Glioblastoma Recurrence after Cediranib Therapy in Patients: Lack of "Rebound" Revascularization as Mode of Escape

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Abstract
Recurrent glioblastomas (rGBM) invariably relapse after initial response to anti-VEGF therapy. There are 2 prevailing hypotheses on how these tumors escape antiangiogenic therapy: switch to VEGF-independent angiogenic pathways and vessel co-option. However, direct evidence in rGBM patients is lacking. Thus, we compared molecular, cellular, and vascular parameters in autopsy tissues from 5 rGBM patients who had been treated with the pan-VEGF receptor tyrosine kinase inhibitor cediranib versus 7 patients who received no therapy or chemoradiation but no antiangiogenic agents. After cediranib treatment, endothelial proliferation and glomeruloid vessels were decreased, and vessel diameters and perimeters were reduced to levels comparable to the unaffected contralateral brain hemisphere. In addition, tumor endothelial cells expressed molecular markers specific to the blood–brain barrier, indicative of a lack of revascularization despite the discontinuation of therapy. Surprisingly, in cediranib-treated GBM, cellular density in the central area of the tumor was lower than in control cases and gradually decreased toward the infiltrating edge, indicative of a change in growth pattern of rGBMs after cediranib treatment, unlike that after chemoradiation. Finally, cediranib-treated GBMs showed high levels of PDGF-C (platelet-derived growth factor C) and c-Met expression and infiltration by myeloid cells, which may potentially contribute to resistance to anti-VEGF therapy. In summary, we show that rGBMs switch their growth pattern after anti-VEGF therapy—characterized by lower tumor cellularity in the central area, decreased pseudopalisading necrosis, and blood vessels with normal molecular expression and morphology—without a second wave of angiogenesis. Cancer Res; 71(1); 19–28. ©2011 AACR.

Introduction
Abundant angiogenesis with microvascular proliferation and tumor necrosis caused by severe hypoxia are diagnostic criteria for glioblastoma (GBM; refs. 1–4). GBM vessels are glomeruloid, dilated, and tortuous, with abnormalities in pericyte coverage and basement membrane, which leads to abnormal vascular function (e.g., increased permeability; refs. 2, 5–7). GBM angiogenesis is driven mainly by VEGF-A (referred to as VEGF here onward) signaling via its tyrosine kinase receptor VEGFR2/KDR (8–10). Blockade of VEGF with bevacizumab (a VEGF-specific antibody; Genentech) or of VEGF receptors with the pan-VEGFR tyrosine kinase inhibitor cediranib (AstraZeneca) was associated with rates of objective radiographic responses in phase II trials in patients with recurrent GBM (rGBM; refs. 11, 12).

In both preclinical and clinical settings, the benefits of cediranib monotherapy for gliomas are typically transient and followed by an apparent increase in tumor burden (11, 13). In addition, imaging studies suggest that GBM progression may not be visible on contrast-enhanced MRI (14, 15). Moreover, preclinical studies suggest a "wave" of tumor revascularization after discontinuation of anti-VEGF therapy, which might accelerate tumor relapse (16, 17).

To gain insight into the changes that occur in rGBMs after anti-VEGF therapy, we analyzed brain tissues obtained at autopsy. We studied the central area versus the infiltrating edge of rGBM tissues from patients treated with cediranib at recurrence after surgery, chemotherapy, and radiation. As a control, we studied in a similar manner the autopsy tissues from a group of rGBM patients who received either no treatment or surgery and/or chemo- and radiotherapy. In addition, we explored the...
changes in molecular, cellular, morphologic profiles, and micro-environmental factors that might be responsible for diffuse infiltrating behavior and resistance to cediranib therapy.

**Materials and Methods**

**Tissue specimens**

Formalin-fixed, paraffin-embedded (FFPE) samples from 5 patients with rGBM—enrolled in a phase II clinical trial of cediranib (11, 13)—underwent postmortem examination. These samples were compared with (i) the initial diagnostic paraffin blocks (available from 4/5 rGBM patients) prior to cediranib therapy and (ii) 7 autopsy samples from GBM patients who had received either no treatment \((n = 1)\) or chemo- and radiation therapy \((n = 6)\). All samples were obtained from the Neuropathology Laboratory at Massachusetts General Hospital after obtaining informed consent and Institutional Review Board (IRB) approval.

**Histologic analysis**

For all analyses, tissues were selected to contain macroscopically identifiable rGBM and its infiltrative edge in the same block. These FFPE samples were stained with hematoxylin and eosin (H&E). In this study, the end of the block (section) that contains tumor with no recognizable normal intervening brain structure is referred to as the “central area” and the “infiltrative area” is the opposite end of the block and includes identifiable brain structures and infiltrating glioma. Each autopsy (average 20 blocks per case) was reviewed by 2 neuropathologists (M.S., P.K.A.) to ensure that blocks selected for the study are morphologically representative of the entire tumor. Samples were stained with standard H&E. The entire H&E slide was scanned using a Scanscope scanner (Aperio Technologies). Regions of interest were selected at the interface of tumor center and infiltrative edge. Cellular density was estimated as the fraction of surface area occupied by tumor nuclei (highlighted by hematoxylin) in the central and the infiltrative regions using an in-house threshold-based segmentation of the nuclei in H&E images using Matlab software (The MathWorks). Foci of pseudopalisading necrosis, that is, dense accumulation of tumor nuclei surrounding foci of necrosis and used as diagnostic criteria of glioblastoma by WHO classification (18) were counted in 30 high-power fields (400× magnification) in the central area of the tumor.

**Immunohistochemical, immunofluorescence, and FISH analyses**

Five micrometer thick FFPE sections were immunostained following manufacturers’ recommendations and standard protocols with antibodies against the following antigens: CD31, Ki67 (both prediluted), collagen IV (1:300), and transferrin receptor (CD71, 1:100; all Dako); αSMA (1:100; Sigma); VEGFR2 (1:250), platelet-derived growth factor receptor (PDGFR) α (1:100), and PDGFRβ (1:100; all Cell Signaling); CD68 (1:100; Thermo Fisher Scientific); nestin (Abcam; 1:500), SDF1α (1:40; Biovision); CXCR4 (1:200) and PDGF-C (1:100; R&D Systems); and MDR1 (1:90; Abcam). For detection of apoptosis, we used the ApopTag kit (Millipore) following manufacturer’s instructions. Double immunofluorescence (IF) staining was performed for nestin with CD34 (Abcam; EP373Y; dilution 1:100) and for PDGFRβ (Cell Signaling; 3169; dilution 1:100) with CD34 (Becton Dickinson; clone MY10; dilution 1:50). Following secondary antibodies were used: Cy5-conjugated goat anti-mouse [Invitrogen; Alexa Fluor 647 (AF-647); dilution 1:50] for nestin, fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit (Vector Fi-1000; dilution 1:50) for CD34, FITC-conjugated goat anti-rabbit (Vector Fi-1000; dilution 1:50) for PDGFRβ and Cy5-conjugated goat anti-mouse (AF-647; dilution 1:50) for CD34. Slides were hybridized with EGFR probe according to a standard FISH protocol.

Semi-quantitative analysis of all immunohistochemical stains was performed by 2 investigators (E.d.T. and M.S.) who independently scored the intensity of staining of all proteins in tumor cells and endothelial cells using a scale from 0 (no staining) to 3 (strong staining). Microvessel density, diameter, perimeter, and the surface covered by the vascular space was estimated in at least 5 random fields of central or infiltrative area or normal tissue on CD31-stained sections using a customized analysis software tool compatible with Imagej (http://rsb.info.nih.gov/ij/; 100 vessels at 200× magnification per section). PDGFRβ expression in endothelial cells was evaluated to detect the “ectopic” expression of this marker in endothelial cells of GBM or other cancers (13, 19). Collagen IV labeling was used to quantify the basement membrane thickness (which is abnormally thick in GBMs; ref. 6) using a segmentation algorithm (Matlab). The profile around the vessels was fit to an exponential decay function (1):

\[
F = A \left( e - \frac{x}{L} \right) + C \quad (A)
\]

where \(F\) is the fraction of collagen IV positive area at \(x\) distance \((1–10 \mu m)\) from the vessels; \(L\) is the characteristic length that correlates with basement membrane thickness. A total of 1,000 cells counted in the areas of the highest Ki-67 expression or apoptosis; and indices were calculated as a percentage of positive cells.

**Statistical analysis**

Data are expressed as mean ± SEM. The principal statistical test was the \(t\) test (2-tailed with unequal variance). We considered a value of \(P < 0.05\) to be statistically significant.

**Results**

**Patient characteristics and response to cediranib treatment**

All 5 rGBM patients studied received cediranib (starting dose of 45 mg/kg/d) for at least 2 cycles (range: 56–232 days). Cediranib showed some radiographic activity in all of these patients: 4 showed partial response (P1, P2, P4, and P5) and 1 a stable disease (P3) based on MRI performed at 28 days (Table 1 and Supplementary Fig. S1). At the “end-of-study” MRI scan when compared with day 28 MRI, 2 patients showed stable disease by imaging but were progressing clinically (P1 and P4), 2 patients (P2 and P5) showed increased tumor volume by T1 post–contrast MRI, and 3 patients (P1, P2, and P4) showed significantly increased FLAIR (fluid-attenuated inversion...
recovery) signal suggestive of infiltrating disease (Table 1 and Supplementary Fig. S1). Four of the 5 patients received no other treatment after cediranib discontinuation. One patient (P2) received further anti-VEGF treatment with bevacizumab (3 cycles) and CPT-11 (irinotecan; 1 cycle). Examination of the specimen from this patient showed a similar pattern to the other 4, thus data from all 5 patients are presented. The 5 rGBM patients had a total survival of 161, 259, 175, 186, and 226 days, respectively, measured from the time of the first cediranib dose. Of interest, the median overall survival (OS) for the 31 patients enrolled in the phase 2 study was 227 days (177–293 days; ref. 11). Clinicopathologic data of control cases are summarized in Table 2.

Cediranib-treated rGBMs contain structurally normal brain vessels

The analysis of rGBM autopsy specimens offered several lines of evidence that rGBM vessels in cediranib-treated patients resembled the normal brain vessels. First, microvascular proliferation and glomeruloid vessels, which are diagnostic features of GBM, were virtually undetectable in autopsy tissues from the patients treated with cediranib (Figs. 1 and 2). As expected, these features were abundantly present in all initial biopsies prior to cediranib therapy in study subjects as well as in control autopsies. Second, the diameter and perimeter of the rGBM vessels in central areas of the tumors were significantly lower in cediranib-treated autopsy specimens compared with control specimens (P < 0.05; Fig. 1) as well as those from the patient’s initial diagnostic GBM biopsy sample (data not shown). The diameter and perimeter of the vessels in the infiltrative edge of GBMs were comparable between the 2 groups and close to those of vessels of the normal uninvolved brain (Fig. 1). Third, the thickness of basement membrane components—as defined by collagen IV immunostaining—was significantly reduced in the central areas of the cediranib treated compared with control patients (P < 0.05; Fig. 1).

Overall, central areas of control cases show broad variability of vascular parameters. In contrast, tumor vascular parameters in cediranib-treated patients are relatively uniform.

### Table 1. Patient characteristics: cediranib-treated patients

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Days on study</th>
<th>Time between last cediranib dose and death, d</th>
<th>Response at 28 d</th>
<th>Response at end of study</th>
<th>Reason for interruption</th>
<th>Other treatment</th>
<th>Age, y</th>
</tr>
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<tbody>
<tr>
<td>P1</td>
<td>232</td>
<td>27</td>
<td>PR</td>
<td>SD</td>
<td>Clinical progression</td>
<td>No</td>
<td>72</td>
</tr>
<tr>
<td>P2</td>
<td>226</td>
<td>110a</td>
<td>PR</td>
<td>PD</td>
<td>Suspected progression</td>
<td>CPT-11</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>by MRI</td>
<td>+ bevacizumab</td>
<td></td>
</tr>
<tr>
<td>P3</td>
<td>170</td>
<td>16</td>
<td>SD</td>
<td>PR</td>
<td>Infection</td>
<td>No</td>
<td>53</td>
</tr>
<tr>
<td>P4</td>
<td>133</td>
<td>42</td>
<td>PR</td>
<td>SD</td>
<td>Clinical progression</td>
<td>No</td>
<td>42</td>
</tr>
<tr>
<td>P5</td>
<td>56</td>
<td>105</td>
<td>PR</td>
<td>PD</td>
<td>Toxicity</td>
<td>No</td>
<td>46</td>
</tr>
</tbody>
</table>

Abbreviations: PR, partial response (drop in >50% of the initial tumor volume); SD, stable disease; PD, progressing disease (increase by 25%).

aAlthough the last dose of cediranib was 110 days prior to death, this patient continued to receive antiangiogenic therapy (bevacizumab) until he died.

bRelative response compared with day 28 MRI.

### Table 2. Patient characteristics: patients with no antiangiogenic therapy (controls, after review of original charts and autopsy reports)

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Final pathologic diagnosis</th>
<th>Treatment</th>
<th>Age, y</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>GBM</td>
<td>Radiation (60 Gy), gross total resection</td>
<td>72</td>
</tr>
<tr>
<td>C2</td>
<td>GBM with prominent oligodendroglial component</td>
<td>Radiation (dose unknown) and resection</td>
<td>59</td>
</tr>
<tr>
<td>C3</td>
<td>GBM</td>
<td>BCNU 2 cycles, gross total resection</td>
<td>61</td>
</tr>
<tr>
<td>C4</td>
<td>GBM–NF1 related</td>
<td>Gross total resection, chemotherapy (etoposide, platinum, ARA-c), radiation (50 Gy)</td>
<td>18</td>
</tr>
<tr>
<td>C5</td>
<td>GBM</td>
<td>Resection post–left carotid artery chemotherapy injection</td>
<td>16</td>
</tr>
<tr>
<td>C6</td>
<td>GBM</td>
<td>Multiple resections, radiation (55 Gy), chemotherapy (cisplatin, BCNU)</td>
<td>21</td>
</tr>
<tr>
<td>C7</td>
<td>GBM</td>
<td>No biopsies, no treatment; diagnosis confirmed by autopsy</td>
<td>68</td>
</tr>
</tbody>
</table>

aOriginal charts for patients C2 and C5 could not be obtained. Limited clinical data regarding their treatment were available from their autopsy reports.
The presence of structurally normal vessels in the central area of cediranib-treated rGBMs might represent an increased infiltration by the tumors of brain tissue and/or increased co-option of normal vessels.

Collectively, these morphometric analyses indicate that vessels within cediranib-treated tumors lack characteristics of abnormal GBM vasculature despite cessation of the anti-angiogenic treatment.
Figure 2. Cell density in rGBMs after cediranib treatment. A, GBMs without previous antiangiogenic treatment (control) have a broad spectrum of cell density within the center of the tumor with a sharp drop in nuclear density in the infiltrating edge, where nuclear density is less variable. Cediranib-treated patients show overall decreased cell density in the central area of the tumor compared with the patients not treated with antiangiogenic agent, with a more gradual and less steep drop of cell density in the infiltrating edge. B, low-power and high-power (inserts) H&E examination and nuclear density heat map reveal that central areas of the control cases have strikingly high nuclear density, abundant vascular proliferation (top left insert) and pseudopalisading necrosis (arrowheads). In cediranib-treated tumors, infiltrating and central areas have a similar nuclear density (scale bar, 100 μm). C, Ki-67 index and apoptotic index in the central areas did not show difference proliferation rate or cell death between control cases and cediranib-treated cases. Cediranib-treated rGBMs showed significantly lower number of foci with pseudopalisading necrosis in the central area of the tumor ($P < 0.001$).
Cediranib treatment decreases cellular density of rGBM

The rGBMs of patients who had not undergone antiangiogenic therapy showed marked variability in cellular density within the central area ranging between 2 and $12 \times 10^3$ nuclei/mm$^2$. The cellular density in the central area of GBMs in cediranib-treated patients was lower and ranged between 2 and $5 \times 10^3$ cells/mm$^2$; however, this did not reach statistical significance. In the control cases, the density decreased sharply with transition into the infiltrative area that showed narrower range of cell density. In contrast, the cediranib-treated patients showed cellular densities in the infiltrative edge that were relatively similar to those in the central area (Fig. 2). Within 1 mm around the tumor edge ($\pm 0.5$ mm), mean nuclear density in control cases rapidly dropped from 5,301 $\pm$ 979 to 2,838 $\pm$ 404 nuclei/mm$^2$ whereas mean nuclear density of cediranib-treated cases was 3,202 $\pm$ 302 nuclei/mm$^2$ in the central area compared with 2,326 $\pm$ 271 nuclei/mm$^2$ in the infiltrating edge (Fig. 2). Interestingly, one patient with the longest interval between the last dose of cediranib and death (P5; 105 days) has the cellular density curve pattern similar to the control cases whereas patients with shorter interval between the last dose of cediranib and death (P1, P3, and P4; 27, 16, and 42 days, respectively) and the patient who received bevacizumab until his death (P2) showed relatively flat curves.

There was no difference in proliferation or apoptosis both in the central area and infiltrating edge between cediranib-treated and control GBMs (Fig. 2). Control cases showed significantly higher number of foci with classical pseudopalisading necrosis than cediranib-treated cases ($P < 0.001$; Fig. 2). Therefore, anti-VEGF therapy seems to decrease cellular density in the central area but not the infiltrative edge. Because the proliferation and apoptosis rates and necrosis are not increased after cediranib therapy, this indicates either increased infiltration into the normal brain or that cediranib had direct antitumor effect leading to decrease of cellular density, which was not restored after discontinuation of the treatment.

Molecular changes in endothelial cells in cediranib-treated rGBM

In 4 of 5 cediranib-treated autopsy specimens, PDGFR$\alpha$ and PDGFR$\beta$ became undetectable in endothelial cells and in 1 case, PDGFR$\beta$ expression was detectable only in a fraction of endothelial cells in the central area (Fig. 1D). Specificity of PDGFR$\beta$ expression by endothelial cells was confirmed by double immunohistochemical staining and double immunofluorescent staining using the endothelial markers CD31 and CD34, respectively, and FISH for EGFR in GBM cells (Supplementary Fig. S2). In addition, VEGFR2 immunoreactivity decreased from a staining intensity of the 3+ (scored in the original biopsies) to an intensity of 1+ (data not shown). All control specimens displayed a strong expression of all 3 receptors. Of particular interest, these changes in endothelial phenotype were accompanied by CD71 expression [transferrin receptor, a marker of a functional blood–brain barrier (BBB)] supporting the decreased permeability detected by functional MRI. As expected in the control specimens, CD71 was greatly reduced in the central area of the tumor. Vessel maturation markers—angiopoietin 2 and Tie2—were quantified and found to be generally expressed by the endothelial cells in vessels in central and infiltrative areas and to a lesser extent in the normal brain tissue in both cediranib-treated and control specimen. Expression of MDR, a marker of multidrug resistance, was not changed after cediranib therapy (Supplementary Table S1).

Cediranib-treated GBMs show high levels of PDGF-C, c-Met, and tumor-infiltrating myeloid cells

Although the number of CD68$^+$ tumor-infiltrating myeloid cells in the infiltrating edge was comparable between control and cediranib-treated rGBMs, 2 of 5 cediranib-treated patients showed high numbers of CD68$^+$ cells in the central area of the tumor (P1 and P3; Fig. 3A and B). The rGBMs of these 2 patients progressed rapidly and led to rapid death. Cediranib-treated and control tumor cells showed no difference in expression of SDF1$\alpha$ or CXCR4 in the central or infiltrating areas (Fig. 3C). However, in cediranib-treated tumors, CXCR4 strongly positive myeloid cells were distributed in the similar pattern as CD68$^+$ cells (Fig. 3D).

In addition, in the rGBMs from cediranib-treated patients, PDGF-C was expressed at variable levels in all cases examined, both in initial biopsy and autopsy samples. PDGF-C was focally expressed also in control cases without cediranib therapy. Finally, c-Met expression was detectable in all the rGBM specimens examined. Both tumor cells in the central area of cediranib-treated patients as well as infiltrating cells showed expression of c-Met (Fig. 4A). A trend toward higher level of expression of c-Met and higher number of positive cells was observed for the cediranib-treated specimens when compared with the control specimens, consistent with the potential role of the HGF/c-Met pathway in glioma progression (20). Finally, we measured the expression of the developmental intermediate filament, nestin. Tumor cells and endothelial cells in the central areas were similarly positive for nestin in cediranib-treated and untreated tumors. Interestingly, endothelial cells in the contralateral, uninvolved part of the brain were strongly positive for nestin in cediranib-treated patients but not in control brain tissues (Fig. 4B). Expression of nestin by endothelial cells was confirmed by double immunohistochemical staining and double immunofluorescent staining with the endothelial markers CD31 and CD34, respectively (Supplementary Fig. S2).

Discussion

Proinfiltrating consequences of antiangiogenic therapy have been suggested in a number of preclinical studies of GBM (21, 22). Rubenstein et al. showed in animal models that blocking VEGF signaling by an antibody increases co-option and growth of satellite tumors (21) and Du et al. showed that ablation of hypoxia-inducible factor 1 alpha (HIF1$\alpha$) signaling leads to co-option and deep invasion of gliomas in mice (23). Casanovas et al. have shown revascularization and increased invasiveness after anti-VEGFR2 antibody therapy (24). Recently, Paez-Ribes et al. have demonstrated that orthotopically implanted GBM cells under a VEGFR-selective kinase inhibitor treatment as well as VEGF$^+/+$ GBM cells show significantly increased inva-

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Figure 3. Tumor-infiltrating myeloid cells, SDF1α and CXCR4 in cediranib-treated rGBM. A and B, quantification of CD68+ cells show similar numbers of macrophages in the central and infiltrating areas of control cases. Tumors from cediranib-treated patients showed a trend toward higher numbers of macrophages in the central area, whereas the numbers of macrophages in the infiltrating areas are similar to control cases. Immunohistochemistry for CD68 also reveals close association between macrophages and blood vessels (B; inserts). Scale bar, 50 μm. C, although we did not observe difference in expression of SDF1α (C) or CXCR4 (D) in tumor cells, control cases showed few CXCR4 tumor-associated macrophages (D, left; arrow) whereas cediranib-treated tumor contained many perivascular CXCR4+ cells in the central area of the tumor (D, right; arrows).
Although our results are consistent with the "switch" to a more invasive growth of human rGBMs after VEGF blockade, we found no evidence for a "rebound" revascularization. Although MRI findings in patients have suggested increased invasion as an adaptive response to bevacizumab (15, 26–28) and cediranib (13, 14), the effects of antiangiogenic monotherapy on rGBM vasculature in patients remain largely unknown. de Groot et al. showed in biopsies of patients with rGBM after bevacizumab therapy that abnormal FLAIR areas contained glioma cells diffusely infiltrating with a lack of glomeruloid vascular proliferation (15). Our data did not show evidence of a switch into alternative proangiogenic pathway as an escape mechanism of recurrence under antiangiogenic therapy and despite the significant time interval between the last dose of cediranib and death, vascular morphology within the central tumor was comparable with those of a normal brain in terms of vascular density, diameter, basement membrane thickness, and expression of PDGFRα, PDGFRβ, CD71, VEGFR-2, and Tie2. Furthermore, we observed decrease of cell density in the central area of the tumor, despite proliferation and apoptotic rates being similar to the tumors without antiangiogenic therapy. The striking decrease of pseudopalisading necrosis after cediranib therapy might be explained by vascular normalization after initial treatment diminishing hypoxic events leading to this characteristic type of GBM necrosis (13).

The molecular pathways that drive GBM progression through anti-VEGF therapy in patients remain largely unknown. We detected PDGF-C and c-Met as 2 possible candidates. We also detected nestin expression in endothelial cells in both tumor and contralateral brain vessels in patients treated with cediranib. Nestin is a marker of undifferentiated cells. This novel finding could imply that sustained interruption of the VEGF signaling might globally change the phenotype of all endothelial cells. Further studies need to be undertaken in order to explore this potential relationship between VEGF signaling and developmental cytoskeletal proteins. Resistance to anti-VEGF therapy has also been linked to myeloid cell infiltration in preclinical models of solid tumors (23, 29) and in lymphomas in patients (30). Our data suggest that CXCR4+ tumor-associated macrophages infiltrate the tumor, possibly in response to transient increase in SDF1α that correlated with rGBM progression during cediranib therapy (13). However, our analyses did not show a significant difference in tissue SDF1α expression between cediranib-treated and control GBMs at the time of autopsy. Despite providing direct evidence for significant structural changes in rGBMs after antiangiogenic therapy, this study has certain implications.

**Figure 4. Expression of c-Met and nestin in cediranib-treated rGBM.** A, diffuse c-Met expression was seen in several tumor cells as well as blood vessels (arrow) within the tumor areas after cediranib treatment. The blood vessels in the contralateral brain tissue (not involved by the tumor) of cediranib-treated patients also expressed c-Met (right, arrow) whereas this was not seen in control autopsy cases (inset), where vessels in the brain tissue not involved by the tumor were negative. B, nestin expression in the central area of the tumor (top left and right) is present in the tumor cells and vessels in both cediranib-treated and control tumors. Of note, vessels were also nestin positive in the normal contralateral part of the brain in cediranib-treated patients (bottom right). This phenomenon was not seen in the control specimen (bottom left).
limitations. The small number of autopsy samples and the natural heterogeneity of the disease warrants further studies to confirm these findings. Our study also emphasizes the importance of autopsy evaluation and brain banking as the ultimate clinicopathologic correlation of a drug effect as well as a crucial step in connecting the clinical and preclinical studies.

In summary, our study provides first in-human morphologic evidence that anti-VEGF treatment changes the growth pattern of rGBMs in patients with decreased microvascular proliferation, loss of pseudopalisading necrosis and diffuse spread into the adjacent normal brain (Fig. 5). Our results show that instead of switching to alternative angiogenesis pathways, rGBMs exhibit a more infiltrative phenotype after antiangiogenic therapy.

Addendum

While this article was being processed for publication, two reports demonstrated that glioblastoma stem cells undergo endothelial differentiation and form a substantial proportion of the new vessels in gliomas (see Ricci-Vitiani et al., Nature 468, 824–8 2010; and Wang et al., Nature 468, 829–33, 2010). These findings describe a new mechanism for tumor vasculogenesis, which is not blocked by VEGF inhibitors or VEGFR2 silencing. These stem cells are nestin-positive, consistent with our evidence for nestin expression in glioblastoma- and normal brain-associated endothelial cells in glioblastoma patients previously treated with anti-VEGF agents. In addition, another report of a clinical trial of bevacizumab with chemoradiation showed no apparent improvement in overall survival compared to chemoradiation alone (Lai et al., J Clin Oncol, published online on Dec 6, 2010; doi: 10.1200/JCO.2010.30.2729), which is consistent with the restoration of a blood-brain barrier phenotype in glioblastoma vessels after anti-VEGF therapy described in our report.

Disclosure of Potential Conflicts of Interest

E. di Tomaso: Current employment, Novartis. P.Y. Wen: commercial research grant, AstraZeneca. A.G. Sorensen: employment, American College of Radiology Image Metrix; commercial research grant, Siemens, Medical Solutions, General Electric Health Care; Glaxo-Smith Kline, Novartis, Exelixis, Schering Plough, AstraZeneca, Takeda/Millennium; consultant/advisory board, Epix, Genentech, Regeneron, Millennium, AstraZeneca, Mitsubishi, Merrimack, Olea Medical, Siemens Medical Solutions, Lantheus, and Biogen-Idec. T.T. Batchelor: commercial research grant, Millennium, AstraZeneca,
Schering-Plough; speaker honorarium, Roche, Schering-Plough; consultant/advisory board, Acceleron, Exelixis, Imclone, EMD - Serono, Schering-Plough.
R.K. Jain: commercial research grant, AstraZeneca, Dyax, MedImmune; speaker honorarium, Genzyme and Aplylam; consultant/advisory board, AstraZeneca, Dyax, Millennium, Regeneron, Genzyme, Morphosys, and SynDevRx; ownership interest, SynDevRx. The other authors disclosed no potential conflicts of interest.

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References

Correction: Glioblastoma Recurrence after Cediranib Therapy in Patients: Lack of "Rebound" Revascularization as Mode of Escape


Reference

Glioblastoma Recurrence after Cediranib Therapy in Patients: Lack of "Rebound" Revascularization as Mode of Escape
