Viral Vector-targeted Antiangiogenic Gene Therapy Utilizing an Angiostatin Complementary DNA

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ABSTRACT

Despite recent advances in neurosurgery, radiation, and chemotherapy, the prognosis of patients with malignant gliomas remains dismal. Based on the observations that solid tumor growth is angiogenic dependent, and gliomas are among the most angiogenic of all tumors, therapeutic strategies aimed at inhibiting angiogenesis are theoretically attractive. Angiostatin, an internal peptide fragment of plasminogen, has been shown to potentially inhibit endothelial proliferation in vitro and tumor growth in vivo. Long-term systemic delivery of proteins, however, poses a number of difficult logistic and pharmacological problems and may not be necessary or optimal for treating locally aggressive tumors such as gliomas. We now demonstrate that retroviral and adenoviral vectors that transduce the angiostatin cDNA can be used to inhibit endothelial cell growth in vitro and angiogenesis in vivo. Vector-mediated inhibition of tumor-associated angiogenesis results in increased apoptotic tumor cell death, leading to inhibition of tumor growth. These studies support a potential role of vector-mediated transduction of the cDNA encoding angiostatin as a potential novel therapeutic strategy for the treatment of malignant brain tumors and confirm the antitumor activity of angiostatin and the concept of dormancy therapy.

INTRODUCTION

Malignant gliomas are among the most well-vascularized of all human tumors, secondary to the ability to stimulate new blood vessel growth (angiogenesis) in the brain, an organ that normally has a mitotically quiescent vascular system. Some of the molecular mechanisms underlying brain tumor-mediated angiogenesis have been elucidated and include overexpression of VEGF, basic fibroblast growth factor, and PDGF, as well as down-regulation of endogenous inhibitors of angiogenesis such as thrombospondin (1-5).

With the hypothesis that solid tumor growth is “angiogenesis-dependent” comes the realization that angiogenesis itself might be a potential therapeutic target. A number of relatively weak inhibitors of angiogenesis have been demonstrated to be capable of inhibiting the growth of experimental brain tumors, including agents such as pentostatin, heparin, hydroxyurea, and TNP-470 (6-13). Angiogenesis inhibition may, therefore, represent a potentially powerful new approach to cancer therapy.

Recently, O’Reilly and co-workers (22) have described an internal peptide fragment of plasminogen, found in the serum and urine of mice with Lewis lung carcinoma, that has potent antiangiogenic properties. This protein, called angiostatin, has significantly greater tumor growth-inhibitory properties than most antiangiogenic drugs described previously. Unfortunately, relatively high doses of protein are needed to control tumor growth after systemic administration.

Systemic delivery of such proteins may not be the most efficient and/or efficacious method for treating locally aggressive tumors such as gliomas. We now demonstrate how a strategy we have previously termed “targeted antiangiogenesis” can be used to deliver a cDNA that encodes angiostatin into tissue at risk for local tumor growth, resulting in high local concentrations of angiostatin and effective inhibition of tumor-induced angiogenesis and tumor growth.

MATERIALS AND METHODS

Cell Lines and Animals. The RT2 glioma cells (kindly provided by Dr. G. Yancey Gillespie, University of Alabama-Birmingham, Birmingham, AL) and U87MG human glioblastoma cells (American Type Culture Collection, Rockville, MD) were maintained in DMEM containing 10% FBS. HUVE cells were purchased from American Type Culture Collection (Rockville, MD) and maintained in Medium 199 containing 10% fetal bovine serum, 100 µg/ml heparin, and 30 µg/ml endothelial cell growth supplement (Collaborative Biomedical Products, Bedford, MA). Five-week-old male Swiss nude mice (Nu/Nu) were purchased from Taconic (Germantown, NY), and adult male Fischer 344 rats (150-175 g) were purchased from Charles River Laboratories (Wilmington, MA). Animal studies were done in accordance with guidelines of the Animal Care and Use Committee of Dana-Farber Cancer Institute.

Plasmid Construction. A cDNA coding for the mouse angiostatin was amplification by PCR using the mouse plasminogen cDNA as the template and two oligonucleotide primers (5'-ACGAAGCTTGGATCCATGGACCATACTGCTC-3' and 5'-ACGTCTAGAGGATCCATGGCTACGTATGCGTAATCCCGAACATGCTGATGGTATGGGCGAACATCCACAACAACA-3') corresponding to the amino acid residues 1-6 and 461-466 of mouse plasminogen, respectively. The 1.4-kb mouse angiostatin cDNA, including seven peptide and the first four triple loop structures (Kringle regions) of plasminogen, was fused to the hemagglutinin cDNA (function as an immunological tag) and cloned into the pRC/CMV vector (Invitrogen). This plasmid was then digested with HindIII/XbaI, blunt ended, and ligated into the blank EcoRI site of pMV7, a retroviral expression vector (14).

Northern Blotting. Total RNA (10 µg) prepared by guanidine isothiocyanate-cesium chloride extraction was electrophoresed in a 1% denaturing agarose gel and transferred to nitrocellulose filters. Filters were hybridized to mouse angiostatin cDNA, neomycin 3' phosphotransferase cDNA (neor), and human β-actin horseradish peroxidase-conjugated probes.

Immunoprecipitation. After growing cells in methionine-free media for 20 h, cells were labeled with 20 µCi/ml of [35S] protein labeling mix (DuPont NEN, Boston, MA). Culture supernatants and cell lysates were collected and immunoprecipitated with mouse monoclonal anti-HA antibody (Boehringer Mannheim, Indianapolis, IN).

Proliferation Assay (Conditioned Media and Coculture Assay). HUVE cells (104) were plated in six-well plates, and culture supernatants of RT2, RT2-neo, and RT2-Agst glioma cells (104) were added to 1 ml of the normal HUVE growth medium. In the coculture assay, RT2, RT2-neo, and RT2-Agst glioma cells (2 x 105) were added to the upper chamber of cell culture inserts, and HUVE cells (104) were plated in the lower chamber. Three days later, HUVE cells were labeled with 2 µCi of [3H]thymidine, and incorporation into DNA was quantitated 24 h later.

Retroviral Infection Assay. HUVE cells (104) were exposed to normal HUVE growth medium, and culture supernatants from PA317 cells or PA317 cells were transfected with pMV7 (PA317-neo) or pMV7-Agst (PA317-Agst). Three days later, HUVE cells were labeled with 2 µCi of [3H]thymidine, and incorporation into DNA was quantitated by scintillation counting.

Animal Studies: Subrenal Capsule Assay. All of the experiments were performed as described previously (12). Five-week-old male Swiss nude mice...
their largest diameter and then at least five thin slices were made from each half of the resected tumor and used for quantitative immunohistochemistry. The mean averages from at least five of these multiple sections were used for the quantitative assays, and the pictures used in the figures were representative of the average section. Positive staining was detected by substrate reaction with diaminobenzidine. Sections were counterstained with methyl green and mounted in permount. Microvessels were counted under ×200 fields on areas of tumor with the highest density of vascular staining. The labeling index of PCNA was calculated as a percentage of positive nuclei to total cells under ×400 magnification.

**In Situ Apoptotic Cell Detection Assay.** To detect apoptotic cells, paraffin-embedded sections were processed using the Apo Tag Plus In Situ Apoptosis Detection Kit Peroxidase (Oncor, Gaithersburg, MD). The apoptotic index was estimated by the percentage of positive staining cells visualized under light microscopy at ×400.

**Recombinant Adenovirus.** The angiostatin cDNA (Agst) was cloned into the XhoI-NorI site of a shuttle plasmid, Ad.CMV-ßgal (15). The resulting shuttle plasmid, pCMV-Agst, was cotransfected into 293 cells with the pMJ17 plasmid containing the adenoviral type 5 genome (kindly provided by Frank Graham, McMaster University, Hamilton, Ontario, Canada) by calcium phosphate coprecipitation as described previously (16, 17). Recombinant adenovirus was isolated from a single plaque, expanded in 293 cells, and purified by double cesium gradient ultracentrifugation. The titer of purified adenovirus was determined in a spectrophotometer at 260 nm and by plaque assays.

**RESULTS**

**Expression of Agst-transfected RT2 Cells.** Norther blot analysis from polyclonal populations of RT2 rat glioma cells stably transduced by pMV7-Agst or the control pMV7 vector demonstrated neo-specific mRNA in both populations of retrofectants, whereas angiostatin mRNA could only be detected in the pMV7-Agst transfectants (Fig. 1A). A mouse monoclonal antibody against HA detects a protein of M, ~70,000–80,000, consistent with angiostatin, in the cell lysates and culture supernatants of the pMV7-Agst transfectants (Fig. 1B). It should be noted that the observed molecular weight of the mouse angiostatin is larger than human angiostatin secondary to the presence of the preactivation peptide, HA tag, and two glycosylation sites in the mouse angiostatin compared with only one such site in human angiostatin.

**Biological Activity of Angiostatin.** To assess the biological activity of the vector-encoded protein and to assess the effect of vector transduction of endothelium, HUVE cells were directly transduced by pMV7-Agst or the control vector. A 30–40% decrease in mitogen-stimulated HUVE cell proliferation in the pMV7-Agst transduced cells compared with control or mock-transduced cells was observed (Table 1). Because in vivo gene transfer will probably result in transduction of tumor cells as well as endothelial cells, we were interested in evaluating whether pMV7-Agst transduction of glioma cells adjacent to endothelial cells could mediate inhibition of endothelial cell proliferation. To experimentally address this question in vitro, retroviral-transduced RT2 glioma cells were plated in the upper chamber of a dual cell culture system, whereas mitogen-stimulated

<table>
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<th>Agst vector</th>
<th>Direct transduction</th>
<th>Coculture</th>
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<tr>
<td>pMV7-neo</td>
<td>100.0 ± 10.4%</td>
<td>100.0 ± 11.9%</td>
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<tr>
<td>pMV7-Agst</td>
<td>63.6 ± 4.5%</td>
<td>48.3 ± 4.8%</td>
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<tr>
<td>Ad.CMV-ßgal</td>
<td>100.0 ± 17.9%</td>
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<tr>
<td>Ad.CMV-Agst</td>
<td>24.9 ± 5.2%</td>
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*Results are expressed as percentage of control vector-transduced HUVE cells.

a p < 0.005 compared with controls.

b RT2 cells directly transduced.

c p < 0.001 compared with controls (Student's t test).

d HUVE cells directly transduced.
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Fig. 2. Growth of retroviral-transduced RT2 glioma xenografts in the subrenal capsule. RT2 cells (2 x 10^5) transduced by either pMV7.neo or pMV7.Agst were surgically implanted into the subrenal capsule of Nu/Nu mice. Two weeks later, animals were sacrificed, and the kidneys were harvested. A, whole mounts of freshly harvested kidneys from pMV7.neo (middle) or pMV7.Agst (far right) transduced tumors. Kidneys from sham-operated animals (far left) are included for comparison. B, quantitative assessment of size (volume and weight) and gross vascularity of pMV7.neo and pMV7.Agst-transduced, 14-day-old subrenal capsule gliomas (n = 5; values represent the mean; bars, SD). C, quantitative assessment of microvasculature, proliferative, and apoptotic indices of pMV7.neo and pMV7.Agst-transduced, 14-day-old gliomas in the subrenal capsule (n = 5; values represent the mean; bars, SD). FVIII RAg, FVIII receptor antigen. D, microphotographs of immunohistochemical stained tumor sections for FVIII antigen (F Vili RAg) and for apoptotic cells as detected by the TUNEL assay in pMV7.neo and pMV7.Agst-transduced, 14-day-old subrenal RT2 gliomas.

HUVE cells were plated in the bottom chamber. We observed a 70% inhibition of HUVE cell proliferation when cocultured with pMV7.Agst-transduced glioma cells compared with HUVE cells cocultured with pMV7-transfected glioma cells. Additionally, conditioned, filtered media from pMV7.Agst-transduced glioma cells induced a 50–70% inhibition of HUVE cell proliferation compared with conditioned media from pMV7-transduced glioma cells (data not shown). Treatment of conditioned media with HA antibody, prior to exposure to HUVE cells, abolished most of the inhibitory effect of the pMV7.Agst conditioned media (data not shown). These data are consistent...
with the hypothesis that tumor cells transduced by the therapeutic vector secrete biologically active angiostatin.

**In Vivo Tumor Growth.** Transduction of RT2 cells by pMV7-Agst or control vector did not change the morphology or growth characteristics of these cells **in vitro** (data not shown). To evaluate growth characteristics of Agst-transduced RT2 glioma cells **in vivo**, we microsurgically implanted vector-transduced RT2 cells into the subrenal capsule of nude mice. As an avascular potential space, the subrenal capsule will only support the growth of tumors if angiogenesis is induced. Thus, this is a sensitive system for evaluating inducers and inhibitors of angiogenesis **in vivo**, as determined by tumor growth and vasculature. Two weeks after the implantation of RT2-neo or RT2-Agst cells (2 × 10⁶), animals were sacrificed, and tumor size and vascularity were assessed. The resultant RT2-neo control gliomas were large, red, and hypervascular, whereas RT2-Agst gliomas were small and white with few visible surface blood vessels (Fig. 2A). The mean tumor volumes in the control vector and Agst-transduced group were 718 mm³ and 147.0 mm³, respectively, and the mean tumor weights were 0.66 and 0.14 g, respectively (P < 0.005; Fig. 2B). In addition, macroscopic assessment of the angiostatin-transduced tumors demonstrated a significant reduction in the number and density of blood vessels compared with that seen in the control vector-transduced tumors (P < 0.001; Fig. 2B). Significantly fewer microvessels were observed in the RT2-Agst tumors as determined by direct microvessel counting of FVIII receptor antigen-stained cells (Fig. 2, C and D). In contrast, the apoptotic index of the pMV7-Agst-transduced tumors was 2–3-fold higher than the control tumors, despite the fact that the proliferative indices, as determined by PCNA staining, were nearly identical (Fig. 2, C and D).

To ensure that the angiostatin-mediated antiangiogenic and antitumor effect we observed was not a phenomenon specific to the subrenal model, we also evaluated tumor growth in a subdermal and intracranial model. Consistent with the results in the subrenal capsule model, the growth of pMV7-Agst-transduced subdermal tumors was inhibited by 70% relative to control vector-transduced tumors (T/C = 0.284; Fig. 3A), and rats bearing angiostatin-transduced intracerebral tumors survived significantly longer than animals bearing control vector-transduced intracerebral tumors (P < 0.009; Fig. 3B).

**Adenoviral Vector-mediated Antiangiogenesis.** The fact that retroviral transduction **in vivo** is an inefficient process prompted us to explore alternative angiostatin gene delivery strategies. Replication-deficient adenoviral vectors are efficient **in vivo** delivery vectors capable of transducing dividing as well as nondividing cells. Thus, direct injection of a angiostatin-expressing adenoviral vector into an area of brain at risk for tumor progression might result in a zone of high concentration of angiostatin. To evaluate this strategy of "targeted angiostatinogenesi," we constructed an adenoviral vector carrying the early CMV promoter upstream to the angiostatin cDNA minicasette (Ad.CMV-Agst; Fig. 4A). Northern and Western analyses failed to demonstrate angiostatin mRNA or protein in parental and control vector-transduced RT2 cells, whereas Ad.CMV-Agst-transduced cells expressed high levels of Agst mRNA and protein (Fig. 4, B and C).

To evaluate the biological activity of the Ad.CMV-Agst-encoded angiostatin protein, we performed **in vitro** assays similar to those described for the retroviral vectors (Table 1). Consistent with the results described above, direct HUVE cell transduction by Ad.CMV-Agst (multiplicity of infection, 100) and nontransduced HUVE cells cocultured with cells transduced by Ad.CMV-Agst (multiplicity of infection, 100) resulted in a 70% inhibition in cell proliferation compared with transduction by Ad.CMV-Lac Z (Table 1).

To assess whether Ad.CMV-Agst could inhibit human glioma growth and angiogenesis **in vivo**, U87MG human glioblastoma cells (10⁶) were implanted into the subrenal capsule of nude mice, followed by injection of Ad.CMV-Lac Z (5 × 10⁸ pfu) or Ad.CMV-Agst (5 × 10⁸ pfu). Three weeks later, animals were sacrificed, and tumors were evaluated. The mean tumor volumes in Ad.CMV-Lac Z- and Ad.CMV-Agst-transduced tumors were 275 and 47 mm³, respectively, and the mean tumor weights were 0.25 and 0.039 g, respectively (P < 0.05; Fig. 5A). In addition, the Ad.CMV-Agst-transduced U87MG tumors were significantly less vascular than the control vector (P < 0.001; Fig. 5A). Although there was no significant difference in proliferative indices between Ad.CMV-Agst-transduced and control tumors, the apoptotic index in Ad.CMV-Agst-transduced tumors was 3-fold higher than that of control tumors (Fig. 5B).

For the purposes of evaluating the effect of Ad.CMV-Agst on the survival of nude mice bearing intracerebral human gliomas, Ad.CMV-Lac Z (5 × 10⁶ pfu) or Ad.CMV-Agst (5 × 10⁸ pfu) were stereotactically injected directly into preestablished (6 days prior) intracerebral U87MG glioblastomas. As can be seen in Fig. 6A, animals treated with Ad.CMV-Agst survived significantly longer than those treated with the control vector (P < 0.05, log-rank test). Not surprisingly, intracranial U87MG glioblastomas transduced by Ad.CMV-Agst demonstrated significantly lower vascular densities and higher apoptotic indices compared with control tumors, despite having similar proliferative indices (Fig. 6B). These data are consistent with the hypothesis that adenoviral-mediated transduction of the angiostatin gene results in inhibition of angiogenesis, ultimately leading to increased tumor cell death without significant effects on tumor cell proliferation.
Fig. 4. Angiostatin-expressing adenoviral vector (pMV7-Agst). A, schematic representation of replication-deficient adenoviral vectors based on adenovirus serotype 5 with deletions of the Eia and Elb genes and insertional mutagenesis of E3. The Eia/b region has been replaced by homologous recombination with a minigene cassette consisting of the SV40 polyadenylation signal and the CMV early promoter driving expression of the ß-galactosidase gene or angiostatin cDNA in Ad.CMV-LacZ and Ad.CMV-Agst, respectively. B, Northern analysis of RT2 cells transduced with vector vehicle alone, Ad.CMV-LacZ, or Ad.CMV-Agst. Filters were probed with ß-actin (far left), ß-galactosidase (middle), or angiostatin (far right)-specific probes. C, immunoprecipitation of 35S-labeled parental, Ad.CMV-LacZ, or Ad.CMV-Agst-transduced RT2 cell lysates and supernatants using an anti-HA monoclonal antibody.

DISCUSSION

It has been more than 25 years since the hypothesis that solid tumor growth is angiogenesis dependent was first proposed (18). Although the mechanisms underlying tumor-mediated angiogenesis are complicated and diverse, there is a growing belief in the "balance hypothesis" of angiogenesis, which states that new blood vessel growth is under homeostatic control via an interplay between endogenous inducers and inhibitors of endothelial cell proliferation, invasion, migration, and capillary tube formation (19). Under normal physiological conditions in the adult, the "angiogenesis switch" is off secondary to the predominance of angiogenesis inhibitors over inducers, except...
in discrete situations such as in the female menstrual cycle, and during wound healing.

Through a series of genetic mutations, tumors begin to overexpress inducers of angiogenesis and/or down-regulate inhibitors. In gliomas, for example, it has been demonstrated that glioblastoma cells overexpress VEGF in situ via a hypoxia-inducible promoter (2). Thus, as gliomas begin to outgrow their pre-existing blood supply, tumor cells become relatively hypoxic, leading to production of VEGF, resulting in stimulation of endothelial migration and proliferation into the area of hypoxia. Additionally, it has been shown that gliomas overexpress both the A and B chain of PDGF, whereas tumor-associated endothelium selectively expresses the β-receptor, suggesting a type of autocrine feedback loop for tumor-associated angiogenesis (20). Finally, it can be demonstrated that thrombospondin, one of the more potent natural inhibitors of angiogenesis, is transcriptionally induced by wild-type p53, whereas mutant p53 is defective in transcriptional control of thrombospondin (21). Because a significant percentage of high-grade gliomas are mutated for p53, loss of thrombospondin may represent another derangement of the normal homeostatic controls on angiogenesis. Thus, in accordance with the “balance hypothesis of angiogenesis,” overexpression of VEGF and PDGF and down-regulation of thrombospondin may tip the scale in favor of turning the angiogenic switch on in these tumors. Consistent with this model is the idea that the angiogenic switch could be turned off again if either the inducers of angiogenesis could be down-regulated and/or inhibitors of angiogenesis could be induced. This is in essence the rationale behind the strategy of antiangiogenesis.

In the search for new and potent inhibitors of angiogenesis, O’Reilly and co-workers made the observation that metastatic tumor growth was suppressed in mice with large Lewis lung carcinomas, but upon removal of the primary tumor, metastatic growth rapidly ensued (22). They demonstrated that this effect was the result of a circulating fragment of the plasminogen protein, called angiostatin, that contained potent antiangiogenic activity (22). Metastatic tumor growth could still be suppressed in animals after removal of the primary tumor if they were systemically treated with angiostatin. Once angiostatin treatments were terminated, however, tumor growth quickly ensued. The explanation for this was demonstrated in later studies, where it was shown that metastatic deposits were present in the lung throughout the angiostatin treatments; however, their growth was suppressed secondary to a high rate of tumor cell apoptosis, presumably as a result of angiostatin-mediated inhibition of angiogenesis. Once inhibition of angiogenesis was relieved, the apoptotic index of the tumor cells returned to that of nontreated cells, and tumor growth was seen. This effect of angiogenesis inhibition has come to be known as “dormancy therapy” (23, 24).

A potential practical problem to the clinical implementation of dormancy therapy may be the requirement for long-term, systemic drug delivery, a particularly vexing problem for a protein drug like angiostatin. Viral vector-mediated targeted antiangiogenesis may offer a solution to this problem for the treatment of localized areas of pathological angiogenesis such as seen in diabetic retinopathy, rheumatoid arthritis, and locally invasive but nonmetastatic tumors like gliomas. In the studies reported here, we demonstrate significant inhibition of tumor-associated angiogenesis after transduction of the tumor cells themselves or the surrounding normal tissue at risk for tumor invasion/progression. Additionally, we demonstrate that this viral vector-mediated inhibition of angiogenesis results in increased tumor cell apoptosis (with no direct effect on the rate of tumor cell proliferation) and tumor growth inhibition in a manner consistent with dormancy therapy.

Targeted antiangiogenesis did not cure the animals in this study but merely prolonged their survival by slowing tumor growth. Although a potential explanation for the lack of tumor cures is that an immunological antiviral response attenuated the effectiveness of our vectors, we do not believe this occurred for several reasons: (a) all of the subrenal tumor experiments were performed in immunodeficient mice; (b) we have recently demonstrated that the immunological response to adenoviral vectors is significantly attenuated within the confines of the central nervous system, generally considered an
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Fig. 6. Effects of Ad.CMV-Agst on intracerebral human glioblastoma xenografts. U87MG (10⁶) cells were stereotactically implanted into the right caudate nucleus of Nu/Nu mice. Six days later, Ad.CMV-LacZ (5 × 10⁸ pfu) or Ad.CMV-Agst (5 × 10⁸ pfu) were stereotactically injected into the growing tumor mass using the same stereotactic coordinates originally used for tumor implantation. A, Kaplan-Meir survival curve for the two viral vector-treated groups. The difference in survival is statistically significant by log-rank analysis (P < 0.05). B, immunohistochemistry demonstrating the density of tumor-associated endothelium as determined by FVIII antigen (F VIII RAg) staining and the presence of apoptotic cells as determined by the TUNEL assay in serial sections of intracerebral U87MG tumors treated with either the Ad.CMV-LacZ or Ad.CMV-Agst vectors.

In summary, these studies support a potential role of vector-mediated transduction of the cDNA encoding angiostatin as a potential novel therapeutic strategy for the treatment of malignant brain tumors and confirm the antitumor activity of angiostatin and the concept of dormancy therapy. Additionally, with the growing data demonstrating a superadditive or synergistic effect of antiangiogenic agents in combination with other antiangiogenic agents and/or in combination with genotoxic drugs (chemotherapy) and radiation, the ultimate use of targeted antiangiogenesis may be in combination with other antitumor treatment modalities.

REFERENCES

immunological sanctuary.³ We have demonstrated prolonged adenoviral-mediated transgene expression months after injection of adenoviral vectors into the brain of immunocompetent rats. Thus, whether the lack of complete tumor control is ultimately a function of the limitations of angiostatin on this particular tumor type, the strategy of antiangiogenesis itself, and/or the inefficiency of the current generation of the retroviral and adenoviral vectors used in these studies cannot be known with certainly at this time. Nevertheless, it is fair to hypothesize that as viral (and nonviral) vector technology improves and more efficient, higher expressing vectors are developed, we will see an improvement in the effectiveness of targeted antiangiogenesis.

³ M. J. Parr, unpublished data.
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