Molecular Mechanisms Underlying Effects of Epidermal Growth Factor Receptor Inhibition on Invasion, Proliferation, and Angiogenesis in Experimental Glioma

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Abstract

Purpose: Epidermal growth factor receptor (EGFR) signal transduction pathways are implicated in malignant glioma aggressiveness and promote tumor cell invasion, proliferation, and angiogenesis. Nevertheless, response to EGFR tyrosine kinase inhibitor gefitinib (Iressa, ZD1839) has been disappointing in clinical trials. One potential explanation may come from the diversity of molecular alterations seen in gliomas. To validate that hypothesis, we have investigated responses to gefitinib on various tumor parameters in human malignant gliomas that exhibited different molecular alterations.

Experimental Design: We used a panel of six human malignant gliomas from established xenografts characterized for their genetic (EGFR, PTEN, TP53, and CDKN2A) and molecular (EGFR, PTEN, ERK, and Akt) alterations. Tumors were treated with gefitinib (1 or 10 μmol/L) for prolonged periods (8 or 16 days) in an organotypic brain slice model that allowed quantification of invasion, proliferation, and angiogenesis.

Results: In nontreated tumors, EGFR amplification was associated with profuse tumor cell invasion. After treatment, invasion was inhibited in tumors with EGFR amplification in a dose-dependent manner. Treatment had only antiproliferative effect in two of three tumors with EGFR amplification. Tumors with PTEN loss were resistant to treatment. We did not observe shrinkage of the tumors after treatment. None of the tumors had mutations of the EGFR kinase domain. Gefitinib had similar antiangiogenic effect in all of the tumors.

Conclusions: Gefitinib reduces cell invasion in EGFR amplified tumors. PTEN loss of expression seems to be a determinant of resistance. Interestingly, inhibition of angiogenesis by gefitinib seems independent on the EGFR genetic status of the tumors.
To better understand the therapeutic challenge raised by EGFR tyrosine kinase inhibition in glioblastoma multiforme, we made use of a panel of six human malignant glioma xenografts selected for their heterogeneity in genetic alterations as source of the tumor cell (28). In contrast to the majority of glioblastoma multiforme cell lines, xenografts conserve their genomic amplification of EGFR over time (29). We made use of a technique that we have recently designed, based on the implantation of human glioblastoma multiforme cells into brain slices maintained over long periods in organotypic cultures, to seek the effects of gefitinib on tumor growth and cell migration as well as angiogenesis in vitro (30, 31).

Materials and Methods

**Molecular and genetic alterations in tumor xenografts.** Six already established subcutaneous malignant glioma xenografts were used as the tumor cell source (28, 32). Xenografts were maintained by serial transplantation into the scapular area of nude mice. Animals were maintained under clean room conditions and received sterile rodent food and water ad libitum in accordance with institutional guidelines for the care. These tumors have previously been characterized for five main genetic alterations: EGFR amplification, Pten mutations, TP53 mutations, CDKN2A mutations, and loss of heterozygosity from chromosome 10. The six tumors have been selected for their different genetic alteration profiles (Table 1).

EGFR gene, protein, and downstream signaling pathways were further analyzed from subcutaneous xenografts. EGFR, Akt, phosphorylated Akt, ERK, phosphorylated ERK, and tubulin expressions were obtained using Western blot analysis on total proteins from fresh tumor xenografts. Fresh tumor xenografts were lysed in 150 mmol/L NaCl, 50 mmol/L Tris-HCl (pH 8), 1% Triton X-100, 4 mmol/L phenylmethylsulfonyl fluoride, 2 mmol/L aprotinin, 5 mmol/L EDTA, 10 mmol/L NaF, 10 mmol/L Na3VO4 for 30 min at 4°C. Lysates were clarified by centrifugation at 10,000 × g for 10 min at 4°C and protein concentrations were determined using the Bradford assay (Bio-Rad). Proteins were separated on 4% to 12% polyacrylamide gel (NuPAGE Novex 4-12% Bis-tris gel; Invitrogen) and electrophoretically transferred onto a polyvinylidene difluoride membrane (Millipore). Membranes were blocked for 1 h at room temperature in T-TBS [132 mmol/L NaCl, 20 mmol/L Tris-HCl (pH 7.6), 0.05% Tween 20] supplemented with 5% nonfat dry

**Table 1. Genetic alterations of xenografts**

<table>
<thead>
<tr>
<th>No.</th>
<th>Xenografts</th>
<th>EGFR</th>
<th>Pten</th>
<th>TP53</th>
<th>CDKN2A</th>
<th>10p</th>
<th>10q</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>ODA-4-GEN</td>
<td>Amp</td>
<td>-</td>
<td>-</td>
<td>LOH</td>
<td>LOH</td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>GBM-1-HAM</td>
<td>Amp</td>
<td>-</td>
<td>Mut</td>
<td>HD</td>
<td>LOH</td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>GBM-17-ROM</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>LOH</td>
<td>LOH</td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>GBM-14-RAV</td>
<td>Amp</td>
<td>-</td>
<td>Mut</td>
<td>LOH</td>
<td>LOH</td>
<td></td>
</tr>
<tr>
<td>T5</td>
<td>TG-17-GIR</td>
<td>Amp</td>
<td>-</td>
<td>Mut</td>
<td>HD</td>
<td>LOH</td>
<td></td>
</tr>
<tr>
<td>T6</td>
<td>GBM-9-THI</td>
<td>-</td>
<td>Mut</td>
<td>-</td>
<td>HD</td>
<td>LOH</td>
<td></td>
</tr>
</tbody>
</table>

Note: As shown previously (32), genetic alterations detected in xenografts were remarkably stable over time. All of the tumors had alterations corresponding to the most malignant gliomas from a genetic point of view, particularly LOH from chromosome 10, a hallmark of glioblastoma.

Abbreviations: LOH, loss of heterozygosity; Pten, phosphatase and tensin homologue tumor suppressor gene; CDKN2A, cyclin-dependent kinase inhibitor 2A; Amp, genomic amplification; Mut, mutation; HD, homozygous deletion.

*Not altered.

**Translational Relevance**

Targeting epidermal growth factor receptor (EGFR) was considered as one of the most promising therapeutic strategy in malignant gliomas. However, first clinical trials with inhibitors of EGFR such as gefitinib (Iressa) have shown disappointing results. Moreover, mechanisms of responsiveness remain unclear. We therefore go back to the bench and investigate the type of biological response of gefitinib in experimental gliomas (effect on proliferation, invasion, and angiogenesis) and correlate the response to the tumor molecular status. We used a panel of molecularly characterized human glioma xenografts implanted in an organotypic brain slice model that allows the quantification of all the aspects of glioma growth. The main finding is that gefitinib should be regarded as an anti-invasive drug in a subgroup of malignant gliomas that exhibit EGFR amplification. Furthermore, antiangiogenic effect was independent of tumors molecular status. For future clinical trials, tumor molecular status should be determined before patient selection. As EGFR inhibitors act mainly as anti-invasive drugs in malignant gliomas, they should be used in combination with other therapeutic modalities such as radiotherapy.

Receptor activates intracellular signaling cascades, including the mitotic ras/mitogen-activated protein kinase and the anti-apoptotic phosphatidylinositol 3-kinase/Akt signal pathways (11–13). In a clinical setting, EGFR amplification has been associated with poor prognosis in glioblastoma multiforme, although this remains controversial, and also with resistance to radiotherapy and chemotheray (14–17).

Given these strong implications in glioblastoma multiforme aggressiveness, inhibition of EGFR appeared to be a promising antiglioma therapy. Small molecules that block tyrosine kinase activity of the receptor have been developed (10, 18). Gefitinib (Iressa, ZD1839) is a commercially available EGFR tyrosine kinase inhibitor that has shown striking activity in a subgroup of patients with non-small cell lung cancer (19), which exhibited an activating mutation of the tyrosine kinase domain (20). Yet, first clinical trials with gefitinib in glioblastoma multiforme showed only modest activity despite a few occurrences of tumor stabilization (21, 22). Attempts to correlate EGFR status of the tumors and response to gefitinib failed and mechanisms of responsiveness in glioblastoma multiforme remain unclear (22, 23). The biology of EGFR in malignant gliomas is likely different from lung cancer, as no mutations have been found in EGFR kinase domain (24). Furthermore, other genetic alterations influence the response to treatment, particularly Pten mutations, observed in up to 40% of glioblastoma multiforme (17, 25). Tumor suppressor Pten is a protein phosphatase that negatively controls the antiapoptotic phosphatidylinositol 3-kinase/Akt pathway (26), an alternative pathway activated by EGFR. Coexpression of EGFRvIII and Pten may be associated with responsiveness to EGFR tyrosine kinase inhibitors (27), although this is controversial (21).
Molecular Status of Gliomas and Response to Gefitinib

Fig. 1. EGFR and PTEN expression profiles of the six human malignant glioma xenografts (T1-T6), A, Western blots of EGFR and PTEN. T1, T4, and T5 EGFR amplified tumors (see Table 1 for details on genetic alterations) had EGFR overexpression. Note that T3 had truncated receptors. T2, T5, and T6 had low levels of PTEN expression. T1 and T6 had PTEN mutation (Table 1). T2 had a low level of PTEN expression without PTEN mutation. T6 had EGFR overexpression and PTEN loss. Tubulin was used as a control for gel loading. B, activation of EGFR downstream signaling pathways. Western blots of Akt and phosphorylated Akt (P-Akt) and ERK and phosphorylated ERK (P-ERK) of the six xenografts. Tubulin was used as a control for gel loading.

Molecular Status of Gliomas and Response to Gefitinib

Molecular Status of Gliomas and Response to Gefitinib

EGFR small interfering RNA knockdown. ON-TARGET plus small interfering RNA (siRNA) SMART pools against EGFR (EGFR siRNA) and scrambled controls (control) were purchased from Dharmacon RNAi Technologies (Thermo Fisher Scientific). RNA interference experiments were done on two human glioblastoma cell lines: U-87 MG (U87) and plates, above 1 ml. brain slice medium (pH 7.65): MEM-21575-022 complemented with 10% FCS, 1% penicillin-streptomycin, 25 mmol/L HEPES, 1% N2 supplement (all from Life Technologies/Invitrogen), 10% d-glucose, and 100 mg bovine serum albumin (all from Sigma).

After 2 days of brain slice culture, fragments of established tumor xenografts were prepared and implanted into the thickness of the slice. nude mice bearing subcutaneous xenografts were sacrificed when tumor bulk reached a diameter larger than 1 cm. After lethal anesthesia, tumors were sterilely retrieved and transferred into brain slice medium. In the culture room, under an operating microscope, areas of necrosis and hemorrhage were identified and discarded. Fresh tumor fragments were subsequently cut into small tumor pieces of 0.2 mm in diameter with the extremities of two 26-gauge needles. Small pieces were deposited onto the slice surface, at the junction of the cortex and the corpus callosum, and gently pushed with the tip of a needle, until the tumor piece was enveloped by the slice parenchyma. For each set of experiments, and for each of the 6 xenografts, 24 to 36 tumor-bearing slices were prepared.

Two days after implantation, treatment was started with gefitinib (Iressa, ZD1839) prepared in DMSO or with control DMSO alone. Gefitinib was added to the brain slice culture medium at a concentration of 1 or 10 μmol/L. Medium with gefitinib or control medium with the same amounts of DMSO (0.1%) was changed every 2 days for 8 days (4 cycles of treatment) or 16 days (8 cycles of treatment). All of the brain slice cultures were done in triplicate in independent experiments. Brain slice cultures were stopped at day 8 or 16 post-implantation, fixed in 4% paraformaldehyde for 4 h at 4°C, and removed from the membrane, and used for tumor growth analysis.

Tumor growth analysis. Standard fluorescent immunohistochemistry was carried out on tumor-bearing slices as described previously (30). Briefly, slice cultures were fixed using 4% paraformaldehyde in PBS at 4°C for 4 h. Slices were rinsed several times in PBS and then incubated for 1 h in PBS containing 10% normal goat serum and 0.6% Triton X-100 (Sigma). They were incubated overnight at 4°C with monoclonal antibody against human vimentin (1:400; NeoMarkers), Ki-67 (MIB-1; 1:50; Immunotech), and/or against CD31 (1:100; Pharmingen). Anti-vimentin antibody did not cross-react with mouse vimentin and was specific for human tumor cells in the brain slices. After three washes in PBS, they were then incubated with a PBS solution containing anti-rabbit or anti-mouse immunoglobulin conjugated to cyanidium-Cy3 or fluorescein-FITC (1:500; Jackson ImmunoResearch). The slices were mounted in Vectashield containing 4',6-diamidino-2-phenylindole (Vector Laboratories).

For quantitative analysis, images obtained from fluorescence microscopy were digitalized and processed using the KS400 3.0 image analysis software (Zeiss). The invasion area was defined as the area where isolated tumor cells intermingled with host cells. Two parameters were used to measure tumor invasion: (a) maximal distance of tumor cell migration and (b) percentage of tumor invasion defined as invasion area / (invasion area plus bulk tumor area) * 100 (34). Tumor cell proliferation was assessed byKi-67 (MIB-1) labeling index. Around the tumor, vascular density was measured on CD31 immunostained vessels as described previously (33).

Dosage of human vascular endothelial growth factor in supernatants. Mouse brain slices injected with fragments of human tumors (T1-T6) were treated with gefitinib at a concentration of 10 μmol/L, or with DMSO alone, every 2 days. Conditioned brain slice supernatants were collected at day 8. The concentration of human vascular endothelial growth factor in the brain slice supernatants was measured with an ELISA (Quantikine ELISA kit) according to the manufacturer's instructions (Quantikine; R&D Systems). All measurements were done in duplicate.

EGFR small interfering RNA knockdown. ON-TARGET plus small interfering RNA (siRNA) SMART pools against EGFR (EGFR siRNA) and scrambled controls (control) were purchased from Dharmacon RNAi Technologies (Thermo Fisher Scientific). RNA interference experiments were done on two human glioblastoma cell lines: U-87 MG (U87) and
Three of the tumors had *EGFR* amplification (T1, T4, and T5) and three did not (T2, T3, and T6; Table 1). *EGFR* amplification was associated with *EGFR* overexpression at the protein level (Fig. 1). One of the tumors with *EGFR* amplification (T5) had overexpression of truncated-mutants *EGFR* (Fig. 1). One tumor (T6) had *EGFR* overexpression without *EGFR* amplification.

**EGFR status and tumor invasion.** Sixteen days after implantation into brain slices, the three tumors (T1, T4, and T5) with *EGFR* amplification exhibited profuse intraparenchymal invasion, whereas the three others (T2, T3, and T6) were much less invasive (65 ± 15% versus 14 ± 11%, respectively; *P* < 0.001; Fig. 2). T5, the tumor with *EGFR* amplification and truncated-mutant *EGFR*, had the strongest invasion ability. To give a comparison, long distance cell migrations of T5 were more than four times the distances seen in T6 (925 ± 136 versus 174 ± 35 μm, respectively; *P* < 0.001).

In contrast, *EGFR* status of the tumor was not associated with tumor cell proliferation (MIB-1 labeling index 15 ± 4% for *EGFR* amplified tumors versus 13 ± 3% for *EGFR* nonamplified tumors; *P* = 0.17).

Tumor invasion was not associated with other genetic alterations tested: TP53 mutations (*P* = 0.93), PTEN mutations (*P* = 0.29), or CDKN2A mutations (*P* = 0.56).

**Differential effects of *EGFR* tyrosine kinase inhibition on invasion and proliferation, with reference to molecular status.** In tumors with *EGFR* amplification (T1, T4, and T5), treatment with gefitinib for 16 days strongly decreased tumor invasion in a dose-dependent manner (Figs. 3 and 4A). In tumors without *EGFR* amplification, treatment with gefitinib had no effect on invasion. Two of these tumors had PTEN mutations (T2 and T3) and one (T5) had low PTEN expression (Fig. 1).

In contrast, under the same conditions, treatment with gefitinib only decreased cell proliferation in two of three tumors with *EGFR* amplification (T1 and T5) in a dose-dependent manner (Figs. 3 and 4A). In tumors without *EGFR* amplification, treatment with gefitinib had a differential effect: a strong anti-invasive effect but no antiproliferative effect even at a high concentration of 10 μmol/L. This tumor had a high level of Akt activation (Fig. 1B). In tumors without *EGFR* amplification, treatment with gefitinib had no effect on
cell proliferation. For all of the six tumors, we never observed shrinkage of the tumor after treatment. None of the six tumors had mutations of the EGFR tyrosine kinase domain.

**Antiangiogenic effect of gefitinib treatment regardless the genetic status of the tumor.** In the brain slice organotypic model, the vasculature, although not functional, can be modified by tumors or treatment (33). Accordingly, endothelial cells organized in a vascular network were enriched in the area surrounding the tumor implants. Treatment with gefitinib for 8 days decreased vascular density in all of the six tumors (Fig. 5). Vascular inhibition was 66 ± 9%. There were no significant differences between tumors whether or not they had EGFR amplification (65 ± 5% versus 68 ± 10%, respectively; P = 0.72). Nevertheless, human vascular endothelial growth factor levels decreased in supernatants of cultures after treatment only in the three cases of EGFR amplified tumors (26 ± 2 pg/mL for EGFR amplified and 176 ± 33 pg/mL for EGFR nonamplified tumors when treated with gefitinib versus 368 ± 76 and 173 ± 24 pg/mL for untreated controls, respectively; P < 0.001).

**Effects of EGFR siRNA knockdown on invasion, proliferation, and vascular network of U87 and GL15 tumors implanted into brain slices.** To determine the antitumor effects of siRNA against human EGFR, U87 and GL15 human glioblastoma cell lines were transfected with EGFR siRNA or scrambled controls before implantation into brain slices. In Western blot analysis, GL15 expressed higher levels of EGFR and PTEN than U87 cells (Supplementary Fig. S6A). Three days after EGFR siRNA transfection, depletion of EGFR was confirmed by Western blot analysis, which showed a dramatic decrease of EGFR expression in both GL15 and U87 cells (Supplementary Fig. S6A), an effect maintained up to 7 days (data not shown). After implantation, GL15 scrambled control tumors were more invasive than U87 scrambled control tumors (4.66 ± 1.26% versus 0.52 ± 0.34%; P < 0.001). More importantly, GL15 EGFR siRNA tumors displayed a significant decrease in tumor invasion and proliferation when compared with GL15 scrambled control tumors (4.66 ± 1.26% versus 2.39 ± 0.92%; P < 0.01 and 10.9 ± 2% versus 5.95 ± 1.45%; P < 0.01, respectively; Supplementary Fig. 6B and C). In contrast, EGFR siRNA had no effect on the invasion of U87 tumors or on the proliferation of U87 tumor cells. Interestingly, when we looked at surrounding tumor vasculature, there was no difference between EGFR siRNA tumors and scrambled control tumors (6.1 ± 1.1 versus 6.1 ± 1.4 mm; P = 0.98).

**Fig. 3. EGFR genetic status of the tumors and anti-invasive effect of gefitinib.** After implantation of small fragments (0.2 mm in diameter) of the six human malignant glioma xenografts (T1-T6) into organotypic brain slice cultures, tumors were repeatedly treated (every 2 d) for prolonged periods (16 d) with control-DMSO or 1 or 10 μmol/L gefitinib added to the slice medium. Cultures were then stopped and fixed and tumor cell immunostaining with human vimentin antibody was carried out. A, representative photomicrographs shown are tumor-bearing slices with EGFR amplification (T1, T4, and T5) after control-DMSO (left), 1 μmol/L (middle), and 10 μmol/L (right) gefitinib treatments. There was a dose-dependent inhibition of tumor cell invasion by gefitinib for tumors with EGFR amplification. B, in contrast, tumors without EGFR amplification (e.g., T6 tumor, a tumor with PTEN mutation) were resistant to treatment even at a high concentration. For quantification of anti-invasive effects, see Fig. 4. Bar, 400 μm.
**Discussion**

In this study, we show that response to gefitinib was both dependent on and independent of genetic and molecular alterations. Existence of **EGFR** amplification was a good predictor for an effect of treatment on tumor cell invasion, whereas one of three of these tumors escaped its antiproliferative action. **PTEN** loss was associated with resistance to gefitinib even at a high concentration. Finally, antiangiogenic effect was independent of tumor genetic alterations, although the treatment decreased vascular endothelial growth factor secretion only in **EGFR**-amplified tumors.

**Targeting EGFR may be instrumental on malignant glioma cell migration.** Tumor cell invasion of the brain parenchyma is a hallmark of malignant gliomas. The spreading of tumor cells around the surgical cavity explains the recurrence of these tumors after resection. Among molecular mechanisms of glioblastoma multiforme invasiveness, growth factors and their downstream signaling pathways play an important role, particularly transforming growth factor-α, the main ligand of EGFR in the brain (35, 36). It has been shown that constitutively activated mutant **EGFRvIII** increases molecular effectors of tumor invasion (37) and favors the autocrine loop transforming growth factor-α/EGFR (38), making glioblastoma multiforme cells more motile. We indeed observed that tumors with **EGFR** amplification were much more invasive than those without amplification. In keeping with this relation, inhibition of the EGFR pathway induced a strong, dose-dependent, anti-invasive effect on these tumors.

Drugs that inhibit invasion may, therefore, be clinically relevant, ideally if they are given early on in treatment. In this setting, a combination of EGFR tyrosine kinase inhibitors with...
post-resection radiation therapy could be an interesting approach because it has been shown that radiation therapy paradoxically enhances glioblastoma multiforme cell migration (39). Moreover, gefitinib radiosensitize glioblastoma multiforme cells experimentally (40).

EGFR inhibition induces tumor stabilization in a subgroup of glioblastoma multiforme. We never observed shrinkage of experimental tumors after prolonged treatment even at a high concentration. This may be in keeping with the observation that gefitinib and erlotinib (Tarceva, OSI-774), another EGFR tyrosine kinase inhibitor, seemed to act mainly as cytostatic agents in glioblastoma multiforme clinical trials (10, 21, 22). In phase II trials, the majority of patients who “responded” to treatment achieved stable disease rather than true regression.

This lack of regression is clearly at variance compared with the positive results obtained in patients with non-small cell lung cancer (20). One interesting difference between the molecular correlates of gefitinib action in the two conditions is the lack, in glioblastoma multiforme, of the EGFR kinase domain mutations that have been associated with gefitinib susceptibility in lung cancer. This type of mutation had not either been observed in another series of xenografted glioblastoma multiforme (41) and in large series of molecular studies in patients (24, 27, 42). Mechanisms of action of gefitinib may, therefore, be different in the two types of cancer. As a consequence, provisional selection of patients with malignant gliomas for gefitinib treatment should not be based on such EGFR mutations.

A high concentration is required to obtain antitumor effects. Gefitinib is a highly selective inhibitor of EGFR that blocks kinase activity of the receptor in vitro at a concentration as low as 0.1 nmol/L (43). Pharmacokinetics data in humans have shown that plasma concentrations resulting from clinically relevant doses are in the range of 1 μmol/L, a much higher concentration than required to block EGFR (44). In a recent work, it was observed that EGFR phosphorylation was not inhibited in tumor tissue resected from patients who were undergoing therapy with gefitinib or erlotinib (42). One reason for this discrepancy between in vitro and in vivo results may be the difficulty for gefitinib to cross the blood-brain barrier (45) although it has been shown that gefitinib could accumulate in glioblastoma multiforme tissue (46), and one may consider that the blood-brain barrier is severely disrupted in tumors. Nevertheless, our experiments concur to the demonstration that therapeutic concentrations of gefitinib are high, as—despite our use of an in vitro model—the concentration needed to obtain full anti-invasive and antiproliferative effects in responding tumors was in the 10 μmol/L range. It can be argued that EGFR amplification (47) and EGFRVIII expression (27) are in the same time molecular determinants of response and, paradoxically, factors of relative resistance to treatment. Indeed, gene amplification of the target has been associated with acquired resistance to imatinib mesylate (STI571, Gleevec) in chronic myelogenous leukemia (48) and EGFRVIII expression was experimentally associated to a relative resistance to gefitinib in glioblastoma multiforme (49).

Taken together, these results suggest that higher doses or new generation of EGFR tyrosine kinase inhibitors may be required to effectively inhibit EGFR phosphorylation in patients.

Antiangiogenic activity of gefitinib is independent of tumor molecular alterations. Attempts to correlate EGFR molecular status of the tumors to EGFR inhibitors clinical response has been disappointing. One explanation may derive from the role of EGFR pathways in angiogenesis. EGFR pathways induce up-regulation of expression of angiogenic factors (such as vascular endothelial growth factor and interleukin-8) by tumor cells (50). Additionally, transforming growth factor-α and EGF act as proangiogenic factors through direct effects on endothelial cells. In the present study, EGFR kinase inhibition resulted in significant inhibition of angiogenesis whatever the genetic and molecular status of the tumor. Although human vascular endothelial growth factor levels were decreased only in EGFR amplified tumors, this result suggests that, in our conditions, gefitinib acts directly on endothelial cells, which are not subject to genetic alterations.

Conclusion

Our results focus on complex relationships between tumor molecular alterations and the type of biological response. In this study, we showed that gefitinib should be regarded as an anti-invasive drug in a subset of glioblastoma multiforme. Future developments in subsequent clinical trials should include appropriate patient selection based on molecular characterization of the tumors. Moreover, xenografts used in organotypic brain slice model could help in the experimental development of more effective combinations between EGFR inhibitors and conventional chemotherapy or other targeted therapies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank Valerie Fong for editing the article.

References


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