Inhibition of Growth of C6 Glioma Cells in Vivo by Expression of Antisense Vascular Endothelial Growth Factor Sequence

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ABSTRACT

Tumor angiogenesis involves a combination of events including the production of inhibitors, proteases, and angiogenic factors that have a chemotactic and mitogenic effect on endothelial cells. Vascular endothelial growth factor (VEGF) is an endothelial cell-specific mitogen that promotes angiogenesis in solid tumors, including brain tumors such as astrocytomas. As an approach to the development of new strategies for gene therapy of brain tumors, we have interrupted the VEGF/VEGF receptor paracrine pathway in an attempt to inhibit angiogenesis and thereby control tumor growth. Rat C6 glioma cells were transfected with a eukaryotic expression vector bearing an antisense-VEGF cDNA. Stable transfectants were observed to express reduced levels of VEGF in culture under hypoxic conditions. When implanted s.c. into nude (nu/nu) mice, growth of the antisense-VEGF cell lines was observed to be greatly inhibited compared to control cells, despite the fact that they have a faster division time in vitro. Analysis of these tumors revealed that they have fewer blood vessels and a higher degree of necrosis, which is a plausible explanation for the reduced tumor size. We believe antisense-VEGF can be successfully used to control tumor growth and may provide the basis for the development of antiangiogenic gene therapy.

INTRODUCTION

VEGF is a powerful mitogen for vascular endothelial cells, both in vitro (1) and in vivo (2). In addition, VEGF has the property of inducing vascular permeability in vivo, hence its alternative name, vascular permeability factor (3). VEGF exists as a homodimer of approximately Mr 46,000, composed of one pair of four possible monomers, which appear to be generated by differential splicing of the VEGF gene transcript (1, 4). There are two known receptors for VEGF, both of which are protein tyrosine kinases. VEGF-R1, originally named FLT (5), and VEGF-R2 (also known as flk-1 (6), NYK (7), and KDR (8)), are structurally related to each other, and both belong to the so-called Class III protein tyrosine kinase receptors, along with the receptors for PDGF (9, 10). The ligands for these receptors are also structurally related, being members of the "cysteine knot" family of proteins to which PDGF and VEGF belong.

There is mounting evidence that VEGF and its receptors play pivotal roles in angiogenesis: that which occurs during normal embryonic development (6, 11) and that which also occurs as a consequence of the requirement of solid tumors to develop their own vascular beds (11). Three lines of evidence point to the central role played by VEGF in tumor angiogenesis: (a) the detection of high levels of VEGF expression in palisading cells around regions of necrosis in a number of solid tumor systems, coupled with the rapid induction of VEGF when tumor cells are grown under hypoxic conditions (2, 12–14), links VEGF expression in tumor cells and a physiological stimulus (hypoxia) that might promote its expression. The detection of elevated levels of both of the receptors of VEGF in tumor blood vessels suggests that VEGF may be having an impact upon the growth of these tumor endothelial cells; (b) injection of antibodies against VEGF markedly reduce the in vivo growth of s.c. injected tumor cells, which are known to produce robust tumor angiogenesis (15); and (c) introduction into tumor endothelial cells of a dominant-negative version of the VEGF-R2 by means of retroviral transfer markedly reduced tumor size (16). Taken together, these data present a formidable case for the notion that VEGF and its receptors play an important role in the development of tumor vasculature and that interference of this paracrine circuit might provide an excellent opportunity for therapeutic intervention, particularly in situations where tumors might otherwise be difficult to treat.

Brain tumors are responsible for approximately 2% of all cancer deaths. Malignant glial neoplasms comprise 40–50% of brain tumor cases and are the fourth most significant form of malignancy in terms of life years lost (17). Astrocytomas are the most common type of human brain tumor and are classified according to malignancy as astrocytomas, anaplastic astrocytomas, and glioblastoma multiforme. The progression of low to high grade is characterized by an increase in neovascularization, focal necrosis, and cellular proliferation. Current treatments, such as surgery and chemotherapy, only provide short-term management of the disease (17, 18). Hence, the development of new primary and adjuvant treatments for glioma is vital.

Analysis of human gliomas has shown that they are highly heterogeneous, although they may be of the same malignancy classification. Furthermore, the cellular profile of each individual tumor is also highly heterogeneous in that they contain cells that are at varying stages of malignancy and have growth factor/receptor expression profiles that can differ markedly (19, 20). Therefore, to develop an effective strategy of gene therapy for glioma, a process common to all the tumors must be targeted. As glioblastoma multiforme is one of the most highly vascularized solid tumors in terms of vasoproliferation, endothelial cell hyperplasia, and endothelial cell cytology (21) the process of angiogenesis represents a suitable target for gene therapy.

We have used the rat C6 glioma cell line, an established model for human glioma (22), to assess the feasibility of disrupting the VEGF/VEGF receptor pathway of angiogenesis by antisense-VEGF expression. The in vivo growth of C6 cell lines expressing antisense-VEGF was demonstrated to be significantly suppressed due to a decrease in the number of blood vessels within tumors and a subsequent increase in the degree of necrosis. The use of antisense-VEGF gene therapy presents an exciting avenue of research into new adjuvant therapies for human glioma and provides the basis for the further development of antiangiogenic gene therapy strategies.

MATERIALS AND METHODS

Antisense-VEGF Vector Construction. The mouse VEGF165 cDNA of 650 bp was initially isolated from the mouse colon by PCR. The clone was demonstrated to encode a functional VEGF because it could induce vascular permeability in the Miles Assay and bind to VEGF-R2 in a receptor-binding...
assay. The VEGF<sub>rat</sub> cDNA was subcloned into the pEF-BOS eukaryotic expression vector. The cDNA clone runs antisense relative to the EF-1α promoter.

**Rat C6 Glioma Culture Conditions.** Rat C6 glioma cells (CCL 107; American Type Culture Collection, Rockville, MD) were routinely cultured in RPMI 1640/5% NCS (heat-inactivated) and 2 mM L-glutamine in a humidified atmosphere of 5% CO<sub>2</sub>. Prior to calcium phosphate transfection, the cells were grown in DMEM/5% NCS and 2 mM L-glutamine with 10% CO<sub>2</sub>.

**Calcium Phosphate Transfection.** Rat C6 cells were grown in DME/5% NCS in 10-cm plastic dishes to 60% confluence. The calcium phosphate transfection was performed as described previously (24) using 20 μg of the antisense-VEGF construct DNA (or pEF-BOS vector alone) and 2 μg of the neomycin-resistance (pgk-neo) plasmid. The cells were then subjected to glycerol shock (5 ml DME/10% NCS/15% glycerol) for 4 min at room temperature and then washed in culture medium and incubated as described. After 48 h or when the cells were confluent, they were trypsinized from the plates and replated at a dilution of 1:20 in selection medium containing Neomycin G418 (1.2 mg/ml). Cell death was observed after 5 days in culture, and discreet colonies were apparent by 7 days post-selection. Individual colonies were then isolated and grown in 24-well culture plates. Genomic DNA and total RNA were then isolated from these colonies, and PCR and RT-PCR analysis was performed. Clones demonstrated to express the antisense-VEGF construct were then recloned by growing single cells in 96-well plates and the RT-PCR analysis was repeated.

**PCR Analysis.** PCR was used to determine which rat C6 clones were successfully transfected with the antisense-VEGF construct or control vector construct. PCR was performed on genomic DNA isolated from rat C6 glioma cells and individual clones of transfected cells using a sense primer that corresponds to the 3' terminal sequence of the VEGF cDNA insert (5' GGG ATC CTC ACC GCC TCG GCT TGT CAC A-3') and an antisense primer that corresponds to the region of the polyadenylation sequence of the pEF-BOS cloning vector (5' GTC CCA CTT GGT GAC CCT-3'). The PCR reaction was performed using standard protocols with 35 cycles of 1 min at 95°C and 2 min at 72°C. Clones demonstrated to be positive by this technique were then analyzed for their ability to express the antisense construct by RT-PCR. The RT-PCR reactions were performed on total RNA isolated from the positive clones using a reverse transcription system kit (Promega Corporation, Madison, WI) as described by the manufacturers with the same PCR primers as described above.

**Southern Blot Analysis.** Genomic DNA was isolated from cultured rat C6 glioma cells and then digested with the restriction enzyme EcoRI and electrophoresed on 1% agarose gels. The DNA was transferred onto Hybond N nylon membrane (Amersham Corp., Buckinghamshire, United Kingdom) and hybridized with the VEGF cDNA in 1 mM EDTA/0.5 M NaHPO<sub>4</sub> (pH 7.2) and 7% SDS at 65°C (25) for 16 h. The membranes were washed in 0.1X SSC/0.1% SDS at 65°C. cDNA probes were radiolabeled with [α-<sup>32</sup>P]cCTP (Amersham) according to the random primer method (26). Northern blots were also routinely hybridized with a glyceraldehyde-3-phosphate dehydrogenase cDNA probe as a control for the quantity of RNA loaded onto the gels.

**Western Blot Analysis.** Cell supernatants were collected from rat C6 cells or transfected cells grown in medium supplemented with 100 μM CoCl<sub>2</sub> at 0, 4, 8, and 24 h. Supernatants were concentrated by heparin-Sepharose-CL4B (5 ml/100 μl of beads; Pharmacia) by incubation at 4°C for 4–16 h with rotation. Following this, the beads were washed with 100 mM sodium phosphate (pH 4.36, 2.03, 1.07, and 0.1 g/L) and eluted with 1X Laemmli sample buffer (6 X 4 ml). Subsequently, the cDNA was electrophoresed on 1% agarose and transferred onto nylon membranes. The filters were prehybridized in 1 mM EDTA/0.5 M NaHPO<sub>4</sub> (pH 7.2) and 7% SDS at 65°C (25) for 16 h. Hybridization was performed with the isolated VEGF cDNA in the above solution also at 65°C for 16 h. The filters were then washed in 0.1X SSC/0.1% SDS at 55°C and then at a higher stringency of 0.1X SSC/0.1% SDS at 65°C. cDNA probes were radiolabeled with [α-<sup>32</sup>P]dCTP (Amersham), according to the random primer method (26). Northern blots were also routinely hybridized with a glyceraldehyde-3-phosphate dehydrogenase cDNA probe as a control for the quantity of RNA loaded onto the gels.
Fig. 3. Northern blot analysis of the expression of antisense-VEGF cDNA and the relative induction of endogenous VEGF in C6 glioma cells and the antisense-VEGF cell lines grown under hypoxic culture conditions. Cells were grown in culture medium supplemented with 100 μM CoCl₂ to simulate hypoxia, and total RNA was isolated at 0 h (a), 4 h (b), 8 h (c), and 24 h (d). Following hybridization with the VEGF cDNA, the membrane was rehybridized with a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA control (lower panel).

Fig. 4. Western blot analysis of VEGF expression in conditioned medium from cells grown with or without 100 μM CoCl₂ to simulate hypoxia and supernatants collected at 0 h (a) and 24 h (b).
frozen in liquid nitrogen, and stored at —70°C until required. Frozen sections of 7 μm were cut serially through the entire tumor. Alternate sections were stained with either hematoxylin and eosin or by indirect immunoperoxidase with the rat antimouse PECAM monoclonal antibody (PharMingen, San Diego, CA), which detects vascular endothelial cells in the tumors. For immunoperoxidase, sections were fixed in acetone (10 min at —20°C) and rinsed in PBS, as was performed following each of the incubation steps. Endogenous peroxidase activity was blocked by incubating the sections in 3% hydrogen peroxidase. The sections were then incubated with PBS/10% mouse serum for 30 min at room temperature, followed by 1 h at room temperature with the rat antimouse PECAM monoclonal antibody diluted in PBS/10% mouse serum. A peroxidase-conjugated rabbit antimouse immunoglobulin secondary antibody (DAKO, Glostrup, Denmark) diluted in PBS/10% mouse serum was then added to the sections for 30 min at room temperature. The substrate solution of 0.06% (w/v) diaminobenzidine-0.03% (v/v) H2O2 in PBS was then added to the sections for 5 min at room temperature. The sections were counterstained with Harris’ hematoxylin.

**Tumor Necrosis and Vascularization.** The percentage of necrosis present in each tumor section was measured using the Flinders MD-30 image analysis system (Leading Edge Technology, South Australia, Australia), and calcula-

**Fig. 5.** Photomicrographs of the cell lines cultured under standard conditions as described in “Materials and Methods.” a, rat C6 glioma; b, C6 vector-transfected control; c, anti-19 (×40).
Fig. 6. The growth of C6 glioma control cells and C6 antisense-VEGF cell lines (anti-19, anti-20, and anti-24) as a function of time. Cells were passaged at $10^5$ cells (time = 0 h) and allowed to grow under standard culture conditions over a period of 160–180 h. The cell number was determined by hemocytometer counting. The total cell number is presented as the mean of duplicate experiments.

**RESULTS**

**Cloning of the Antisense-VEGF cDNA.** The VEGF insert was cleaved from pBluescript (KS−) by XbaI digestion and cloned into the XbaI restriction site of the eukaryotic expression vector, pEF-BOS. Restriction enzyme mapping performed on DNA from transformed clones demonstrated the presence of the VEGF165 cDNA insert cloned in the antisense orientation in the pEF-BOS vector (Fig. 1).

**Rat C6 Glioma Cells Expressing Antisense-VEGF.** Following transfection of rat C6 glioma cells with the antisense-VEGF construct (or vector alone control) and subsequent antibiotic selection, 40 individual clones were isolated and grown in 24-well culture plates. PCR analysis of DNA isolated from these clones revealed that 60% of clones had the antisense-VEGF construct. Of these positive clones, 85% of clones were also shown to express the antisense-VEGF insert as assessed by RT-PCR analysis. A selection of these clones were then re-cloned at the level of one cell/well, and the RT-PCR analysis was repeated to confirm expression of the cDNA. Three antisense-VEGF cell lines were then selected for further analysis and will be referred to as anti-19, anti-20, and anti-24. Three individual clones of C6 cells transfected with vector alone were also analyzed, and the data presented is from vector control clone 8.

Southern blot analysis was performed on genomic DNA of the three antisense-VEGF clones to assess copy numbers of the integrated cDNA (Fig. 2). Genomic DNA was digested with EcoRI, and the membranes were hybridized with the VEGF cDNA insert. It was evident that each of the antisense-VEGF cell lines had multiple copies of the insert cDNA, with anti-19 having the highest copy number, followed by anti-24 and then anti-20.
Response of the Antisense-VEGF Cell Lines to Hypoxia. Control rat C6 glioma cells and transfected cell lines were grown under hypoxic culture conditions, and total RNA was isolated at 0, 4, 8, and 24 h of culture. Northern blot analysis was performed to determine the level of induction of endogenous VEGF expressed in these cell lines (Fig. 3). This experiment also demonstrated the level of expression of the antisense-VEGF cDNA. It is evident that anti-19 expressed antisense-VEGF at a greater level than anti-24, with the lowest level expressed by anti-20 (Fig. 3). Induction of endogenous VEGF expression was observed in normal C6 cells following 24 h of hypoxia (4.2 kb; Fig. 3). However, at this time point, endogenous VEGF mRNA expression was not observed in the anti-19 cell line. After 24 h of hypoxia, endogenous VEGF mRNA was expressed in both anti-20 and anti-24 cell lines, yet at a lower level than in normal C6 cells. An additional product (approximately 2.4 kb) was also observed, with anti-19 and anti-20 clones grown under hypoxia, and may represent splice products. These results were also confirmed by Western blot analysis whereby the conditioned medium of each of the cell lines (C6, C6 vector control, anti-19, and anti-20 at the same time points RNA was extracted) was assessed for VEGF expression (Fig. 4). VEGF was detected in conditioned medium from C6 cells after 24 h of hypoxia, while it remained undetectable in anti-19. There was also detectable VEGF secreted by anti-20, although at a lower level than by C6 cells.

In Vitro Growth Properties of Antisense-VEGF Cell Lines. The antisense-VEGF cell lines appeared phenotypically indistinguishable from normal C6 glioma cells and C6 transfected vector alone cells (Fig. 5). However, the growth rates of the different antisense-VEGF cell lines differed markedly from the control cells (Fig. 6). The division time determined for C6 vector alone control cells was 20 h (Fig. 6), which is identical to that of normal C6 cells (data not shown). The growth rates of three individual clones of the C6 vector control cells were determined and shown to have identical growth patterns (data not shown). Anti-19 cells grew at a similar rate to control cells (19 h). However, the other two antisense-VEGF cell lines grew far more rapidly than controls, with anti-20 and anti-24 having division times of 12 and 13 h, respectively (Fig. 6). This result was also evident from soft agar colony assays in which anti-19 resulted in a similar number of colonies as control cells, while anti-20 and anti-24 resulted in a 3-fold greater number of colonies (data not shown).

In Vivo Growth of the Antisense-VEGF Cell Lines. Control rat C6 glioma cells and antisense-VEGF cell lines were s.c. injected into nude mice, and tumor volumes were measured daily for the duration of the experiments. The in vitro growth rates of the tumors arising from the normal and C6 vector alone control cells were identical; therefore, all of the results shown will be of the vector-alone cells because their growth has not been reported previously, whereas C6 cell growth has been reported by several laboratories. Tumor growth was detectable and measurable for control C6 cells by 12 days postimplantation, while the antisense-VEGF cell lines had yet to give rise to tumors (Fig. 7). Examination of mice at 16 days postimplantation revealed that the control C6 cells had produced tumors of 1.65 ± 0.37 (SE) cm³, while none of the antisense-VEGF lines had produced visible tumors. At day 19, the control cells had resulted in tumors of 3.48 ± 0.87 (SE) cm³, while the antisense-VEGF cell lines had only just begun to give rise to tumors of 0.08 ± 0.06 (SE) cm³, 0.09 ± 0.03 cm³, and 0.27 ± 0.05 cm³ for anti-19, anti-20, and anti-24, respectively. At 21 days postimplantation, the control tumors measured 4.75 ± 0.85 (SE) cm³, and the control section of the experiment was terminated. At this time point, however, the tumors from the antisense-VEGF cell lines had not grown significantly from day 19; therefore, the monitoring of the growth of these tumors was continued until day 25. At this time point, the tumors from the antisense-VEGF cell lines had remained at almost the same size as those measured at day 19. Anti-19, anti-20, and anti-24 cell lines produced tumors of 0.18 ± 0.05 (SE) cm³, 0.26 ± 0.06 cm³, and 0.37 ± 0.06 cm³, respectively (Fig. 7).

Tumor Necrosis. Sections of tumors were analyzed for their degree of tissue necrosis. Once again, the vector control C6 cells produced results indistinguishable from normal C6 cells. The results presented in Figs. 8 and 9 demonstrate that there is a higher degree of necrosis in the tumors of the antisense-VEGF cell lines in comparison to tumors produced by control C6 cells. Control C6 tumors had an average necrosis of 9.3 ± 2.5% (SE) and
Fig. 8. Photomicrographs of tumor sections stained with hematoxylin and eosin demonstrating an increase in necrosis in the tumors derived from the antisense-VEGF cell lines compared to control C6 glioma cells. a, C6 vector control; b, anti-19 (×40).

6.8 ± 4.7%. In contrast, the antisense-VEGF cell lines were significantly more necrotic, ranging from 14.9 ± 2.1% (SE) for anti-19 (tumor #3) through to 35.0 ± 13.2% for anti-20 (tumor #2) (Fig. 9).

Tumor Vascularization. The average number of blood vessels observed in the tumors derived from the antisense-VEGF cell lines were significantly lower (P < 0.03 for each antisense-VEGF cell line compared to control) than in control tumors (Fig. 9 and Fig. 10). Control C6 tumors had an average of 10.3 ± 1.8% (SE) and 11.2 ± 1.7% blood vessels/field (0.6 × 0.4 mm) per tumor (Fig. 10). The number of vessels observed in the antisense-VEGF derived tumors were often up to 50% lower than controls, as observed with anti-19 (tumor #3) and anti-24 (tumor #3). The highest level of vascularization was observed with the anti-20 (tumor #4) tumor, which had an average of 7.5 ± 1.2 vessels/field/tumor. However, this was still a 30% reduction in vessels compared to control tumors. It is important to note that the tumor of anti-20 (tumor #4) was also the least necrotic of the tumors (Fig. 10) and was observed to have grown into adjacent muscle tissue.

DISCUSSION

It is now evident, as with other cancers, that a number of specific growth factors play significant roles in the promotion of the growth, progression, and invasive competence of astrocytomas. Thus, while a potential therapy based around the interruption of paracrine and/or autocrine growth factor pathways that impinge upon the tumor cells themselves might well prove to be a successful antitumor approach, it would be difficult to find a pathway that is universally applicable to all tumors, due to the wide variation in the extent to which such growth factor-mediated pathways are important to the growth of such tumors.

Exploiting the ubiquity of tumor angiogenesis as a suitable target for therapy has been proposed previously to be an important concept for antitumor therapy (29). This concept has recently been the subject of renewed interest in the development of new therapeutic strategies. A significant body of evidence is accumulating in favor of the notion that VEGF and its receptors play an important role in the development of solid tumors, such as those derived of glial origin. This pivotal role seems not to be a direct effect upon the tumor cells themselves, but rather it appears to be important to the process of tumor angiogenesis. Three lines of evidence have elegantly demonstrated that this growth factor and its receptors are excellent targets for the development of antitumor strategies based on the inhibition of tumor angiogenesis. These are: the demonstration that VEGF is inducible by rendering cells hypoxic (2, 12–14); the inhibition of in vivo tumor growth by antibodies against VEGF (15); and the transient inhibition of tumor
growth by infection of tumor blood vessels with retroviruses bearing dominant-negative mutants of the VEGF-R2 (16) receptor.

In this study, we have added a new dimension to these observations and demonstrated that the inhibition of VEGF expression in the rat glioma cell line, C6, results not only in the severely impaired growth of these tumors in vivo but also in markedly reduced vascularization of the tumors and increased levels of necrosis. The inhibition of tumor growth is even more significant in light of the increased growth rate

![Fig. 10. Photomicrographs of tumor sections stained with a rat antimouse PECAM monoclonal antibody by indirect immunoperoxidase. A diaminobenzidene substrate system was used to visualize antibody binding and is represented by the brown precipitate. Tissue sections were counterstained with Harris’ hematoxylin. There is a significant reduction in the number and size of the blood vessels in the tumors of antisense-VEGF cell lines. a, C6 control cells (×40); b, anti-19 (×40); c, C6 control cells (×100); d, anti-19 (×100).]
of two of these antisense-VEGF cell lines in vitro. Furthermore, the reduced levels of VEGF produced by the antisense-VEGF-transfected C6 cell lines resulted in a decrease of both the number and the thickness of tumor blood vessel walls, while levels of necrosis, and presumably the various metabolites released by necrotic tissues, were significantly increased. This observation suggests that VEGF is the principle mediator of tumor angiogenesis and that the absence of a VEGF response cannot be adequately compensated for by other angiogenic factors, such as basic fibroblast growth factor or PDGF.

Interestingly, much of the angiogenesis that was observed in the tumors derived from the antisense-VEGF-transfected C6 cells appeared to be located in small clusters of vessels, which may have been derived from one or a small number of vessels that had locally escaped the suppression of VEGF production. Whether these clusters of vessels indicated the presence of clones of C6 cells that had lost the ability to produce the antisense transcript and had, therefore, regained the capacity to recruit new blood vessels, could not be judged in this study.

It has been reported recently that VEGF suppresses the cell surface expression of E-selectin and V-CAM on endothelial cells both in vivo and in vitro (30). It might be anticipated, therefore, that a diminution of eosiophil adherence (inter alia) to these endothelial cells would result from the tumor-associated production of VEGF. Our preliminary observations assessing the degree of eosiophil infiltration of both control C6 and antisense-VEGF-transfected tumors (data not shown) fail to support this notion, and it seems unlikely that the production of VEGF is a strategy by which the tumors seek to avoid destruction by the host immune system. Rather, we favor the notion that VEGF is the primary motive force for the recruitment of blood vessels in tumors, at least in this model system.

Intriguingly, our data also hint at the possibility that VEGF may have an autocrine effect upon the C6 cells themselves. We have demonstrated that expression of antisense-VEGF results in a halving of cell division times versus control vector-transfected cells, suggesting that the VEGF expressed by C6 may be inhibiting their growth. As individual clones of vector control cells were analyzed, the difference in growth rates cannot be attributed to clonal variation within the original C6 cells. There is no evidence at present to suggest that C6 cells possess receptors for VEGF. Indeed, data from other laboratories suggest that C6 do not express either VEGF-R1 or VEGF-R2 (13, 31). Moreover, the expression of VEGF-R1 and VEGF-R2 has been perceived to be the sole domain of endothelial cells and their precursors; thus, our observations will require further work to establish the possible involvement of a VEGF autocrine loop in the C6 glioma system. However, our findings do demonstrate that the inhibition of VEGF is sufficient to control tumor growth in vivo by the suppression of tumor neovascularization. The antisense-VEGF strategy offers a new avenue of gene therapy development as an adjuvant treatment for human glioma.
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