Intracranial Inhibition of Platelet-derived Growth Factor-mediated Glioblastoma Cell Growth by an Orally Active Kinase Inhibitor of the 2-Phenylaminopyrimidine Class[1]

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ABSTRACT

Glioblastoma multiforme is the most common primary human brain tumor, and it is, for all practical purposes, incurable in adult patients. The high mortality rates reflect the fact that glioblastomas are resistant to adjuvant therapies (radiation and chemicals), the mode of action of which is cytotoxic. We show here that an p.o.-active small molecule kinase inhibitor of the 2-phenylaminopyrimidine class may have therapeutic potential for glioblastomas. STI571 inhibits the growth of U343 and U87 human glioblastoma cells that have been injected into the brains of nude mice, but it does not inhibit intracranial growth of ras-transformed cells. Studies on a broad panel of genetically validated human and animal cell lines show that STI571 acts by disruption of the ligand:receptor autocrine loops for platelet-derived growth factor that are a pervasive feature of malignant astrocytoma. The cellular response of glioblastoma cells to STI571 does not appear to involve an apoptotic mechanism.

INTRODUCTION

Primary tumors of the central nervous system account for less than 1.5% of all of the cancer cases reported in the United States each year. However, the majority of these cancers are either glioblastoma multiforme or anaplastic astrocytoma and thus, for all practical purposes, incurable. These infrequent tumors are the third leading cause of cancer-related death among men 15–54 years of age and the fourth leading cause of death for women 15–34 years of age. Moreover, primary brain tumors are the most common solid tumor of childhood and the second leading cause of cancer death in children after leukemia (1, 2).

Most malignant tumors are curable only when surgery or radiotherapy are used together with adjuvant chemotherapy. Unfortunately, there has been little progress in brain tumor chemotherapy during the past 25 years. At most, 15–20% of all brain tumor patients currently benefit from chemotherapy, and the gains are measured in added months rather than added years of life. Historically, chemotherapy for gliomas has used cytotoxic drugs, the mode of action of which is cell cycle independent (3, 4). However, fundamental insights into signal transduction and cell cycle control generated during the past decade are being translated into a new generation of signal transduction/cell cycle inhibitors. These drugs can be very selective in action and may provide opportunities to attack brain cancers on a qualitatively new front.

One of these new drugs is STI571, a small molecule kinase inhibitor of the 2-phenylaminopyrimidine class (Fig. 1A). This p.o.-active pharmaceutical targets the activated Abl oncoprotein and certain members of the subgroup III receptor tyrosine kinase family, including the receptors for PDGF.[7] Other non-receptor tyrosine kinases, serine/threonine kinases, and growth factor receptors (EGF, insulin-like growth factor, insulin, and fibroblast growth factor) are at least two orders of magnitude more resistant to the action of STI571 than the Abl oncoprotein and the PDGF receptors (5). Preclinical studies with STI571 on the growth of BCR-abl-positive cells (5) culminated in clinical trials on chronic myelogenous leukemia that are yielding encouraging results (6). A broad body of literature suggests that gliomas might constitute another target for STI STI571.

The PDGF A and B ligand genes are expressed in virtually all glioma cell lines and in fresh surgical isolates of human malignant astrocytoma (7–9). To complement ligand expression, PDGF receptor genes are also expressed in malignant astrocytoma. Expression of the PDGF β receptor mRNA is detected within tumor cells (10) and endothelial cells of hyperplastic capillaries within the tumors (8). The PDGF receptor α subunit is overexpressed in virtually all cultured glioma cell lines and in fresh surgical isolates of human malignant astrocytoma (7–9). Overexpression of the α receptor gene can be detected even within low-grade astrocytoma (9). Thus, overexpression of the α receptor appears as an early event in the "progressive" pathway to astrocytoma. Because the α receptor subunit is the universal PDGF receptor, sensitive to all three isoforms of PDGF (11, 12), it follows that activation of PDGF α receptors through an autocrine loop may be a pervasive feature of malignant astrocytoma.

PDGF autocrine loops could, in theory, be completely incidental to the disease. Alternatively, the closure of PDGF autocrine loops could be an early initiating event that becomes irrelevant to the malignant phenotype as the tumor progresses to acquire additional genetic lesions. However, a broadening base of data indicates that PDGF receptor autocrine loops initiate the transformation process and then continue to contribute to the transformed phenotype of malignant astrocytoma cells. Evidence for an initiating role in glioblastoma comes from studies showing that a retrovirus expression vector encoding PDGF B-chain, the universal PDGF ligand, can induce monoclonal or oligoclonal astrocytoma after injection into the brain of newborn mice (13). Evidence for a maintenance role in the transformed phenotype is derived from studies showing that disruption of ligand:receptor complex can inhibit PDGF receptor autophosphoryl-

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ation and revert the transformed phenotype of glioblastoma cell lines that have been in culture for many years. Growth inhibition and reversion of the transformed phenotype have been achieved with suramin, neutralizing antibodies to PDGF, and with dominant-negative mutations of either PDGF ligand or PDGF receptor (14–18).

Collectively, these data suggest that PDGF receptors might be a therapeutic target for glioblastoma multiforme. However, the agents that have thus been shown to function as antagonists of PDGF receptor activation are unlikely to be of practical value in the treatment of patients with brain cancer. Even in cell culture studies, some glioblastoma cell lines are refractory to PDGF antagonists that act at the outer cell surface (*i.e.,* suramin and anti-PDGF antibodies), presumably because significant levels of functional PDGF:receptor complex can be formed within the cell cytoplasm (15, 19). In clinical applications, this cellular compartmentation problem would be exacerbated by the blood-brain barrier. Within current limitations of gene transfer technology, prospects for therapy with dominant-negative mutations of PDGF ligand or PDGF receptor are likewise bleak. In studies presented here, we show that STI571 selectively disrupts PDGF receptor autocrine loops and has therapeutic potential for malignant astrocytoma.

MATERIALS AND METHODS

Cell Culture. A panel of BALB/c 3T3 cells transformed with c-sis, v-sis, PDGF-A, Ha-ras, v-src, or SV40 was described previously (17). This panel includes also Ha-ras-transformed human bladder carcinoma cells (EJ), human cervical carcinoma cells (HeLa), human colon carcinoma cells (SW480), and two independent human astrocytoma cell lines that express PDGF ligand: receptor autocrine loops (17). A functional role for PDGF autocrine loops in maintaining the transformed phenotype of the two human astrocytoma cell lines (U87 and U343) as well as the cells transformed by c-sis, v-sis, and PDGF-A was established by genetic means. The transformed phenotype of these cells was reverted by dominant-negative mutations of a PDGF ligand, whereas the cells transformed by downstream oncoproteins or by SV40 were unaffected (17). All cell lines were maintained in DMEM (Life Technologies, Inc.) supplemented with 10% BCS, 2 mM glutamine, 10,000 units/ml penicillin/streptomycin (Life Technologies, Inc., Gaithersburg, MD) in a 10% CO_2 incubator at 37°C. Cells are subcultured every 2–4 days by treatment with 0.25% trypsin.

PDGF Receptor Tyrosine Kinase Inhibitor. STI571 was synthesized by Novartis Pharmaceuticals and has been described previously (5, 20). For *in vitro* assays and for the initial studies on tumor growth in nude mice, STI571 was solubilized and delivered in DMSO. For later studies on intracranial tumor growth, STI571 was diluted in water and administered by oral gavage as indicated.

Clonogenic Growth Assay. Approximately 10^3 cells were plated into 60-mm dishes (Falcon) using DMEM/10% BCS. After 24 h, the serum-supplemented medium was removed, and cells were shifted to PDGF-free culture conditions by using 5% PPP as a supplement to DMEM in place of serum. STI571 was added to the PPP-supplemented medium as indicated, and thereafter, medium was renewed every 2 days. At day 11, colonies were fixed and stained with methylene blue.

Cell Proliferation Assay. Cells were plated into 60-mm culture dishes and processed as for the clonogenic assays above. At day 11, cells were harvested by trypsin digestion and counted using either a hemocytometer or a Coulter counter.

Flow Cytometry. Approximately 10^5 cells were plated into DMEM with 10% BCS. After 24 h (day 1), the medium was changed to DMEM/5% PPP with or without STI571. Cell cycle distribution was analyzed by propidium iodide staining and flow cytometry, using an EPICS752 (Coulter Corp) with the 488-nm laser.

s.c. Tumor Growth. For all *in vivo* experiments, male NCr Nude mice (Taconic, Germantown, NY), 4–6 weeks of age, were used. All animal studies were performed under the approval of Dana-Farber Cancer Institute Animal Research Committee in accordance with federal, local, and institutional guidelines (Protocol number: 98-041). Pooled colonies of ras-transformed BALB/c

3T3, v-sis-transformed BALB/c 3T3, U87, and U343 were trypsinized and resuspended in DMEM and counted. By using a tuberculin syringe and a 27-gauge hypodermic needle, 10^7 cells (0.1 ml) were injected into the s.c. tissue of the right flank region. Five days after cell implantation (day 1), STI571 was started at a dose of 50 mg/kg/day, delivered by i.p. injections in two daily doses. Experimental sets for U343 and U87 consisted of 10 mice for the control and 10 mice for STI571-treated groups. For ras-transformed BALB/c 3T3 and v-sis-transformed BALB/c 3T3, five mice for each experimental set were studied. Tumor growth was followed by measuring perpendicular tumor diameters. Tumor volumes were calculated using the formula $\pi \times L \times D^2/6$, where L is the longest diameter and D is the diameter at right angles to it. According to the animal research protocol, mice having tumors in excess of 2 cm were sacrificed; otherwise, tumor measurements were continued until day 30.

Intracranial Tumor Growth. For intracranial implantation, 10^6 cells in a volume of 10 μl of PBS were injected into the right frontal hemisphere, using a stereotactic fixation device (Stoelting, Wood Dale, IL). After a recovery period of 5 days, animals began receiving either STI571 or vehicle through an orogastric tube daily. Animals were examined daily by a veterinarian who was blind to the study and sacrificed as directed when symptoms of intracranial tumor growth became evident.

TUNEL Staining and Immunoblotting. TUNEL stains on tumors removed from mice treated or not with STI571 for 1 week were performed after fixation with 4% paraformaldehyde in PBS and cryostat sectioning using the Apoptosis Detection System, Fluorescein (Promega Corp., Madison, WI) according to the manufacturer's instructions. For the generation of protein extracts, tumors were allowed to grow to approximately 0.5×0.5 cm in size then the animal was treated (+) or not (−) with 50 mg/kg STI571 via i.p. injection. Tumors for immunoblots were harvested 2 h after treatment, and extracts were generated immediately. PDGF receptor was immune-precipitated from 1 mg of protein, resolved on a 7.5% polyacrylamide gel, and transferred to Immobilon, and the resulting blot was probed as indicated and as described previously (17)

Statistical Analysis. Statistical analysis for the s.c. tumor volumes was carried out by Student's *t* test. For the survival rates of the intracranial tumor implanted mice, Kaplan-Meier analysis using the STATVIEW software package was used. $P < 0.05$ was considered to be significant.

RESULTS

STI571 Selectively Inhibits the Growth of Cells That Express PDGF Receptor Autocrine Loops. BALB/c 3T3 cells express both α and β PDGF receptor subunits, and they require an exogenous source of PDGF for optimum growth in culture. In previous studies on the action of PDGF ligand dominant-negative mutations, we transformed wild-type BALB/c-3T3 cells through stable expression of PDGF ligand cDNAs (17). As controls, we also transformed these 3T3 cells with oncoproteins that function downstream of receptor tyrosine kinases, notably H-ras, v-src, and SV 40. As an initial evaluation of STI571, we conducted a dose-response assay on this panel of well-characterized BALB/c 3T3 cell lines.

The chemical structure of STI571 is shown in Fig. 1*A*. As shown in Fig. 1*B*, the mitogenic response of wild-type 3T3 cells to ectopic PDGF is inhibited by STI571 at concentrations as low as 3 μM. The 3T3 cells transformed by PDGF A or PDGF B proliferate in the absence of ectopic PDGF, because expression of the ligand cDNAs closes a PDGF autocrine loop. However, proliferation of PDGF A/B 3T3 cells under these conditions is again inhibited by low (3 μM) concentrations of STI571. The H-ras, v-src, and SV40 3T3 cells grow well in PDGF-free medium (DMEM + 5% PPP). However, STI571 does not inhibit the growth of these cells until much higher concentrations of the drug are added to the culture medium. Thus, the biological "action spectrum" of STI571 on these well-characterized 3T3 cells duplicates the action spectrum of the PDGF dominant-negative mutations noted in our previous studies (17).

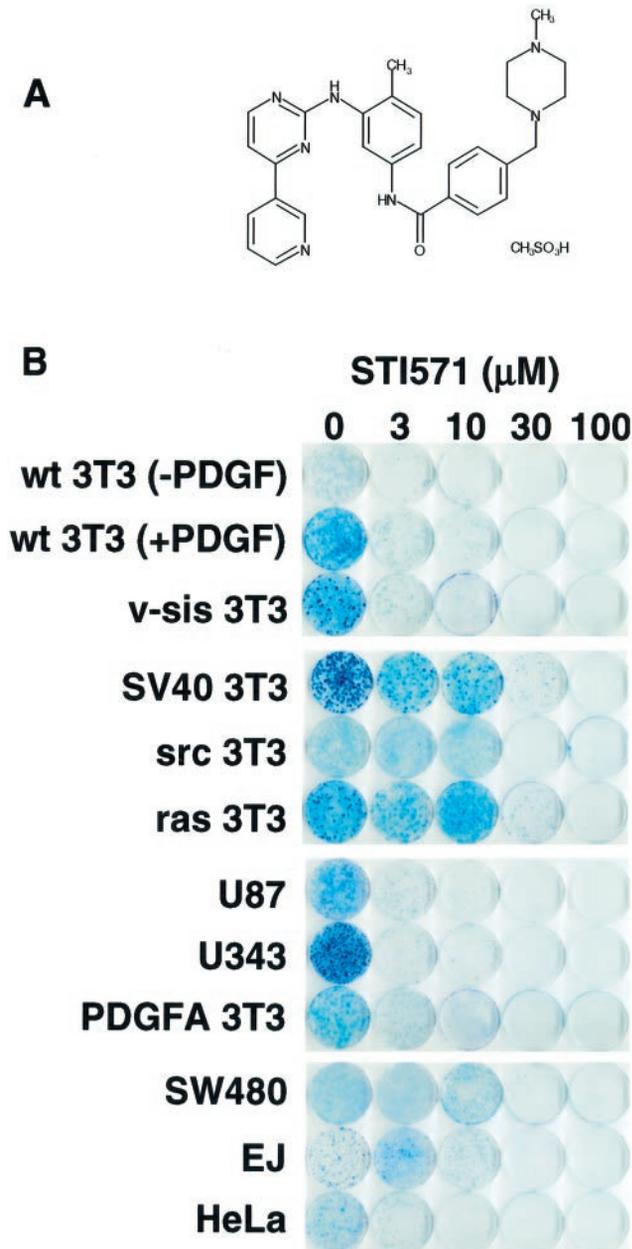In our previous studies, we also examined the action of dominant-

Fig. 1. Dose-response analysis of STI571 by clonogenic growth assay. *A,* the chemical structure of STI571. *B,* cells were plated into 60-mm diameter tissue culture dishes as described in "Materials and Methods." After 24 h, the cell culture medium was changed to DMEM-5% PPP supplemented with STI571 as indicated. Culture medium was changed on alternate days. On day 11, the cultures were fixed and stained with methylene blue. The plates shown here are typical of experiments performed in triplicate.

negative PDGF mutations on a panel of human tumor cell lines (17). Accordingly, we tested the action of STI571 on this same panel of human cell lines. For a frame of reference, we included cultures of BALB/c 3T3 cells transformed by the *PDGF A* gene. As indicated (Fig. 1*B*), all of these human cell lines grow well in PDGF-free medium. Two different glioblastoma cell lines (U343 and U87) are growth inhibited by STI571 at low (3 μM) concentrations. Under identical conditions, a human bladder carcinoma (EJ) and a human colon carcinoma (SW 480), both of which express an activated *ras* gene, are relatively resistant to STI571. HeLa cells are sensitive to STI571, a characteristic that would not be predicted from conventional wisdom regarding the tissue distribution of PDGF receptors and the cellular origins of HeLa. However, as noted in our previous

studies, the HeLa cell stocks in our laboratory are growth inhibited by dominant-negative mutations of PDGF ligand formation (17). Thus, human cells that are sensitive to STI571 are identical to the human cells that respond to genetic disruption of PDGF autocrine loops. Conversely, the human cells that are resistant to STI571 are likewise unaffected by genetic agents that disrupt PDGF autocrine loops (17).

A "Therapeutic Window" for STI571. The clonogenic dose-response assays provide a rapid qualitative assessment of sensitivity to STI571. A more quantitative assessment of drug sensitivity was conducted using lower concentrations of the drug and monitoring actual cell number. As shown (Fig. 2), cell lines that express PDGF autocrine loops are inhibited by concentrations of STI571 as low as 1.5 μM. By contrast, cells transformed by downstream oncoproteins were insensitive to STI571 at concentrations as great as 10 μM.

STI571 Selectively Inhibits Tumor Formation from Cells That Express PDGF Receptor Autocrine Loops. A subset of the transformed cell lines that were screened *in vitro* for sensitivity to STI571 was inoculated s.c. into NCr nude mice. Five days after cell implantation (day 1), treatment with STI571 was initiated at a dose of 50 mg/kg/day, delivered by i.p. injection in two daily doses of 25 mg/kg each. As shown (Fig. 3), this drug regimen inhibited the formation of
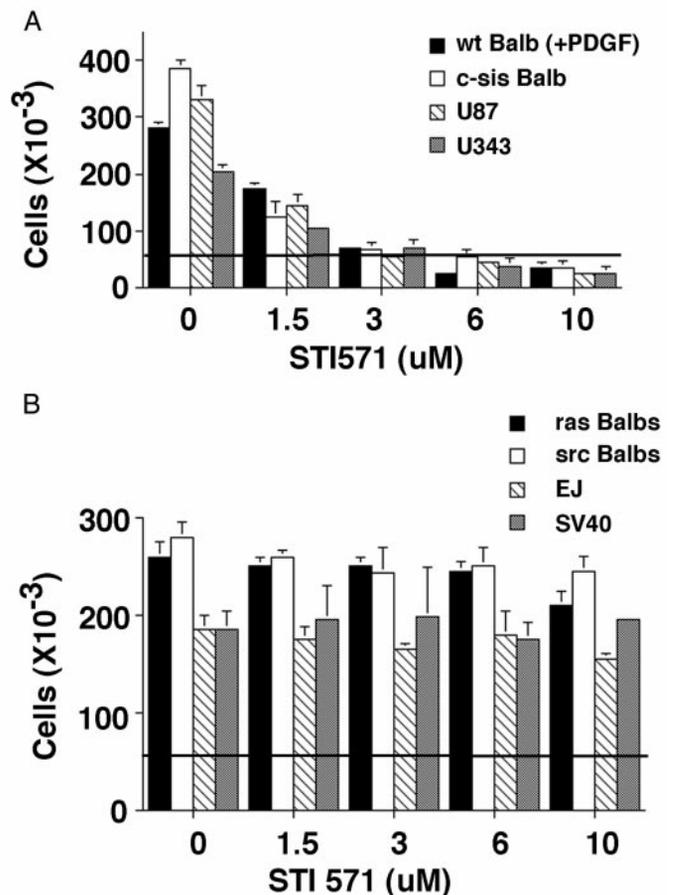

Fig. 2. A "therapeutic window" for STI571 in culture. A group of well-characterized BALB/c 3T3 cell lines and human carcinoma cell lines was plated into 60-mm tissue culture dishes at 50,000 cells/plate. After 24 h, the cell culture medium was changed to DMEM-5% PPP supplemented with STI571 as indicated. On day 11, cells were harvested by trypsin digestion and counted. *A,* cell lines that responded to treatment with STI571: BALB/c 3T3 cells (■), c-sis-transformed BALB/c 3T3 cells (□), U87 glioblastoma cells (▨), and U343 glioblastoma cells (▦). *B,* cells that failed to respond to STTI571 treatment:ras-transformed BALB/c 3T3 cells (■), src-transformed BALB/c 3T3 cells (□), EJ bladder carcinoma cells (▨), and SV40-transformed BALB/c 3T3 cells (▦). The *horizontal line* in both reflects the starting (plated) population. Results were reproduced in at least three independent experiments. Results from a typical experiment performed in triplicate are shown; *bars,* SE.
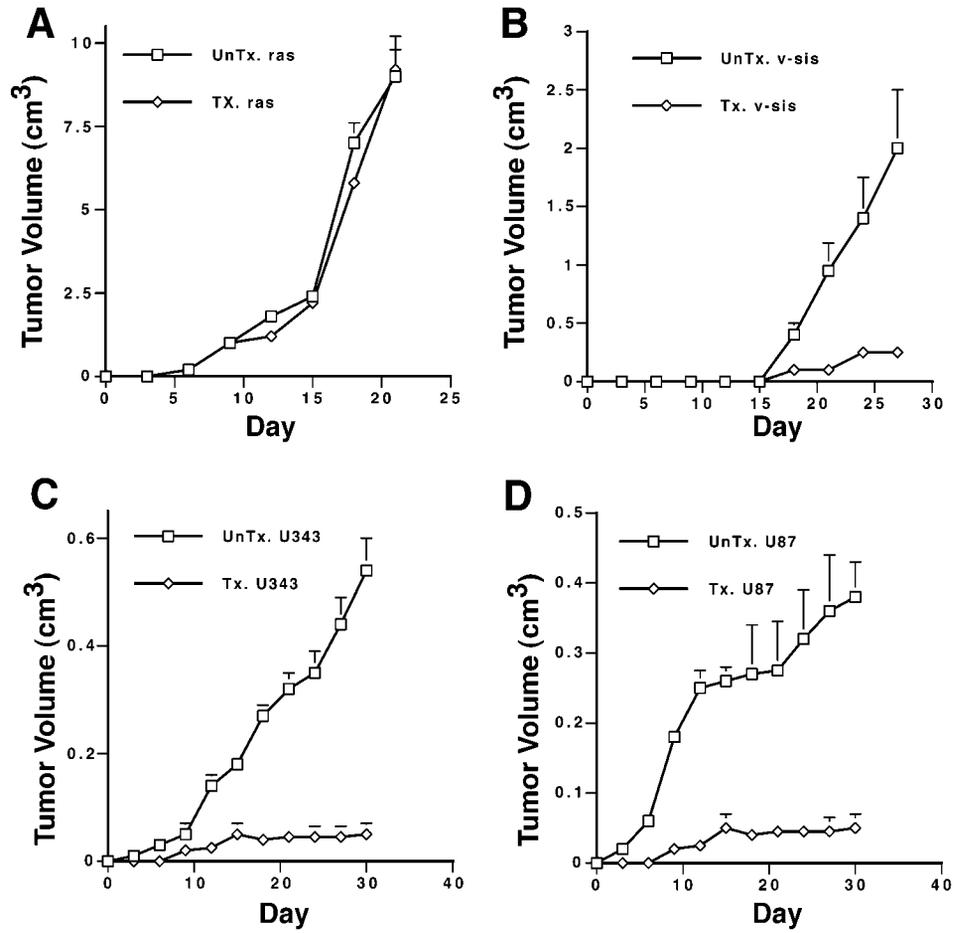
Fig. 3. Inhibition of tumor growth in NCr nude mice by STI571. Two independent lines of transformed BALB/c 3T3 cells [ras (*A*) or v-sis (*B*)] and two independent glioblastoma cell lines [U343 (*C*) or U87 (*D*)] were inoculated into male NCr nude mice (16 each) as described in "Materials and Methods." At 5 days after inoculation, the mice began receiving STI571 (□, 50 mg/kg/day) or vehicle (◇) by twice daily i.p. injections of 25 mg/kg each. Results presented here are representative of three independent experiments.

tumors from PDGF B 3T3 cells and from the two human glioblastoma cell lines U343 and U87. However, STI571 did not inhibit the formation of tumors from ras-transformed 3T3 cells. Thus, the action spectrum of the drug on tumor formation in animals is similar to the action spectrum for growth inhibition *in vitro* in PDGF-free medium. To an outward appearance, mice receiving STI571 appeared healthy throughout the course of the experiment.

Administration of STI571 Inhibits the Autocrine Stimulation of the PDGF Receptor in Glioblastomas. To determine whether we could interrupt the autocrine activation of the PDGF receptor in tumors with the STI571 compound, we injected U87 glioblastoma cells s.c. and allowed tumor formation to approximately 0.5×0.5 cm. The mice indicated were treated once with a single i.p. injection of 50 mg/kg STI571. Tumors were harvested after 2 h, and protein extracts were generated immediately. The PDGF receptors were immuneprecipitated, size fractionated on SDS polyacrylamide gels, and immunoblotted with anti-phosphotyrosine antibody (4G10). Alternatively, the samples were immunoblotted with an antibody targeted to activated (phosphorylated) p85 recognition motifs within PDGF receptors (anti-pY751; Ref. 21). The immunoblots thus generated are of suboptimal appearance because of the significant levels of normal mouse stroma within the tumor samples. Nevertheless, the immunoblots reveal the ability of STI571 to abolish the endogenous tyrosine phosphorylation of the PDGF receptor in these tumors (Fig. 4). STI571, therefore, is able to interrupt the autocrine loop responsible for ectopic stimulation of the PDGF receptor in human glioblastoma.

Oral Administration of STI571 Selectively Inhibits Intracranial Tumor Formation from Cells That Express PDGF Receptor Autocrine Loops. The blood-brain barrier is not fully intact within the microenvironment of a malignant astrocytoma. Nevertheless, any

drug intended for treatment of astrocytoma must contend successfully with anatomical problems of drug delivery unique to primary cancers of the brain. In addition, STI571 is administered by oral delivery in
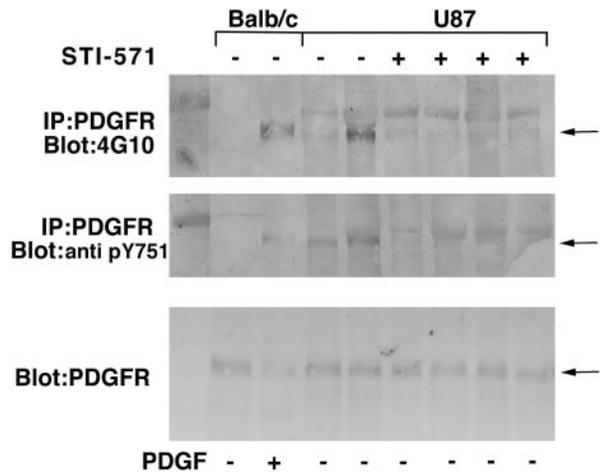

Fig. 4. Inhibition of autocrine stimulation of the PDGF receptor by STI571 *in vivo*. U87 cells were injected into male NCr nude mice as described in "Materials and Methods." Independent tumors were allowed to grow to approximately 0.5×0.5 cm in size, and then the animal was treated (+) or not (−) with 50 mg/kg STI571 via i.p. injection. Tumors were harvested 2 h after treatment, and protein extracts were generated immediately. PDGF receptor was immune-precipitated (*IP*) from 1 mg of total protein extract. *Arrow* to the *right*, position of the PDGF receptor. As controls, Balb/s 3T3 cells in culture were treated as indicated with 30 ng/ml PDGF BB and harvested after 10 min. Protein extracts were generated and subjected to the same immuneprecipitation as above, but 100 μg of total protein were used.
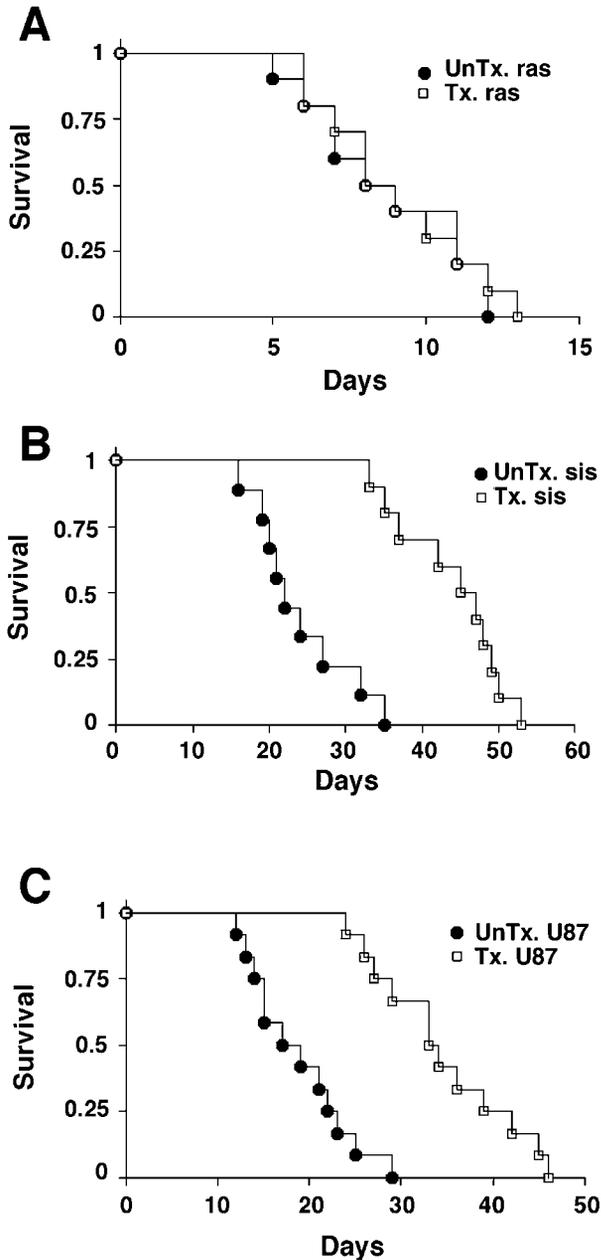
Fig. 5. Oral administration of STI571 inhibits intracranial growth of cells that express PDGF receptor autocrine loops. A stereotactic device was used for intracranial inoculation of cells into male NCr nude mice as described in "Materials and Methods." At 3 days after intracranial injection, the mice began receiving either STI571 (50 mg/kg/day in DMSO in twice-daily doses of 25 mg/kg, A–C, ●) or vehicle (□) by oral gavage as described in "Materials and Methods." Kaplan-Meier cumulative survival plots are shown for ras-transformed 3T3 cells (A, 10 control and 10 drug-treated animals), v-sis-transformed 3T3 cells (B, 10 control and 11 drug-treated animals), and U343 cells (C, 12 control and 12 drug-treated animals). D and E are similar experiments using U8343 (15 treated and 15 control) and U87 (20 treated and 20 control) glioblastoma cell lines, respectively; however, STI571 was administered at 50 mg/kg/day in water in a single daily dose for the duration of the experiment.
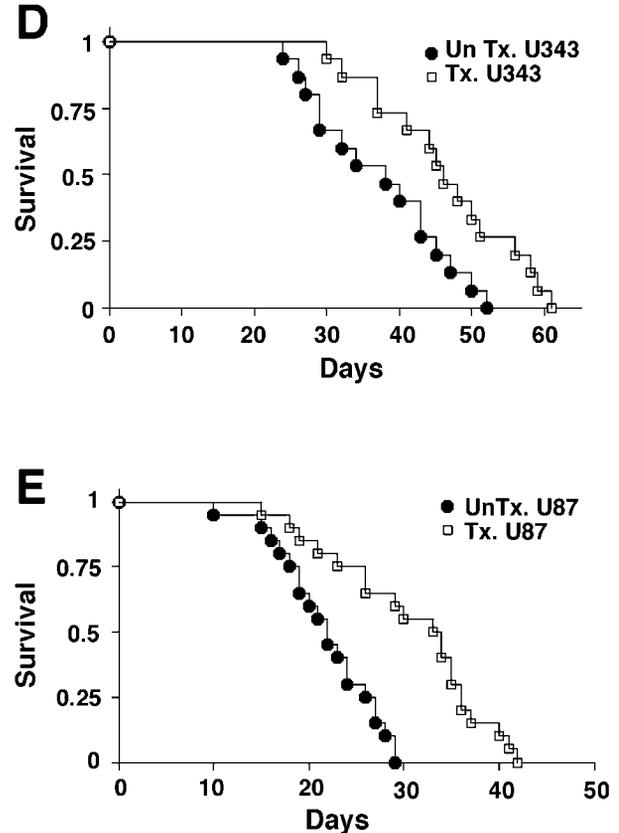
human patients (6). For a more practical assessment of the potential of STI571 as a therapeutic for human brain cancers, we inoculated transformed cells into the cranium of nude mice and delivered the drug through oral gavage. The mice were observed at daily intervals and sacrificed (as directed by a veterinarian blind to the study) when symptoms of tumor growth (seizures or paralysis) became severe. As shown in Fig. 5, oral administration of STI571 prolongs the survival of mice that received an intracranial inoculation of PDGF B (sis) 3T3 cells (Fig. 5B), U87 glioblastoma cells (Fig. 5, C and E), or U343 glioblastoma cells (Fig. 5D). As a negative control, we injected ras-transformed BALB/c 3T3 cells. STI571 could not prolong the survival of mice that had received an intracranial injection of these cells (Fig. 5A). These data suggest that p.o.-administered STI571 can access the cranium at levels sufficient to be of therapeutic value in malignant astrocytoma. Moreover, the target selectivity demonstrated in Figs. 1–3 is retained in the intracranial environment.

STI571 Promotes Growth Arrest Rather Than Apoptosis. To define the cellular basis of STI571-induced growth inhibition, we

conducted a FACS analysis of cells that had been treated with the drug while growing in culture. As positive and negative controls, we used wild-type 3T3 cells (Fig. 6, A and B) grown in the presence or absence of PDGF and ras-transformed BALB/c 3T3 cells (Fig. 6D), respectively. As shown (Fig. 6B), when STI571 is added to wild-type 3T3 cells (growing in the presence of PDGF) they become growth arrested in the G_0-G_1 phase of the cell cycle. The drug-induced growth arrest of wild-type 3T3 cells is identical to that seen when these cells are deprived of PDGF (Fig. 6A). The ras-transformed 3T3 cells grow in the absence of PDGF as noted previously (Figs. 1 and 2) and show no change in their cell cycle distribution profile in the presence of STI571 (Fig. 6D). STI571 treated c-sis-transformed BALB/c 3T3 cells growth arrested in a similar manner as the wild-type cells (Fig. 6C), indicating that STI571 is able to interrupt the PDGF-mediated autocrine loop that allows these cells to grow in the absence of the growth factor.

Against this backdrop, both of the glioblastoma cell lines display a classic "3T3-like" growth arrest in the G_0-G_1 phase of the cell cycle
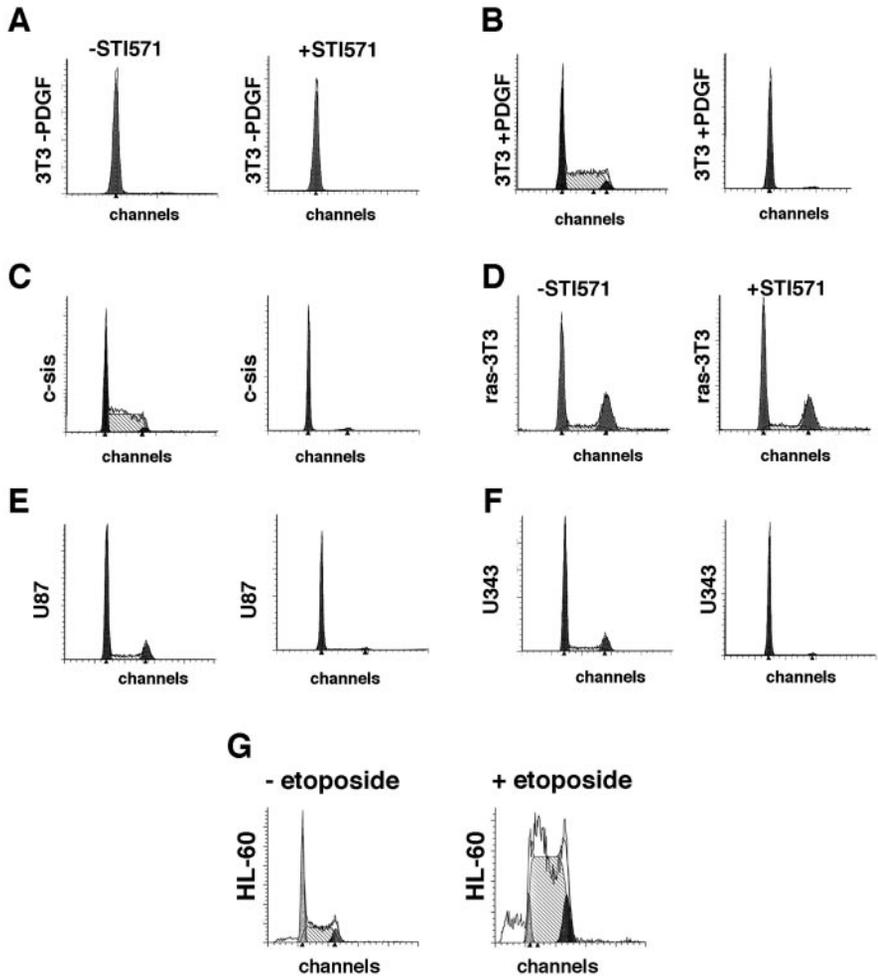
Fig. 6. Inhibition of cell growth by STI571 reflects primarily cell cycle arrest rather than apoptosis. Cells were plated into 60-mm culture dishes as described in "Materials and Methods." After 24 h, the plating medium was changed to DMEM-5% PPP supplemented with STI571 (10 μM) as indicated. All cells were then cultured for 3 days in the presence or absence of STI571, as indicated at the top of the figure and processed for FACS analysis as described in "Materials and Methods." As positive controls, we used wild-type 3T3 cells cultured in the absence (A) or presence (B) of recombinant PDGF (30 ng/ml) as indicated as well as c-sis-transformed BALB/c 3T3 cells (C) grown in the absence of PDGF. As negative controls, ras-transformed BALB/c 3T3 cells, which do not respond to STI571, were used (D). Both U87 (E) and U343 (F) cell lines exhibited similar FACS patterns in response to treatment with STI571. For a positive control of apoptotic FACS signature, we used HL-60 promyelocytic leukemia cells treated or not with etoposide (G). These results are typical of three independent experiments.

when exposed to STI571 (Fig. 6, E and F). Close scrutiny of the glioblastoma FACS profiles reveals no sign of the sub-G_1 cellular debris associated with apoptosis. As a positive control, we used a well-characterized model for drug-induced apoptosis. As shown in Fig. 6G, FACS analysis of HL-60 cells that had been treated with etoposide shows ample evidence of the cellular debris associated with apoptosis. The etoposide-treated cells are also reactive in TUNEL stains (data not shown). Thus, our assays would have detected STI571-induced apoptosis in these studies had it occurred. TUNEL staining of small U87-derived glioblastoma tumor samples excised from STI571-treated nude mice revealed no sign of STI571-induced apoptosis in vivo (Fig. 7). We conclude that the main mechanism of STI571-induced growth inhibition in vitro and in vivo is that of cell cycle arrest, rather than apoptosis.

DISCUSSION

Glioblastoma Multiforme Is the Most Common Primary Human Brain Tumor. Analysis of molecular markers suggests that there are at least two, and possibly more, independent pathways to glioblastoma (22). One pathway involves p53 loss-of-function mutations, in the absence of the EGF receptor amplification. This pathway gives rise to pediatric brain stem gliomas and to the giant cell glioblastomas seen in adults (22, 23). A second pathway usually occurs in older patients with de novo glioblastoma and is characterized by EGF receptor and MDM2 gene amplification without p53 mutations (24). In either route to glioblastoma, overexpression and activation of PDGF receptors may play a prominent role. PDGF receptor autocrine loops are a pervasive feature of glioblastomas (7–10, 13). Experiments in cell culture and mouse model systems suggest that PDGF autocrine loops both initiate (13) and sustain (14, 15, 17–19) the transformed phenotype of glioblastoma cells.

STI571 was developed as an inhibitor of the c-Abl tyrosine kinase. Other non-receptor tyrosine kinases and serine/threonine protein kinases are resistant to STI571. Receptor tyrosine kinases in the EGF, insulin-like growth factor, and fibroblast growth factor families are likewise insensitive to STI571. However, PDGF receptor family tyrosine kinases are as sensitive to STI571 as the original c-Abl target (5, 20). Using a well-characterized panel of murine and human cell lines, we show that STI571 selectively inhibits the growth of cells that are driven by activated PDGF receptors. This selective growth inhibition is observed in both tissue culture and nude mice. Moreover, the drug is p.o. active against intracranial implants of human glioblastoma in nude mice.

The main mechanism of STI571-induced growth inhibition in vitro and in vivo is that of cell cycle arrest, rather than apoptosis. It should be noted, however, that the population of sis 3T3, U343, and U87 cells does decline during an 11-day exposure to STI571 (Fig. 2A). A considerable amount of floating cell debris can be seen in these long-term drug-treated cultures. Thus, over an extended time, disruption of PDGF autocrine loops leads to some degree of cell death, perhaps through nonapoptotic mechanisms or through low levels of apoptosis. In addition, it should be noted that these studies involve immortalized cell lines that have grown in culture for many years. It is possible that primary tumors with PDGF autocrine loops might respond differently to STI571.
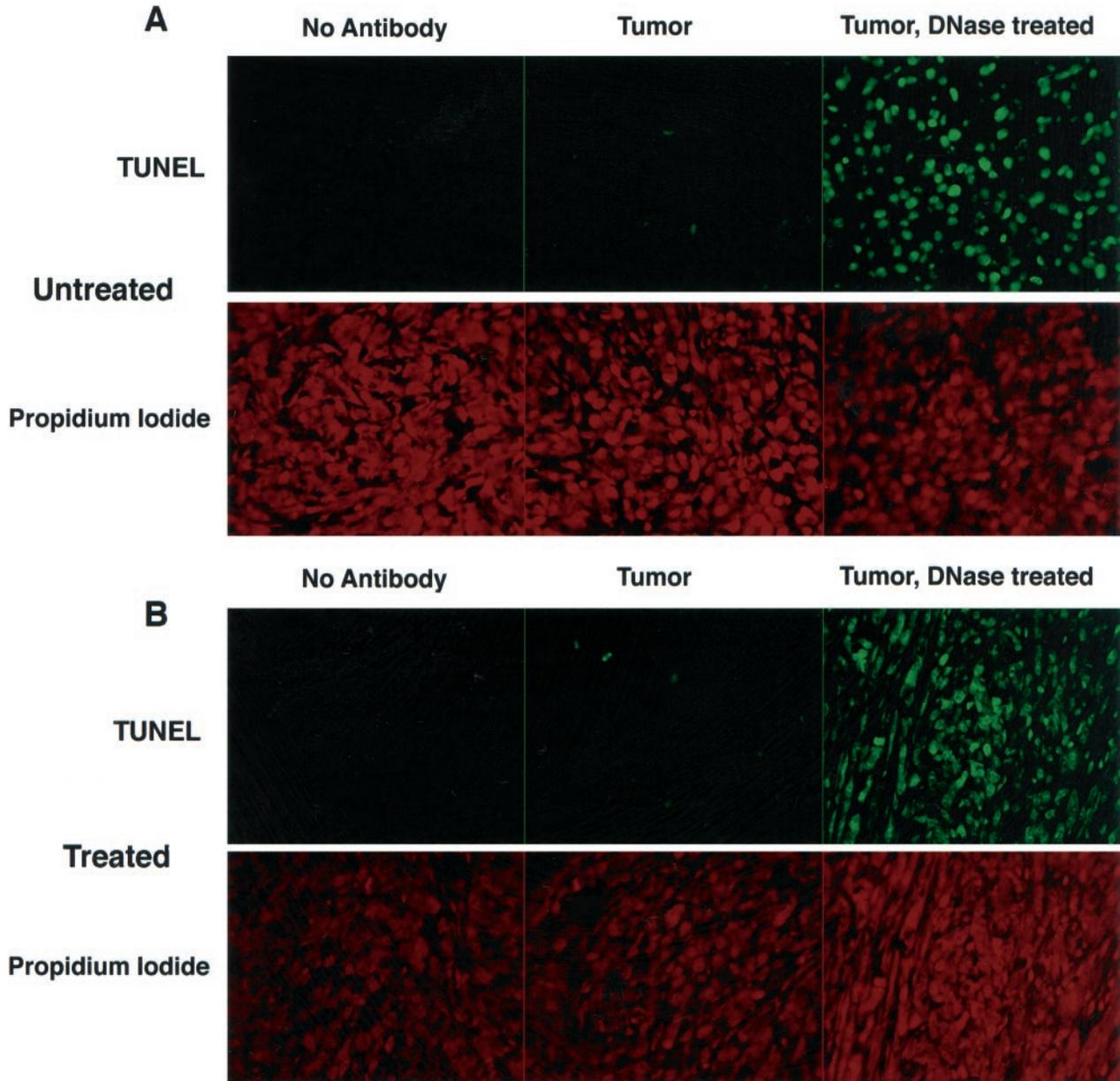
Fig. 7. TUNEL staining of tumors from mice treated with STI571. Tumors were generated by s.c. injection of U87 cells and allowed to progress to approximately 1×1 cm in size. Mice were then treated with vehicle (*A*) or STI571 (*B*, 50 mg/kg/day in saline, i.p.) for 1 week. Tumors were excised, fixed, sectioned, and examined for apoptosis by TUNEL staining. The positive control reflects treatment of the section by DNase I prior to staining.

One concern is that STI571, while inhibiting PDGF receptor signaling, might antagonize other signaling pathways that are required for programmed cell death (apoptosis). For example, the other known target for STI571, c-Abl, is known to facilitate the apoptotic response to DNA damage (25–27). Myeloid cell lines that have been transformed by Bcr-Abl undergo apoptotic cell death rather than growth arrest when exposed to STI571. Thus, STI571 does not inherently antagonize the process of programmed cell death. Rather, the data suggest that cellular responses to STI571 mimic the effect of growth factor withdrawal from factor-dependent cell lines. Factor-dependent myeloid cell lines generally undergo programmed cell death upon factor withdrawal, whereas nonmyeloid lines, such as 3T3, undergo growth arrest.

Obviously, a cytotoxic response would be preferable to a cytostatic response in a cancer therapeutic. However, cytotoxic agents have proven generally ineffective as therapeutics for glioma. Given the minimal toxicity shown by STI571 thus far in clinical trials on chronic myelogenous leukemia, the possibility emerges that STI571, perhaps used in combination with other therapeutic modalities, might be of some benefit to patients with glioblastoma. As time goes on, other treatment modalities might prove to synergize with STI571 by promoting cell killing in addition to growth arrest.

ACKNOWLEDGMENTS

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