Sorafenib Induces Growth Arrest and Apoptosis of Human Glioblastoma Cells through the Dephosphorylation of Signal Transducers and Activators of Transcription 3

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Abstract

Glioblastoma is the most common type of primary brain tumor and is rapidly progressive with few treatment options. Here, we report that sorafenib (≤10 μmol/L) inhibited cell proliferation and induced apoptosis in two established cell lines (U87 and U251) and two primary cultures (PBT015 and PBT022) from human glioblastomas. The effects of sorafenib on these tumor cells were associated with inhibiting phosphorylated signal transducers and activators of transcription 3 (STAT3; Tyr705). Expression of a constitutively activated STAT3 mutant partially blocked the effects of sorafenib, consistent with a role for STAT3 inhibition in the response to sorafenib. Phosphorylated Janus-activated kinase (JAK)1 was inhibited in U87 and U251 cells, whereas phosphorylated JAK2 was inhibited in primary cultures. Sodium vanadate, a general inhibitor of protein tyrosine phosphatases, blocked the inhibition of phosphorylation of STAT3 (Tyr705) induced by sorafenib. These data indicate that the inhibition of STAT3 activity by sorafenib involves both the inhibition of upstream kinases (JAK1 and JAK2) of STAT3 and increased phosphatase activity. Phosphorylation of AKT was also reduced by sorafenib. In contrast, mitogen-activated protein kinases were not consistently inhibited by sorafenib in these cells. Two key cyclins (D and E) and the antiapoptotic protein Mcl-1 were downregulated by sorafenib in both cell lines and primary cultures. Our data suggest that inhibition of STAT3 signaling by sorafenib contributes to growth arrest and induction of apoptosis in glioblastoma cells. These findings provide a rationale for potential treatment of malignant gliomas with sorafenib. Mol Cancer Ther; 9(4); 953–62. ©2010 AACR.

Introduction

Glioblastoma multiforme (GBM), a high-grade glioma (WHO grade IV), is the most common and lethal primary malignant brain tumor, and its prognosis remains very poor with median survival time not exceeding 15 months (1, 2). The majority of glioblastomas develop very rapidly without clinical, radiological, or morphologic evidence from a less malignant precursor lesion (primary glioblastomas). Due to the dismal prognosis of glioblastomas with currently available therapies, there is an urgent need for new treatments based on a better understanding of the molecular basis of malignant progression in this tumor. Despite the genetic heterogeneity of malignant gliomas (3), common molecular alterations are often found in signal transduction pathways. Such pathways include growth factors, phosphoinositide 3-kinase-AKT-mammalian target of rapamycin, and Raf-mitogen-activated protein (MAP)/extracellular signal-regulated kinase kinase (MEK)-MAPK/extracellular signal-regulated kinase (4–6). However, the large heterogeneity and low prevalence of each molecular abnormality have decreased the statistical power of studies seeking to establish their prognostic implications (7). The activity of signal transducer and activator of transcription (STAT) proteins, particularly STAT3, is frequently elevated in a wide variety of solid tumors and hematologic malignancies and is associated with proliferation and maintenance of tumors (8, 9). Thus, STAT3 has emerged as a promising molecular target for cancer therapy (10). Activated STAT3 is expressed in many types of brain tumors, including both low- and high-grade gliomas (11). Inhibition of the STAT3 signaling pathway suppressed proliferation and induced apoptosis in glioblastomas (12, 13). Thus, STAT3 may have an important role in the formation and maintenance of glioblastomas.

Sorafenib (BAY43-9006, Nexavar) is an oral multikinase inhibitor that was originally developed for its inhibitory effect on Raf and receptor tyrosine kinase signaling (14). Recent findings showed that sorafenib inhibited tumor growth and angiogenesis and induced apoptosis through either Raf-MEK-MAPK–dependent...
or Raf-MEK-MAPK–independent pathways, depending on the type of tumors being investigated (15, 16). Sorafenib induces apoptosis in imatinib mesylate–resistant Bcr/Abl human leukemia cells in association with STAT5 inhibition (17). We previously reported that sorafenib induces apoptosis and inhibits cell proliferation associated with the inhibition of STAT3 signaling in medulloblastomas (18). Evaluation of sorafenib from phase I and II clinical trials on several forms of advanced solid tumors showed favorable tolerability and promising clinical antitumor activity (19–21).

Molecularly targeted therapies such as sorafenib, which can disrupt molecular defects in signaling pathways, may provide clinical benefits in the treatment of glioblastomas. Our present results show that sorafenib inhibits cell proliferation and induces apoptosis in two established cell lines (U87 and U251) and two primary cultures (PBT015 and PBT022) of human glioblastomas. The biological effects of sorafenib on glioblastomas are associated with the inhibition of STAT3 signaling as well as the downregulation of cyclin D, cyclin E, and Mcl-1 proteins. Our findings suggest that sorafenib is a promising agent for the treatment of human malignant gliomas.

Materials and Methods

Reagents and Methods

Sorafenib was kindly provided by Onyx and Bayer Pharmaceuticals. Anti–cyclin D1 and anti–cyclin D3 were obtained from Calbiochem. Anti–cyclin E was obtained from BD Biosciences. Anti–cyclin D2 and anti–Mcl-1 were obtained from Santa Cruz. Horseradish peroxidase–labeled anti-mouse and anti-rabbit secondary antibodies were from GE Healthcare. All other antibodies were purchased from Cell Signaling.

Cell lines and primary culture

Established human glioblastoma cell line U87 was obtained from the American Type Culture Collection, and a tumorigenic clone of U251 was generously provided by Dr. Walter Debinski (Wake Forest University, Winston-Salem, NC). Both U87 and U251 cells were maintained in DMEM (with t-glutamine) supplemented with 10% fetal bovine serum and 1% Antibiotic-Antimycotic. The primary cultures (PBT015 and PBT022) were derived from glioblastoma (WHO grade IV) specimens obtained from patients undergoing surgical treatment. Collection of tissue was in accordance with the City of Hope Institutional Review Board–approved protocols. Tumors were graded by the attending neuropathologist in accordance with WHO-established guidelines. Freshly obtained tumor specimens were finely minced and enzymatically dissociated into single cells using 400 μ/mL Collagenase III in DMEM:F12 media. RBCs were lysed using ACK Lyse according to the manufacturer’s instructions. Adherent cells were grown in media consisting of DMEM:F12, 2 mmol/L t-glutamine, 25 mmol/L HEPES buffer, 7% heat-inactivated FCS, 100 U/mL penicillin, and 100 μg/mL streptomycin. PBT015 and PBT022 cells were subsequently maintained in DMEM (with t-glutamine) supplemented with 10% fetal bovine serum and 1% Antibiotic-Antimycotic. All cultured cells were grown in a humidified atmosphere of 5% CO₂ at 37°C.

Proliferation assays

Cell proliferation assays were done with the CellTiter 96 Aqueous One Solution Cell proliferation Assay from Promega, which contains MTS. Each well of a 96-well plate was seeded with 5,000 cells in culture medium. After overnight culture (16 h), the cells were treated with different concentrations of sorafenib and controls were treated with vehicle (DMSO). After 24 or 48 hours of treatment, MTS was added to the cells according to the supplier’s protocol and absorbance was measured at 490 nm using an automated enzyme-linked immunosorbent assay (ELISA) plate reader.

Apoptosis assay

U87, U251, PBT015, and PBT022 cells (2 × 10⁵) were seeded in 60-mm culture dishes in culture medium. The following day, the cells were treated with indicated concentrations of sorafenib for 48 hours. After treatment, all cells including both detached and attached cells were collected and the apoptotic cells were detected by the Annexin V-FITC Apoptosis Detection kit (BD Biosciences). The cells were stained with Annexin V-FITC and propidium iodide (PI) according to the supplier’s instructions. Viable and apoptotic cells were detected by flow cytometry in the Analytical Cytometry Core at City of Hope National Medical Center. Apoptotic cells include both the early apoptotic portion (Annexin V positive) and the late apoptotic portion (Annexin V and PI positive).

Immunoblotting analysis

Twenty micrograms of total proteins were resolved in a 4% to 15% gradient Tris–HCl gel from Bio–Rad. After gel electrophoresis, the proteins were transferred to Hybond-C membranes (Amersham). The membranes were blocked for 1 hour at room temperature in 10% nonfat dry milk in 1× PBS with 0.1% Tween 20, followed by an overnight incubation at 4°C with primary antibodies in 1× PBS with 0.1% Tween 20 with 2% nonfat dry milk. Horseradish peroxidase–labeled anti-mouse or anti-rabbit secondary antibodies were incubated for 1 hour at room temperature. Immunoreactivity was detected with SuperSignal West Pico substrate (Pierce).

Electrophoretic mobility shift assay

For the detection of the DNA-binding activity of STAT3 by electrophoretic mobility shift assay, nuclear protein extracts were prepared using high-salt extraction as previously described (22). Ten micrograms of nuclear protein from control cells or cells treated with 10 μmol/L sorafenib was incubated with ³²P–radiolabeled double-stranded DNA oligonucleotides using a high-affinity variant of the
sis-inducible element (sense strand, 5′-AGC-TTC-ATT-TCC-CTG-AAA-TCC-CTA-3′) derived from the c-fos gene promoter, which binds activated Stat3 and Stat1 proteins (23). Anti-STAT3 polyclonal antibody was used to identify STAT3 in “supershift” assays. For use in supershift assays, 1 μL of the concentrated STAT3 antibodies was preincubated with nuclear protein for 20 minutes at room temperature before the addition of radiolabeled probe (30 min, 30°C) and separation by nondenaturing polyacrylamide gel-electrophoresis and autoradiographic detection.

Plasmids transfections
The constitutively activated STAT3 mutant plasmid (pSTAT3-C) was murine STAT3, which was cloned into pRc/cytomegalovirus vector with a FLAG epitope (24). pSTAT3-C was transfected into VC312 cells by Lipofectamine 2000 (Invitrogen). Stable cell line was selected by G418 and confirmed by immunoblotting analysis.

Statistical analysis
The comparisons between vehicle control (DMSO) and sorafenib-treated groups were done by using Student’s t test. P values of <0.05 are considered as significant.

Results
Sorafenib inhibits proliferation and induces apoptosis in U87 and U251 cells
To characterize the effects of sorafenib on cell proliferation in glioblastomas, we performed dose-response and time course studies in two established cell lines, U87 (Fig. 1A) and U251 (Fig. 1B). Cells were treated with increasing concentrations of sorafenib (2.5, 5, and 10 μmol/L) for 24 and 48 hours. Control cells were treated with the vehicle (DMSO) only. Because previous studies suggest that sorafenib binds to serum proteins (25), all treatments with sorafenib were done in 1% serum to reduce the effect of serum. Sorafenib markedly inhibited proliferation of both U87 and U251 cells in a dose- and time-dependent manner. We next investigated whether sorafenib could induce apoptosis in U87 and U251 cells. After treatment with increasing concentrations of sorafenib (2.5, 5, and 10 μmol/L) for 48 hours, cells were analyzed by Annexin V-FITC/PI staining and flow cytometry. Apoptotic cells shown in Fig. 1C and D included both early apoptotic cells (Annexin V positive) and late apoptotic cells (Annexin V and PI positive). Sorafenib inhibited survival of these tumor cells in a dose-dependent manner. These results show...
that proliferation and survival was greatly reduced for U87 and U251 cells exposed to 10 μmol/L of sorafenib, which is a therapeutically achievable concentration in clinical trials with doses of 400 mg of sorafenib twice daily (26).

**Sorafenib inhibits STAT3 phosphorylation at Tyr705 in U87 and U251 cells**

We investigated the levels of total and phosphorylated STAT3, AKT, and MAPK (p44/42) proteins in U87 and U251 cells after sorafenib treatment. Total protein levels of STAT3, AKT, and MAPK were not significantly changed after 4 or 24 hours of sorafenib treatment (Fig. 2A and B). By contrast, phosphorylation of STAT3 at Tyr705 was reduced at both an early time point (4 h) and a late time point (24 h) following sorafenib treatment (Fig. 2A and B). The inhibition of p-STAT3 (Tyr705) was dose and time dependent. Phosphorylation of AKT was decreased after 24-hour treatments in both U87 and U251 cells. However, phosphorylation of MAPK did not change substantially at either time point. These results indicate that the inhibition of STAT3 signaling is an early response to sorafenib treatment and a common response to sorafenib in both U87 and U251 cells.

**Expression of cyclin D, cyclin E, and Mcl-1 is reduced by sorafenib in U87 and U251 cells**

Because sorafenib strongly inhibits proliferation of U87 and U251 cells (Fig. 1), we investigated the effect of sorafenib on key cell cycle regulators, including D-type and E-type cyclins. Immunoblot analyses were done to determine the expression of cyclin D1/D2/D3 and cyclin E in U87 and U251 cells after 24 hours of sorafenib treatment. Figure 2C shows that sorafenib decreased the expression of cyclin D1/D2/D3 and cyclin E in U87 and U251 cells. These results are consistent with the inhibition of cell proliferation in these tumor cells. The Bcl-2 family of proteins has a key role in survival of normal and tumor cells (27). The expression of three antiapoptotic proteins in this family, Mcl-1, Bcl-xL, and Bcl-2, was investigated after sorafenib treatment. Mcl-1 was decreased in both U87 and U251 cells after sorafenib treatment (Fig. 2D), whereas Bcl-2 and Bcl-xL levels were not inhibited in these cells. These results are consistent with the induction.
of apoptosis by sorafenib (Fig. 1) and implicate the importance of Mcl-1 in this response.

Sorafenib inhibits primary cultures of human glioblastomas

PBT015 and PBT022 cells were isolated from glioblastoma (WHO grade IV) patients and cultured as described in Materials and Methods. To evaluate whether sorafenib has the same effect on low-passage primary cultures as on established cell lines, PBT015 and PBT022 cells were treated with sorafenib in the same manner as U87 and U251 cells. Proliferation of PBT015 (Fig. 3A) and PBT022 (Fig. 3B) cells was inhibited by sorafenib in a dose- and time-dependent manner. Sorafenib also induced the apoptosis of PBT015 (Fig. 3C) and PBT022 (Fig. 3D) cells in a dose-dependent manner after 48 hours of treatment. This inhibitory effect on the proliferation and survival of PBT015 and PBT022 cells is similar to that of U87 and U251 cells (Fig. 1).

Sorafenib inhibits signaling pathways in primary cultures of glioblastomas

We examined the STAT3, AKT, and MAPK (p44/42) signaling pathways after sorafenib treatments in PBT015 and PBT022 cells. Although total protein levels of STAT3, AKT, and MAPK were not significantly changed, phosphorylated STAT3 (Tyr705), AKT (Ser473), and MAPK (Thr202/Tyr204) were dramatically decreased by sorafenib after 4 and 24 hours of treatment (Fig. 4A and B). Although sorafenib did not affect phosphorylated MAPK in the two established cell lines examined (U87 and U251), sorafenib substantially reduced the phosphorylation of MAPK in the two low-passage primary cultures. These results indicate that there are different properties between primary cultures and established cell lines from human glioblastomas. Importantly, inhibition of STAT3 signaling is a common response in both cell lines and primary cultures.

Sorafenib also inhibited the expression of cyclin D1/D2/D3 and cyclin E in PBT015 and PBT022 cells (Fig. 4C). Antiapoptotic proteins Mcl-1 and Bcl-2 were decreased in PBT015 and PBT022 after sorafenib treatment (Fig. 4D), whereas Bcl-xL level was not changed in any of the glioblastoma cell lines tested. However, inhibition of Mcl-1 is a common response in both established cell lines and primary cultures of glioblastomas.

Sorafenib rapidly inhibits STAT3 phosphorylation at Tyr705 and DNA-binding activity

To determine whether the inhibition of STAT3 phosphorylation at Tyr705 is an early event, we treated two established cell lines (U87 and U251) and two primary
cultures (PBT015 and PBT022) of human glioblastomas with 10 μmol/L of sorafenib for 0, 1, 5, 15, and 30 minutes. Immunoblotting analysis (Fig. 5A) showed that the inhibition of phosphorylation of STAT3 at Tyr705 by sorafenib was detected at 5 to 15 minutes in these tumor cells. Phosphorylation of STAT3 on Tyr705 is important for STAT3 dimerization, translocation, and DNA binding (8). Therefore, we evaluated whether sorafenib inhibited the formation of STAT3/DNA complexes in nuclei of tumor cells from glioblastomas. Electrophoretic mobility shift assay was used to detect the effect of sorafenib on the DNA-binding activity of STAT3. Nuclear extracts were prepared from cells treated with 10 μmol/L of sorafenib for 30 minutes. Figure 5B shows that sorafenib inhibited the formation of STAT3/DNA complex in the nuclei of U87 and PBT015.

Expression of constitutively activated STAT3 mutant partially rescues the effects of sorafenib

To further confirm that inhibiting STAT3 activity is critical for the biological effects of sorafenib on glioblastoma cells, we transfected a constitutively activated STAT3 mutant (pSTAT3-C; ref. 24) into U87 and PBT022 cells. Stable cell lines were established by antibiotic selection (G418), and pRC was used as control vector. Cells containing either pSTAT3-C or control vector were treated with 10 μmol/L of sorafenib for 24 hours, and proliferation assays were done. Expression of constitutively activated STAT3 increased the resistance of U87 and PBT022 cells to sorafenib compared with untransfected cells or transfected cells with control vector (Fig. 5C).

Phosphorylation of STAT3 at Tyr705 induced by interleukin-6 is inhibited by sorafenib

The ability of interleukin-6 (IL-6) to directly activate the STAT3 through Janus-activated kinase (JAK) family kinases produces serious unintended consequences in the progression of neoplasia (28). The expression of IL-6 in glioblastoma patients shortened their survival (29). To confirm whether sorafenib also inhibits the phosphorylation of STAT3 at Tyr705 induced by IL-6, tumor cells were treated with 10 μmol/L of sorafenib for 20 minutes and then IL-6 (10 ng/mL) was added to cells for 10 minutes. Immunoblotting assays showed that sorafenib greatly inhibited the phosphorylation of STAT3 induced by IL-6 in both cell lines and primary cultures (Fig. 5D).
Effects of sorafenib on the activities of JAK1, JAK2, and Src in glioblastoma cells

The phosphorylation of STAT3 at Tyr705 is usually mediated by receptor-associated tyrosine kinases, such as the JAKs, or less frequently by nonreceptor tyrosine kinases (Src; ref. 8). Inhibiting phosphorylated STAT3 (Tyr705) by sorafenib was detected at 5 to 15 minutes of treatment (Fig. 5A) in glioblastoma cells. To elucidate how sorafenib causes the dephosphorylation of STAT3 at Tyr705, expression of total and phosphorylated proteins of JAK1, JAK2, and Src were examined after 5, 15, and 30 minutes of sorafenib treatment in both cell lines and primary cultures. Figure 6A shows the effects of sorafenib on the expression of total and phosphorylated JAK1, JAK2, and Src in two established cell lines, U87 and U251, by immunoblotting assays. Expression of phosphorylated Src and total JAK1, JAK2, and Src was not affected by sorafenib. Phosphorylated JAK1 was decreased in a time-dependent manner in both cell lines. Phosphorylated JAK2 was only reduced in U87 cells after 30 minutes of treatment. Interestingly, sorafenib inhibited the phosphorylation of JAK2 in two primary cultures, PBT015 and PBT022, in a time-dependent manner (Fig. 6B). However, phosphorylated JAK1, Src, and total proteins were not affected by sorafenib in these primary cultures. These results indicate that there are intrinsic differences between established cell lines and primary cultures of human glioblastomas.

Sodium vanadate blocks the dephosphorylation of STAT3 at Tyr705 induced by sorafenib

We also tested the possibility that the dephosphorylation of STAT3 induced by sorafenib is contributed by direct effects of protein tyrosine phosphatases (PTP). Tumor cells were pretreated with 0.5 mmol/L sodium orthovanadate, a general inhibitor for tyrosine phosphatases, for 25 minutes and then 10 μmol/L of sorafenib was added to cells for another 30 minutes. Immunoblotting assays showed that sodium vanadate blocked the effects of sorafenib on the dephosphorylation of STAT3 in both cell lines and primary cultures (Fig. 6C). To further confirm the effect of sodium vanadate, we also did the converse experiments, in which the tumor cells were first treated with 10 μmol/L of sorafenib for 5 minutes and then sodium vanadate was added for 30 minutes. Sodium vanadate showed similar inhibition on the dephosphorylation of STAT3 induced by sorafenib (data not shown), comparable with the results using sodium vanadate first (Fig. 6C). Although phosphorylated JAK1 in U87 and U251 cells or phosphorylated JAK2 in...
PBT015 and PBT022 cells was inhibited by sorafenib, sodium vanadate showed little effect on the activities of these two kinases (Fig. 6C). These results indicate a role for tyrosine phosphatases in the mechanism of STAT3 dephosphorylation induced by sorafenib.

**Sorafenib affects the activities of PTPs in glioblastoma cells**

Because sodium vanadate can reverse the dephosphorylation of STAT3 (Tyr705) induced by sorafenib (Fig. 6C), we further investigated the effects of sorafenib on the activities of several tyrosine phosphatases, which include PTPα, PTP1B, and SHP2. Immunoblotting assays with total cell lysates showed that sorafenib increased phosphorylation of PTPα and SHP2 in U87 and PBT015 cells (Fig. 6D). The phosphorylated PTP1B was not affected by sorafenib treatment in comparison with DMSO control (zero concentration). TC-PTP activity was determined by a human TC-PTP activity assay kit (R&D Systems), and its activity was slightly increased by sorafenib treatment in U87 and PBT015 cells (data not shown). These results suggest that PTPs may contribute to dephosphorylation of STAT3 induced by sorafenib.

**Discussion**

In glioblastomas, STAT3 acts as a molecular “hub” to link extracellular signals to transcriptional control of proliferation, cell cycle progression, and immune evasion (30). Because STAT3 plays such a central role in glioblastoma signal transduction, inhibiting STAT3 signaling provides a new opportunity for glioblastoma treatment. Here, we present evidence that sorafenib inhibits proliferation and induces the apoptosis of two established cell lines (U87 and U251) as well as two primary cultures (PBT015 and PBT022) derived from human glioblastomas.
Sorafenib Inhibits Glioblastoma

glioblastomas. Inhibition of cell growth and survival in these glioblastoma tumor cells is associated with the inhibition of the STAT3 signaling pathway. Although there are different growth properties among these tumor cells, inhibition of phosphorylated STAT3 at Tyr705 is common to all cells. The formation of STAT3/DNA complexes in nuclei is consequently decreased by sorafenib in these tumor cells. Furthermore, sorafenib inhibits STAT3 signaling within minutes, and this inhibition persists for at least 24 hours following treatment. Importantly, overexpression of a constitutively activated STAT3 mutant partially blocked the effects of sorafenib.

Phosphotyrosine (pTyr705) in STAT3 mediates dimer formation, which is required for the binding of STAT3 to DNA (8). Our results show that sorafenib inhibited the tyrosine phosphorylation of STAT3 in both established cell lines and primary cultures of glioblastomas. Tyrosine residue in STAT3 is phosphorylated by JAK or Src family kinases in response to cytokines or growth factors. Sorafenib inhibited phosphorylated JAK1 in U87 and U251 cells, whereas it inhibited phosphorylated JAK2 in primary cultures. Sodium vanadate, a general inhibitor of PTPs, reverses the dephosphorylation of STAT3 at Tyr705 induced by sorafenib in both cell lines and primary cultures of glioblastomas. These results indicate that both JAKs and PTPs are involved in the mechanisms of action of sorafenib on dephosphorylation of STAT3.

STAT3 regulates basic biological processes important in tumorigenesis including cell cycle progression, apoptosis, tumor angiogenesis, and tumor-cell evasion of the immune system (8, 9). Key genes in cell cycle control, such as cyclin D1, are regulated by STAT3 (8). Thus, inhibition of STAT3 signaling by sorafenib is likely to contribute to inhibition of cell proliferation. Our data indicate that D-type and E-type cyclins, which are involved in cell cycle control, are common downstream targets for sorafenib in glioblastomas. The expression of cyclin E and three types (D1, D2, and D3) of cyclin D is decreased by sorafenib in a dose-dependent manner. These results are consistent with the inhibition of proliferation by sorafenib in tumor cells from glioblastomas.

Expression of Mcl-1, an antiapoptotic protein, is also regulated by STAT3 signaling (8). Sorafenib has been reported to downregulate the expression of Mcl-1 in several kinds of tumor cells (17, 31). Here, we show that sorafenib inhibited Mcl-1 expression in both established cell lines and primary cultures from human glioblastomas. The STAT3 and Mcl-1 proteins are the only ones inhibited in common among the established cell lines and primary cultures of glioblastomas. Therefore, down-regulation of Mcl-1 through the inhibition of phosphorylated STAT3 may be an important mechanism of action of sorafenib in glioblastomas.

Although sorafenib was originally developed for the inhibition of Raf-MEK-MAPK signaling (14), sorafenib only inhibits the phosphorylation of MAPK/42/44 in two primary cultures, but not in established cell lines (U87 and U251). Thus, the antitumor activity of sorafenib is dissociated from the inhibition of MAPK in established cell lines of glioblastoma. Because sorafenib inhibits vascular endothelial growth factor receptors (14), which are highly expressed in glioblastomas (32), it is possible that sorafenib could inhibit angiogenesis in glioblastomas.

Treatment of glioblastoma is complicated by the blood-brain barrier, which is a physiologic obstacle for delivery of drugs to the central nervous system. Various approaches have been developed for the local delivery of drugs to brain tumors, including convection-enhanced delivery (33). Therefore, the local delivery of sorafenib to the malignant cells in the brain may result in more effective antitumor activity with reduced systemic toxicity. Sorafenib shows good tolerability and promising antitumor activity from clinical trials in several types of solid tumors (19-21). Thus, sorafenib is potentially a promising drug for the treatment of malignant gliomas.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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