We evaluate the effects of adjuvant treatment with the angiogenesis inhibitor Avastin (bevacizumab) on pathological tissue specimens of high-grade glioma. Tissue from five patients before and after treatment with Avastin was subjected to histological evaluation and compared to four control cases of glioma before and after similar treatment protocols not including bevacizumab. Clinical and radiographic data were reviewed. Histological analysis focused on microvessel density and vascular morphology, and expression patterns of vascular endothelial growth factor–A (VEGF-A) and the hematopoietic stem cell, mesenchymal, and cell motility markers CD34, smooth muscle actin, D2-40, and fascin. All patients with a decrease in microvessel density had a radiographic response, whereas no response was seen in the patients with increased microvessel density. Vascular morphology showed apparent “normalization” after Avastin treatment in two cases, with thin-walled and evenly distributed vessels. VEGF-A expression in tumor cells was increased in two cases and decreased in three and did not correlate with treatment response. There was a trend toward a relative increase of CD34, smooth muscle actin, D2-40, and fascin immunostaining following treatment with Avastin. Specimens from four patients with recurrent malignant gliomas before and after adjuvant treatment (not including bevacizumab) had features dissimilar from our study cases. We conclude that a change in vascular morphology can be observed following antiangiogenic treatment. There seems to be no correlation between VEGF-A expression and clinical parameters. While the phenomena we describe may not be specific to Avastin, they demonstrate the potential of tissue-based analysis for the discovery of clinically relevant treatment response biomarkers.

Keywords: angiogenesis, Avastin, bevacizumab, biomarkers, glioblastoma, glioma

Angiogenesis mediated by vascular endothelial growth factor (VEGF) is instrumental to the growth and progression of high-grade gliomas. 1 Avastin (bevacizumab) is a monoclonal antibody that binds to VEGF-A, blocking its receptor interactions. In preclinical trials, Avastin has been shown to inhibit angiogenesis, decrease microvascular density and vascular permeability, and, most importantly, inhibit tumor growth. 2–5 No tissue-based data on the effect of antiangiogenic treatment in patients with glioma have been reported.

Avastin is currently being tested in phase II clinical trials for treatment of recurrent malignant gliomas. We...
have observed a heterogeneous clinical and radiographic response in a cohort of recurrent high-grade gliomas treated with Avastin at our institution. Of interest, some patients experienced multifocal tumor recurrence following treatment with Avastin.

Such variable clinical outcomes underscore the importance of understanding correlations between pathological and clinical features and treatment response. To this end, analysis of tumor tissue is an important tool to validate treatment targets and identify potential biomarkers of treatment response. In this study, we describe the histological features of five cases of recurrent high-grade glioma before and after Avastin treatment.

Materials and Methods

Patients who underwent stereotactic needle biopsy or craniotomy and resection by the Department of Neurosurgery at New York University (NYU) Medical Center following Avastin treatment were retrospectively identified from a collective of patients with recurrent high-grade gliomas treated with Avastin as a salvage therapy at NYU Medical Center.

A retrospective chart and imaging review was conducted including all inpatient hospital charts and outpatient clinical data from the treating neurosurgeons, neuro-oncologists, and radiation oncologist.

Images were retrieved from the electronic archive of the NYU Department of Radiology. A retrospective analysis of the radiological imaging was performed, focusing on the immediate pretreatment and subsequent posttreatment MR imaging. Imaging was performed on a 1.5T system (Avanto, Sonatavision, or Symphony; Siemens, Erlangen, Germany). Conventional MR imaging, including nonenhanced axial T1-weighted spin-echo, axial T2-weighted, axial fluid-attenuated inversion recovery, and postcontrast axial T1-weighted imaging, was analyzed. Gradient-echo planar images were acquired during the first pass bolus of gadopentetate dimeglumine (Magnevist; Berlex Laboratories, Wayne, NJ, USA), injected at a rate of 5 ml/sec. A total of 60 images were acquired at 1-second intervals. Regions of interest (ROIs) were obtained in both abnormally enhancing tissues as well as abnormal, nonenhancing areas of T2 and FLAIR hyperintensity. Paraffin-embedded tumor specimens were obtained from the archives of the Department of Pathology. Sections stained with hematoxylin and eosin (H&E) were retrospectively reviewed for selection of blocks for immunohistochemistry. All immunostains were performed on formalin-fixed, paraffin-embedded tissue using a Ventana automated immunostainer. Primary antibodies included VEGF-A (Santa Cruz Biotechnology, Santa Cruz, CA, USA), CD34 (Ventana, Tucson, AZ, USA), smooth muscle actin (Ventana), fascin (Ventana), collagen IV (Ventana), and D2-40, also known as podoplanin (Signet Laboratories, Dedham, MA, USA).

For comparison of histological features and immunohistochemistry, four control cases of high-grade gliomas before and after adjuvant treatment without Avastin were randomly selected from the archives of the Department of Pathology, Division of Neuropathology at NYU Medical Center.

Histological evaluation was performed by two independent observers (L.F., C.H.C.) using a standard light microscope. Microvascular density was determined by CD34 immunostains as described previously. Briefly, the areas of highest microvascular density on CD34-stained sections were first identified at scanning power. Microvessel counts were subsequently performed in the selected areas with a 20× objective (total magnification 200×). Each stained lumen as well as each CD34-stained single endothelial cell was counted as a microvessel. The results were expressed as the highest number of microvessels within any single 200× microscopic field.

VEGF, CD34, smooth muscle actin, D2-40, and fascin expression in tumor cells was evaluated by estimating the percentage of cells labeled by each stain in the whole tissue section as well as cells labeled in the “hot spot” 40× high-power field in which most cells are labeled as determined at scanning power (1.25×). The percentages were averaged and assigned semiquantitative scores according to the percentage of cells labeled as follows: “0” (no labeling of tumor cells), “1+” (less than 1%), “2+” (1%–10%), “3+” (>10%–50%), and “4+” (>50%). The scores were used for comparison of pretreatment with posttreatment expression levels of each immunohistochemical marker (Table 1).

Results

Clinical

The clinical data are summarized in Table 2. Five patients aged 29 to 59 years (mean, 47 years) at the time of initial diagnosis underwent resection or biopsy of a high-grade glioma before and after treatment with Avastin. Four of these patients had recurrent tumors and underwent prior resections. Histological diagnoses were anaplastic oligoastrocytoma (one patient), glioblastoma (two), anaplastic oligodendroglioma (one), and anaplastic astrocytoma (one). Four patients had a high-grade glioma (WHO grade III or IV) at initial presentation, and one patient experienced secondary progression of a low-grade glioma 5 years after initial pathological diagnosis. The time interval from initial diagnosis to start of Avastin treatment ranged from 1 month to 71 months.

All patients received a total dose of 59.4 Gy of fractionated, external-beam involved-field radiotherapy. Four patients treated with Avastin for recurrent high-grade glioma underwent radiation as part of their initial therapy. All patients previously received chemotherapy, consisting of four cycles of temozolomide (Temodar) and carboplatin either as single-agent treatment or in combination (Table 2). One patient received Avastin as part of the initial chemotherapy protocol concomitantly with radiation.

Karnofsky performance scores were 90 or greater in all patients when Avastin was started. Patients 1–4 had a partial response to treatment by Macdonald cri-
matter, genu of the corpus callosum, and left basal ganglia (Fig. 1c).

In patient 2, the pretreatment scan demonstrated prior bifrontal craniotomy with nodular enhancing tumor at the surgical cavity margins and T2/FLAIR signal abnormalities in the frontal lobes and bilateral anterior temp lobes (Fig. 2a). Posttreatment scan demonstrated an initial interval response to therapy, with a decreased size of the nodular enhancing component. There was also an interval decrease in the perilesional T2/FLAIR hyperintense signal in the frontal lobes (Fig. 2b). Four months later, there was a marked increase in left frontal and temporal lobe enhancement, with interval extension of abnormal T2/FLAIR hyperintense signal and associated mass effect to the left basal ganglia and caudate (Fig. 2c).

In both patients 3 and 4, pretreatment images revealed postoperative resection cavities with a rim of contrast enhancement and surrounding T2/FLAIR signal abnormality, which initially decreased following Avastin treatment. Subsequent interval imaging over the following weeks and months demonstrated progressive enlargement of both the enhancing and nonenhancing portions of the tumor at the margins of the resection cavity consistent with local tumor progression.

Patient 5 had two adjacent peripherally enhancing masses in the right frontal lobe; abnormal T2/FLAIR signal was evident involving the entire right frontal lobe white matter and extending across the midline via the genu of the corpus callosum, internal capsule, insular cortex, with a larger heterogeneously enhancing, and a new mass within the right paramedian frontal lobe, consistent with multifocal recurrence.

<table>
<thead>
<tr>
<th>Histology</th>
<th>Immunohistochemistry</th>
<th>MVD</th>
<th>CD34</th>
<th>VEGF-A</th>
<th>D2-40</th>
<th>Fascin</th>
<th>SMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>Decrease</td>
<td>Increase</td>
<td>Decrease</td>
<td>Increase</td>
<td>Increase</td>
<td>Increase</td>
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<tr>
<td>Patient 2</td>
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<td>Increase</td>
<td>Increase</td>
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<td>Same</td>
<td>Same</td>
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<tr>
<td>Patient 3</td>
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<td>Increase</td>
<td>Same</td>
<td>Same</td>
<td>Same</td>
<td>Same</td>
</tr>
<tr>
<td>Patient 4</td>
<td>Decrease</td>
<td>Same</td>
<td>Decrease</td>
<td>Same</td>
<td>Decrease</td>
<td>Same</td>
<td>Same</td>
</tr>
<tr>
<td>Patient 5</td>
<td>Increase</td>
<td>Same</td>
<td>Decrease</td>
<td>Same</td>
<td>Same</td>
<td>Same</td>
<td>Same</td>
</tr>
<tr>
<td>Control 1</td>
<td>Decrease</td>
<td>Same</td>
<td>Increase</td>
<td>Decrease</td>
<td>Increase</td>
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<tr>
<td>Control 2</td>
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<td>Decrease</td>
<td>Increase</td>
<td>Increase</td>
<td>Increase</td>
<td>Same</td>
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<tr>
<td>Control 3</td>
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<td>Decrease</td>
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<td>N/A</td>
<td>N/A</td>
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<tr>
<td>Control 4</td>
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<td>Decrease</td>
<td>Decrease</td>
<td>Decrease</td>
<td>Same</td>
<td>Increase</td>
</tr>
</tbody>
</table>

Abbreviations: MVD, microvessel density; VEGF-A, vascular endothelial growth factor–A; SMA, smooth muscle actin; N/A, not available.

In the control group, patient age ranged from 42 to 62 years. There were two males and two females. Histological diagnoses before and after adjuvant radiation and chemotherapy were glioblastoma (two), anaplastic oligodendroglioma (one), and anaplastic oligoastrocytoma (one).

**Imaging**

In patient 1, pretreatment scans (Fig. 1a) demonstrated prior left frontoparietal craniotomy and peripheral contrast enhancement around a resection cavity. There was no nodular or mass-like enhancement. After treatment (Fig. 1b), there was persistent minimal peripheral enhancement and increased periventricular and left frontoparietal hyperintense T2/FLAIR signal. Two months later, there was multifocal progression with multiple new enhancing nodules and a dominant right frontal lobe heterogeneously enhancing mass with a marked increase in surrounding T2/FLAIR hyperintense signal consistent with a combination of vasogenic edema and/or infiltrative tumor, extending into the frontal white matter, genu of the corpus callosum, and left basal ganglia (Fig. 1c).

In patient 2, the pretreatment scan demonstrated prior bifrontal craniotomy with nodular enhancing tumor at the surgical cavity margins and T2/FLAIR signal abnormalities in the frontal lobes and bilateral anterior temp lobes (Fig. 2a). Posttreatment scan demonstrated an initial interval response to therapy, with a decreased size of the nodular enhancing component. There was also an interval decrease in the perilesional T2/FLAIR hyperintense signal in the frontal lobes (Fig. 2b). Four months later, there was a marked increase in left frontal and temporal lobe enhancement, with interval extension of abnormal T2/FLAIR hyperintense signal and associated mass effect to the left basal ganglia and caudate (Fig. 2c).

In both patients 3 and 4, pretreatment images revealed postoperative resection cavities with a rim of contrast enhancement and surrounding T2/FLAIR signal abnormality, which initially decreased following Avastin treatment. Subsequent interval imaging over the following weeks and months demonstrated progressive enlargement of both the enhancing and nonenhancing portions of the tumor at the margins of the resection cavity consistent with local tumor progression.

Patient 5 had two adjacent peripherally enhancing masses in the right frontal lobe; abnormal T2/FLAIR signal was evident involving the entire right frontal lobe white matter and extending across the midline via the genu of the corpus callosum. Following resection, radiotherapy, and chemotherapy, including treatment with Avastin, there was increased nodular enhancement within the genu of the corpus callosum, internal capsule, insular cortex, with a larger heterogeneously enhancing, and a new mass within the right paramedian frontal lobe, consistent with multifocal recurrence.
Table 2. Clinical data

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age/Sex</th>
<th>Location</th>
<th>Histological Diagnosis</th>
<th>Initial Adjuvant Treatment</th>
<th>KPS Avastin</th>
<th>Diagnosis to Avastin*</th>
<th>Last Avastin to Surgery</th>
<th>Extent of Resectionb</th>
<th>Adjuvant Chemotherapy</th>
<th>Responsec</th>
<th>Progressiond</th>
<th>Number of Resections</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>29/F</td>
<td>Left frontal</td>
<td>Anaplastic oligoastrocytoma</td>
<td>PBRT</td>
<td>90</td>
<td>2 mos.</td>
<td>77 days</td>
<td>Biopsy</td>
<td>Temozolomide + Avastin (4 cycles)</td>
<td>Yes</td>
<td>Multifocal</td>
<td>3</td>
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<tr>
<td>2</td>
<td>55/F</td>
<td>Left frontal</td>
<td>Glioblastoma</td>
<td>59.4 Gy + carboplatin (4 cycles)</td>
<td>100</td>
<td>26 mos.</td>
<td>16 days</td>
<td>Partial</td>
<td>Irinotecan + Avastin (5 cycles), Avastin alone (3 cycles)</td>
<td>Yes</td>
<td>Multifocal</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>59/M</td>
<td>Left frontal</td>
<td>Anaplastic astrocytoma</td>
<td>59.4 Gy + Temodar (4 cycles)</td>
<td>90</td>
<td>6 mos.</td>
<td>93 days</td>
<td>Gross total</td>
<td>Irinotecan + Avastin (6 cycles), carboplatin + Avastin (4 cycles)</td>
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<td>Local</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>45/M</td>
<td>Right frontal</td>
<td>Anaplastic oligodendroglioma</td>
<td>59.4 Gy + carboplatin (4 cycles)</td>
<td>100</td>
<td>43 mos.</td>
<td>148 days</td>
<td>Subtotal</td>
<td>Irinotecan + Avastin (2 cycles)</td>
<td>Yes</td>
<td>Local</td>
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<td>5</td>
<td>47/M</td>
<td>Right frontal</td>
<td>Glioblastoma</td>
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<td>100</td>
<td>1 mos.</td>
<td>208 days</td>
<td>Partial</td>
<td>Temodar + Avastin (4 cycles)</td>
<td>No</td>
<td>Multifocal</td>
<td>2</td>
</tr>
</tbody>
</table>

Abbreviations: F, female; PBRT, proton beam radiation therapy; mo., month; M, male.

*Diagnosis to Avastin refers to diagnosis with high-grade glioma.

bPartial resection: <50% contrast-enhancing tumor volume, subtotal 50%–99%, gross total 100%.

cDenotes a partial radiographic response to Avastin therapy.

dLocal progression: new contrast enhancement at the margin of the resection cavity; multifocal progression: multilobar, bilateral enhancement or multilobar T2 hyperintense signal.
Histology

Immunohistochemical findings are summarized in Table 1. Cases 1 and 2 are depicted in Figures 3 and 4. In all cases, morphological tumor appearance was similar before and after treatment. The histological diagnoses were identical, except for one patient whose tumor had been diagnosed as anaplastic astrocytoma but had progressed to glioblastoma following adjuvant treatment. Radiation effect consisting of thickened and hyalinized vessel walls was present in all patients. A sarcomatous tumor component consisting of fascicles of spindle cells separating nodules of round tumor cells with moderate amounts of surrounding cytoplasm could be observed in both resection specimens from one patient. There was no morphologically sarcomatous differentiation in the remaining five patients or in the control group.

Vascular Morphology and VEGF Expression

In all cases, blood vessels displayed typical features of highly angiogenic tumors: all had rounded endothelial cells with enlarged nuclei and clustering of blood vessels in groups, often decorated by vascular arcades and/or glomeruloid proliferations. Following treatment with Avastin, the appearance of the vasculature was similarly bizarre in three study and all control group cases. However, the glioblastomas in patients 1 and 2 were outfitted with a network of regularly spaced, thin-walled, sometimes dilated blood vessels that no longer included vascular clusters, arcades, or glomeruloid structures (Fig. 3f). The microvascular density was decreased on the second resection in all cases, except for the tumor of patient 5, where the microvessel count was increased. A variable proportion of tumor cells displayed cytoplasmic staining with VEGF-A. Comparison of VEGF-A expression in tumors before and after Avastin therapy...
demonstrated a relative increase in expression in three cases and a decrease in two cases. Increased VEGF-A expression levels were seen in two cases after treatment without Avastin, whereas the other two cases showed decreased VEGF-A expression. Vascular basement membranes were also outlined by VEGF-A immunostains in all cases (Figs. 3b, 3g, 4b, and 4g).

Collagen IV immunostains were available for analysis in two cases, decorating a continuous ring of basement membrane as an integral part of vessel walls both before and after adjuvant treatment (not shown).

**Mesenchymal and Hematopoietic Stem Cell and Invasion Markers**

A CD34 immunostain labeled an increased proportion of tumor cells, often surrounding blood vessels, following Avastin treatment in patients 1 and 2 (Figs. 3f and 4f). The tumors of the remaining three patients did not have any labeling of tumor cells. Three of four control cases also included tumor cells reactive with CD34. However, the relative proportion of labeled cells was decreased in two cases and unchanged in the third.

Smooth muscle actin immunostains marked pericytes surrounding blood vessels in all tumors (Figs. 3e and 4e), and an estimate of the percentage of CD34-labeled blood vessels also highlighted by smooth muscle actin did not reveal any difference between consecutive resection specimens. There was also cytoplasmic and membranous staining of tumor cells with smooth muscle actin in five cases (Fig. 3j), including two cases of the control group. The percentage of tumor cells stained was increased in patient 1 and in one patient in the control group.

D2-40 (podoplanin) revealed staining patterns similar to smooth muscle actin, decorating the cytoplasm and cell membranes of some tumor cells, with staining accentuated around tumor blood vessels. In contrast...
to smooth muscle actin, all tumors including the control group displayed immunoreactivity for this marker. The proportion of tumor cells labeled was similar or increased following adjuvant therapy in the Avastin group (Figs. 3h and 4h) but decreased in three of four cases in the control group.

Fascin labeled a variable percentage of tumor cells prominently at the invading edge of the tumor, where available for evaluation (Figs. 3c, 3h, 4c, and 4h), but no particular trend in expression levels after treatment was found.

Discussion

Inhibitors of angiogenesis are a promising tool for the treatment of high-grade gliomas. The clinical response to these agents is variable, and presently there are no criteria for optimal patient selection. Furthermore, no data exist on changes that occur in tumors following antiangiogenic treatment. This is perhaps because a neurosurgical procedure with associated morbidity is rarely indicated following salvage therapy for recurrent high-grade gliomas. However, pathological evaluation of tumor tissues from patients before and after novel treatments is a crucial initial step toward understanding their effects. It also may identify components of tumor morphology that aid in patient selection for anti-angiogenic therapy.

Vasculation and VEGF Expression

In this study, we present our preliminary observations of the effects of Avastin on tumor and blood vessel morphology. Tumor cellularity and morphology were similar before and after treatment. Two patients displayed a marked decrease in microvessel density and a striking apparent “normalization” of blood vessels following Avastin therapy, as described by Jain et al.8 After treatment with Avastin, bizarre vascular structures such as arcades and glomeruloid proliferations were no longer present in these specimens. Since we did not observe the same phenomena in control cases of high-grade glioma after similar treatment without Avastin, we postulate this may be an effect of antiangiogenic treatment. Unexpectedly, there was an increase in microvessel counts in one patient who did not have a radiographic response to Avastin. In this case, persistent contrast enhancement on MRI correlated with continuing vascular proliferation histologically.

VEGF immunostains labeled a variable proportion of tumor cells. There was an increase in VEGF immunoreactivity in one case and a decrease in four cases. The increases in VEGF immunoreactivity do not coincide with an increase with microvessel density nor with a lack of radiographic treatment response. Similarly, no correlation is apparent between the percentage of tumor cells labeled prior to therapy and the radiographic response to Avastin treatment.

VEGF also labeled the basement membranes of tumor blood vessels. Vascular basement membranes are well known to play an important role in tumor vascularization: they bind VEGF and may serve as a reservoir of angiogenic factors.9 In addition, in vivo experiments demonstrate that basement membrane sleeves persist after antiangiogenic treatment and serve as a potential scaffold for the sprouting of vascular endothelial cells after completion of anti-VEGF treatment.10,11 Such revascularization occurred as early as 7 days after antiangiogenic treatment in mouse models of lung carcinoma. The intervals between the last Avastin dose and surgical intervention were longer than 1 week in all patients. It is possible that similar revascularization occurred in the interval. Patients 1 and 2 were characterized by bizarre vascular structures before but not after Avastin therapy. If this is an effect of Avastin, the effect persisted for weeks to months.

The possibility that pretreatment VEGF expression may predict clinical response to antiangiogenic treatment should be investigated in larger patient series. In the present study, we did not observe a correlation between pretreatment tumor expression of VEGF and response to bevacizumab, consistent with reports in non-CNS neoplasms. In 169 patients with metastatic colorectal cancer, pretreatment microvessel density and VEGF-A expression on tissue microarrays did not correlate with effects of Avastin treatment.12 To further study the in vivo actions of Avastin, better characterization of VEGF receptor tyrosine kinase expression patterns and downstream signaling cascades may be useful, as has been done in a variety of tumors with variable results. Bone marrow biopsy specimens from patients with acute myeloid leukemia after treatment with Avastin demonstrated reduced VEGF expression without a corresponding change in the expression of phosphorylated activated VEGF receptors.13 In breast cancer patients, Avastin therapy was associated with decreased expression of phosphorylated VEGF receptors without a change in microvessel density or VEGF expression.14

Mesenchymal and Hematopoietic Stem Cell and Invasion Markers

It is interesting to note that three of five patients had multifocal tumor recurrence following Avastin treatment. This observation led to a more detailed immunohistochemical analysis of stem cell, mesenchymal, and tumor invasion markers including CD34, smooth muscle actin, D2-40 or podoplanin, and fascin.

We observed a relative increase in CD34-positive cells following angiogenic treatment. CD34 is a glycoprotein normally expressed by mature endothelial cells, mesenchymal, and hematopoietic progenitor cells, and neural stem cells. It is also expressed in CNS lesions associated with epilepsy, such as gangliogliomas, pleomorphic xanthoastrocytomas, and cortical dysplasia.15-17 In this context, CD34 immunoreactivity was thought to indicate cellular origin from atypically differentiated neural precursors. More recently, expression of CD34 in conjunction with other hematopoietic markers has been reported in a case of medulloblastoma.18 Taken together, this may indicate that expression of such a stem cell marker reflects progression toward a more primi-
tive phenotype. Furthermore, it is possible that CD34-positive cells in gliomas are recruited into the tumor from circulating bone marrow–derived hematopoietic progenitors. In vitro experiments demonstrated that bone marrow–derived CD34-positive cells can develop neural and astrocytic phenotypes. For future characterization of these tumor components, double-staining studies with other stem cell, glial, neural, and endothelial markers may be useful.

Smooth muscle actin labels smooth muscle cells or pericytes that form an integral part of microvascular proliferations in high-grade gliomas. It is also expressed by tumor cells in some glioblastomas and gliosarcomas. The upregulation of this marker in two of our cases may thus represent a transition to a “mesenchymal” phenotype. Alternatively, there may be a causal relationship between inhibition of VEGF and upregulation of smooth muscle actin: in vitro studies on human CD34-positive vascular progenitor cells demonstrated a differentiation into endothelial cells in response to VEGF, whereas addition of platelet-derived growth factor to the same progenitor cell type produces smooth muscle actin–positive cells with contractile properties. It is possible that a blockage of VEGF activity by bevacizumab favors the differentiation of vascular progenitor cells toward a smooth muscle cell phenotype.

D2-40 is an immunohistochemical marker recognizing podoplanin, a transmembrane glycoprotein normally expressed in lymphatic endothelium, mesothelium, and glandular myoepithelial cells. It is also expressed in diffusely infiltrating astrocytic tumors, and D2-40 expression levels correlate with tumor grade. Expression is highest in glioblastoma, and thus D2-40 serves as a marker of malignant progression. Functionally, podoplanin modifies the actin cytoskeleton and mediates cell migration by filopodia formation. As a second marker of tumor cell motility, we performed immunostaining for fascin, an actin-modifying protein implicated in formation of cell filopodia, which is abundantly expressed in many human malignancies, especially high-grade neoplasms. In gliomas, fascin expression correlates with tumor grade and is a putative marker of tumor invasiveness.

While many immunohistochemical markers for glioma invasion are available, we chose these markers for their proven technical reliability and consistency in routine diagnostic pathology. We found the expression of these markers after Avastin was increased in some cases and decreased in others, with similar findings in the control group. We cannot conclude that treatment with Avastin has any effect on the expression of these markers. Of interest, one patient who experienced a diffuse recurrence showed robust upregulation of smooth muscle actin, CD34, fascin, and D2-40 after Avastin. In some cases, antiangiogenic treatment may trigger tumor cell migration to areas with robust vascularization. It is also possible that there is a relationship between increased expression of invasion markers and a diffuse recurrence of disease.

Conclusion

This series demonstrates that treatment effects may be observed in situ on surgical specimens. We observed a decrease in microvesSEL density and a “normalization” of vasculature in some cases, which may be a direct effect of Avastin therapy. We did not observe a correlation between expression levels of VEGF, the biological target of Avastin, and decreased tumor vascularity after treatment. Upregulated expression of hematopoietic stem cell, mesenchymal, and invasive markers may be associated with antiangiogenic treatment. Clearly, future studies on larger patient cohorts are necessary for robust clinicopathological correlations to be observed. Such assessment will be essential to validate therapeutic targets and predict treatment response to novel tumor therapies such as Avastin.

References


