation of supercoiled DNA during DNA synthesis. This is relevant because when topoisoerase I is used in combination with irinotecan (known as CPT-11), a chemotherapeutic agent that inhibits topoisoerase I activity, a synergistic oncolytic effect is achieved. This was proven in vitro and in vivo in an intracranial mouse glioma model. Such therapy led to a significant increase in survival of mice treated with a combination of the oncolytic virus and chemotherapy. The clinical potential of this vector remains to be addressed in human studies.

A new variation of Ad5-Delta24 called Delta24-hyCD was recently created and tested in vivo. In contrast to the original, this vector has been designed to express a humanized form of the Saccharomyces cerevisiae cytosine deaminase gene (or hyCD). The median inhibitory concentration dose of 5-FC required for a complete cytotoxic effect by the Delta24-hyCD virus was fivefold lower than with Delta24 alone in the malignant glioma cell lines U251MG and U87MG. Intratumoral treatment of mice bearing intracranial U87MG xenografts with Delta24-hyCD combined with 5-FC significantly improved survival, confirming that Delta24-hyCD with concomitant systemic 5-FC has shown a significant improvement over Ad5-Delta24 in the context of experimental glioma models.

**Enhanced Transduction in Neoplastic Cells**

The initial step in viral infection is transduction, which is the entry of a virion into the host cell. One strategy for enhancing the tropism of CRAd for gliomas is facilitating viral transduction in neoplastic cells. The entry of adenoviral particles into cells is mediated by interactions between specific cell receptors and viral proteins. Specifically, adenovirus serotype 5, one of the most popular adenoviruses used for oncolytic vectors, requires the expression of CAR by host cells to achieve transduction. Inconsistent and poor expression of CAR in different tumors has been shown to limit the development of an efficient therapy for cancer based on the use of CRAd with wild-type knob domain. Indeed, CAR is widely present in most tissues but poorly expressed in gliomas.

To overcome CAR deficiency in tumors, chimeric vectors derived from different adenovirus serotypes have been developed. Recently, a novel vector derived from a recombinant Ad5 vector containing the canine adenovirus serotype 1 knob was created. This vector was named Ad5-Luc1-CK1, and its effectiveness was compared with that of other chimeric viruses displaying the canine adenovirus serotype 2 knob (Ad5Luc1-C2) or Ad3 knob (Ad5/3Luc1). The canine adenovirus serotype 1 knob altered Ad5 tropism through the use of a CAR-independent entry pathway that was separate from the ones used by other chimeric viruses. The gene transfer efficiency of this novel vector was superior in comparison with other vectors examined in this study when tested in ovarian cancer cell lines. This vector remains to be tested in the context of gliomas.

Conditionally replicative adenoviruses can be retargeted by modifying their capsid proteins to bind with receptors.
that are preferentially expressed on glioma cells. Brain tumors express a series of membrane proteins that include integrins, growth factor receptors, and others (Table 2). Some of these molecules are known to contribute to the neoplastic phenotype; in other cases, the precise function of these receptors remains unclear. In addition to the modification of viral proteins, the ligands or chimeric molecules of glioma receptors that match viral and cell surface molecules can be incorporated into a viral genome as transgenes. Finally, antibodies also are used to enhance viral transduction on neoplastic cells.

Several examples that illustrate the principle of enhanced viral transduction are worth discussing (Table 3). For example, Ad5-Delta24RGD is an adenoviral vector with an insertion of RGD motif into the fiber knob of the Delta24 vector. The insertion of RGD into the adenoviral fiber enhances the viral infection of target cells through the interaction of the inserted motif with αvβ3 integrins that are abundantly expressed in glioma. When tested in brain tumor models, Ad5-Delta24RGD led to higher oncolytic activity than that shown by the original Ad5-Delta24 vector after infection of glioma cells with low expression of CAR through CAR-independent pathways. This vector was tested in vivo in nude mice with subcutaneously injected human malignant glioma IGRG121 xenografts. Intratumoral injection resulted in complete tumor regression in nine of 10 mice and long-term survival in all treated mice compared with controls. The oncolytic activity of this vector was shown to be enhanced by irradiation such that the same therapeutic effect was achieved when a 10-fold lower viral dose was applied.

Another potential target for oncolytic vectors is EGFR. This receptor is ideal for retargeting because it offers various advantages. First, its expression is abundant on gliomas; in most cases, there is amplification of the EGFR gene on these tumors. In addition, once it is bound to its ligand, activation of a PI3K-dependent pathway leads to internalization of the complex. Activation of PI3-K is required for adenovirus entry to host cells. A number of strategies are designed to take advantage of EGFR expression on gliomas. For instance, a bispecific antibody conjugate has been developed that interferes with the binding of vectors to cell receptors but retargets the viral

### Table 2

Receptors found on gliomas as possible targets for CRAds

<table>
<thead>
<tr>
<th>Receptor Type</th>
<th>Expression on Gliomas</th>
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<tbody>
<tr>
<td>PDGFR</td>
<td>PDGFR</td>
</tr>
<tr>
<td>EGFR</td>
<td>EGFR</td>
</tr>
<tr>
<td>IL-13Rα</td>
<td>IL-13Rα</td>
</tr>
<tr>
<td>αvβ3</td>
<td>αvβ3</td>
</tr>
<tr>
<td>αvβ5</td>
<td>αvβ5</td>
</tr>
<tr>
<td>transferrin</td>
<td>transferrin</td>
</tr>
<tr>
<td>IGF-1R</td>
<td>IGF-1R</td>
</tr>
<tr>
<td>expressed by tumor endothelium</td>
<td>expressed by tumor endothelium</td>
</tr>
<tr>
<td>endosialin</td>
<td>endosialin</td>
</tr>
<tr>
<td>FAS-L</td>
<td>FAS-L</td>
</tr>
<tr>
<td>(KDR)/VEGF receptor</td>
<td>(KDR)/VEGF receptor</td>
</tr>
<tr>
<td>E selectin</td>
<td>E selectin</td>
</tr>
<tr>
<td>FLT-1</td>
<td>FLT-1</td>
</tr>
</tbody>
</table>

### Table 3

CRAds with facilitated transduction into glioma cells

<table>
<thead>
<tr>
<th>Adenoviral Vector</th>
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<tr>
<td>Ad5-Delta24RGD</td>
<td>insertion of an Arg-Gly-Asp (RGD) motif into the fiber knob of vector Delta24</td>
<td>deficient Rb pathway &amp; αv integrins</td>
<td>in vivo: on nude mice w/ s.c. human malignant glioma IGRG121 xenografts; intratumoral injection resulted in tumor regression in 9 of 10 mice &amp; long-term survival in all treated; the oncolytic activity was enhanced by irradiation</td>
<td>73,124</td>
</tr>
<tr>
<td>Ad vector retargeted to EGFR</td>
<td>addition of a bispecific antibody conjugate antiCAR-antiEGFR</td>
<td>EGFR &amp; PI3K-dependent pathway</td>
<td>in vitro: EGFR targeting enhanced Ad gene delivery to 7 of 12 established glioma cell lines &amp; to 6 of 8 cultured primary gliomas</td>
<td>94</td>
</tr>
<tr>
<td>double ablated Adv (CAR &amp; integrin binding) defective</td>
<td>addition of bispecific single-chain antibody directed to EGFR &amp; viral fiber knob protein to a CAR &amp; integrin binding defective adenoviral vector</td>
<td>EGFR &amp; PI3K-dependent pathway</td>
<td>in vitro: improved tumor-to-normal brain targeting index 5- to 38-fold compared to native vectors; the retargeted vectors were able to transduce organotypic glioma spheroids w/ efficiencies similar to those of native adenoviral vectors</td>
<td>129</td>
</tr>
<tr>
<td>recombinant Ad5 vector</td>
<td>insertion of transgene coding for a soluble recombinant protein including CAR ectodomain fused w/ human EGF (sCAR-EGF fusion protein)</td>
<td>EGFR &amp; PI3K-dependent pathway</td>
<td>in vitro: the expression of this protein improved gene transfer efficiency on a series of cancer cell lines</td>
<td>26</td>
</tr>
<tr>
<td>FGF2-retargeted adenovirus</td>
<td>FGF2 was utilized as a targeting ligand</td>
<td>FGFR1 up-regulated expression by glioma cells</td>
<td>in vivo: enhanced transduction efficiency of FGF2-retargeted AdV in CAR-negative intracranial gliomas compared w/ AdV alone, w/out evidence of increased angiogenesis</td>
<td>133</td>
</tr>
</tbody>
</table>

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### TABLE 4

<table>
<thead>
<tr>
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<th>Examples of Oncolytic Activity Against Glioma</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Adgfa2TK</td>
<td>HSV-TK gene is driven by the GFAP promoter</td>
<td>GFAP expression in gliomas</td>
<td>in vitro: infection of C6, U251MG glioma cell followed by treatment with ganciclovir revealed cell toxicity</td>
<td>131</td>
</tr>
<tr>
<td>recombinant adenoviral vector</td>
<td>addition of E2F-1 promoter to control the expression of transgenes</td>
<td>activation of E2F by Rb gene mutations or mutations affecting upstream regulators of pRB</td>
<td>in vivo: tumor injection led to eradication of established gliomas with less toxicity to normal tissue than that seen with standard adenoviral vectors</td>
<td>105</td>
</tr>
<tr>
<td>Ad-MK</td>
<td>incorporation of midkine promoter on CRAd</td>
<td>midkine promoter activity on glioma</td>
<td>in vitro: this vector showed strong oncolytic in midkine-positive glioma cells</td>
<td>68</td>
</tr>
<tr>
<td>Adv-MBP-Bax &amp; Adv-MBP-caspase-8</td>
<td>addition of Bax or caspase-8 transgenes under the control of MBP promoter</td>
<td>MBP promoter activity in glioma</td>
<td>in vitro: transfection of these vectors on U251 &amp; U373MG resulted in moderate expression of correspondent transgenes; coinfection resulted in effective induction of apoptosis on tested cells</td>
<td>116</td>
</tr>
</tbody>
</table>

### Use of Tumor-Specific Promoters

Cancer development is accompanied by a series of modifications in gene expression. Indeed, most cancer cells show activated promoter elements that enhance tumor growth and invasion. Such promoters, if properly incorporated into viral genomes, can drive the expression of viral genes and transgenes. The resulting vectors limit their respective gene’s expression and oncolysis to neoplastic cells (Table 4). A series of promoters have been found to be active in gliomas (Table 5).

For example, telomerase is a promoter that is overexpressed in 90% of human cancers. The enzyme has been reported to be active in 10 to 45% of anaplastic astrocytomas and 75 to 89% of GBM tumors, but it has been undetectable in normal brain tissue. Telomerase subunit hTERT recently has been described to be responsive to E2F-1 transcription factor. In one experiment, hTERT subunit promoter was used in a DNA construct for induction of apoptosis in glioma cell lines.

### TABLE 5

<table>
<thead>
<tr>
<th>Promoter elements as candidates for gene therapy for gliomas</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>tissue specific</td>
<td></td>
</tr>
<tr>
<td>glial fibrillary acidic protein (GFAP)</td>
<td>131</td>
</tr>
<tr>
<td>myelin basic protein (MBP) gene promoter</td>
<td>96,97,116</td>
</tr>
<tr>
<td>tumor specific</td>
<td></td>
</tr>
<tr>
<td>nestin promoter</td>
<td>20</td>
</tr>
<tr>
<td>human telomerase RNA (hTR) promoter</td>
<td>12</td>
</tr>
<tr>
<td>human telomerase reverse transcriptase (hTERT) promoter</td>
<td>69</td>
</tr>
<tr>
<td>E2F-1 promoter</td>
<td>105</td>
</tr>
<tr>
<td>midkine promoter</td>
<td>68</td>
</tr>
<tr>
<td>survivin</td>
<td>21,63,67</td>
</tr>
<tr>
<td>COX-2 promoter</td>
<td>62,84,117</td>
</tr>
<tr>
<td>tumor endothelium (KDR)/VEGF receptor promoter</td>
<td>59</td>
</tr>
<tr>
<td>FLT-1</td>
<td>55,99</td>
</tr>
<tr>
<td>E-selectin</td>
<td>59,132</td>
</tr>
<tr>
<td>endoglin</td>
<td>39</td>
</tr>
</tbody>
</table>
gene was driven by the hTERT promoter. This transgene codifies for an autocatalytic caspase capable of inducing apoptosis independent of the upstream, initiator caspases.\textsuperscript{10} The hTERT/rev-caspase-6 construct induced apoptosis in hTERT-positive malignant glioma cells, but not in hTERT-negative astrocytes and fibroblasts. In an in vivo assay, the growth of subcutaneously injected tumors in nude mice was suppressed by treatment with the hTERT/rev-caspase-6 construct.\textsuperscript{70}

The midkine promoter has been shown to be selectively active in many malignant tumor types, including gliomas.\textsuperscript{1,6,98,115,126,142–144} In the case of glioma, the midkine promoter activity was two times higher in midkine-positive cells than in midkine-negative primary normal brain cells. Midkine promoter–based CRAds (Ad-MK) was developed to test this promoter. This vector showed strong oncolytic activity in midkine-positive glioma cells but did not exhibit cytotoxicity in midkine-negative primary normal brain cells. When tested in an in vivo experiment, Ad-MK eradicated midkine-positive glioma xenografts.\textsuperscript{68}

Promoter sequences that are responsive to E2F also are candidates for targeting CRAds to gliomas. Mutations of the $Rb$ gene or mutations that affect upstream regulators of pRB are commonly found in gliomas.\textsuperscript{47,128} These alterations lead to activation of E2F-responsive transcription regulatory sequences such as the E2F-1 promoter. Adenoviral vectors that contain transgenes driven by the E2F-1 promoter have been proven to mediate tumor-selective gene expression. When tested in vivo, these vectors eradicated established gliomas with significantly less toxicity to normal tissue than seen with standard adenoviral vectors.\textsuperscript{105}

Finally, survivin is a member of the inhibitor-of-apoptosis family that is overexpressed by 79.1% in astrocytic tumors. The expression of this gene correlates with tumor grade and is present in 90% of GBMs. Survivin seems to play an important role in the oncogenesis and progression of these tumors.\textsuperscript{21,6,67} This is suggested by its expression pattern and by the fact that patients with survivin-positive astrocytic tumors have significantly shorter overall survival times compared with patients who have survivin-negative tumors.\textsuperscript{65} The activity of this promoter is enhanced by hypoxia,\textsuperscript{116} which is commonly found in rapidly growing tumors like high-grade gliomas. The promoter of the survivin gene has already been proven effective in producing transgene expression in lung cancer.\textsuperscript{8} Recently, the survivin promoter was incorporated into a CRAd. This vector has been proven effective for targeting glioma xenografts in vivo.\textsuperscript{130}

Conclusions

Conditionally replicative adenoviral vectors are innovative and potentially powerful tools that currently are being developed and tested in preclinical and human studies of malignant brain tumors. Selective manipulation of CRAds, by either transductional or transcriptional modification, offers the possibility of selectively targeting tumor tissue but sparing normal and healthy brain. Although preliminary in vitro and in vivo data are encouraging, the full promise of CRAd agents will require rigorous clinical testing in the setting of malignant brain tumors.

References

1. Adachi Y, Lakka SS, Chandrasekar N, et al: Down-regulation of integrin alpha(v)beta(3) expression and integrin-mediated sig-

naling in glioma cells by adenovirus-mediated transfer of antisense urokinase-type plasminogen activator receptor (upAR) and sense p16 genes. J Biol Chem 276:47171–47177, 2001


22. Debinski W, Gibo DM: Molecular expression analysis of restrictive receptor for interleukin 13, a brain tumor-associated can-
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65. Kambara H, Okano H, Chiocca EA, et al: An oncolytic HSV-1 mutant expressing ICPI45.5 under control of a nestin promoter increases survival of animals even when symptomatic from a...


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106. Phuong LK, Allen C, Peng KW, et al: Use of a vaccine strain of measles viruses genetically engineered to produce carcinoen- 


112. Rich JN, Rasheed BK, Yan H: EGFR mutations and sensitivi- ty to gefitinib.


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Address reprint requests to: Maciej S. Lesniak, M.D., 5841 South Maryland Avenue, MC 3026, Chicago, Illinois 60637. email:mlesniak@surgerybsd.uchicago.edu.