SB-431542, a small molecule transforming growth factor-β-receptor antagonist, inhibits human glioma cell line proliferation and motility

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Abstract

Transforming growth factor-β (TGF-β) is a multifunctional cytokine that promotes malignant glioma invasion, angiogenesis, and immunosuppression. Antisense oligonucleotide suppression of TGF-β_2 ligand expression has shown promise in preclinical and clinical studies but at least two ligands mediate the effects of TGF-β in gliomas. Therefore, we examined the effects of SB-431542, a novel, small molecule inhibitor of the type I TGF-β receptor, on a panel of human malignant glioma cell lines. SB-431542 blocked the phosphorylation and nuclear translocation of the SMADs, intracellular mediators of TGF-β signaling, with decreased TGF-β–mediated transcription. Furthermore, SB-431542 inhibited the expression of two critical effectors of TGF-β-vascular endothelial growth factor and plasminogen activator inhibitor-1. SB-431542 treatment of glioma cultures inhibited proliferation, TGF-β–mediated morphologic changes, and cellular motility. Together, our results suggest that small molecule inhibitors of TGF-β receptors may offer a novel therapy for malignant gliomas by reducing cell proliferation, angiogenesis, and motility. [Mol Cancer Ther 2004;3(6):737–45]

Introduction

Despite maximal therapy, the median survival of patients with the most aggressive primary brain tumor, the glioblastoma, remains 10 to 12 months (1). In the year 2003, there will be an estimated 18,300 new primary malignant brain tumor patients in the United States with 13,100 deaths (2). Significant morbidity and mortality from malignant gliomas comes from local invasion of the tumor preventing complete surgical resection (3). Malignant gliomas remain almost universally fatal despite maximal therapy. Current glioma treatments use non-specific modalities that damage DNA. Therapeutic benefits of targeting specific signal transduction pathways in some cancers have pushed rational molecular targeting to the forefront of cutting-edge cancer therapy. The identification and targeting of pathways critical to the phenotype of cancers offers new hope in the treatment of many patients. Prominent among the signal transduction pathways that play a critical role in malignant gliomas is the transforming growth factor-β (TGF-β) pathway (reviewed in refs. 4, 5).

Malignant gliomas express TGF-β ligands and receptors, suggesting the presence of autocrine or paracrine loops (6-8). TGF-β ligands are secreted in latent forms and are activated through cleavage of the carboxyl-terminal region known as the latency-associated peptide (9). Some cancers, such as gliomas, bypass this regulation by secreting the active form of TGF-β (10). Active TGF-β ligands bind to specific cell surface receptors to initiate the formation of an activated heterodimeric serine/threonine kinase receptor complex (11-14). The constitutively active type II receptor phosphorylates and activates the type I receptor which then initiates the intracellular signaling cascade from the cytoplasm to the nucleus by phosphorylating intracellular mediators, predominantly involving a family of proteins called SMADs. SMAD2 and SMAD3 specifically mediate the signals induced by TGF-β and activin (15-19). Phosphorylation of these SMADs induces release from the receptor, relief of auto-inhibitory folding, binding to SMAD4, and translocation to the nucleus where transcription regulation is initiated. TGF-β may act to either promote or inhibit the transcription of specific targets.

TGF-β has been noted to act in a ''paradoxical'' fashion in cancer—in normal epithelial tissues, TGF-β functions as a tumor suppressor through growth inhibition, but in advanced epithelial cancers, TGF-β promotes tumor growth through induction of tumor invasion and neoangiogenesis and suppression of the immune response (20, 21). We and others have shown that malignant glioma cell lines are refractory to TGF-β–mediated growth inhibition yet retain expression of essential elements of the TGF-β signal transduction pathways—the cognate receptors and SMADs (8, 22, 23). Because anti–TGF-β therapies have shown promise in both preclinical and early clinical

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trials (24-27), we sought to determine the benefits of a small molecule inhibitor of TGF-β receptor serine/threonine kinase activity in modulating the *in vitro* phenotype of malignant glioma cultures. SB-431542 is a novel, small molecule ATP-mimetic inhibitor of the kinase activity associated with members of the activin receptor-like kinase (ALK) family—specifically ALK5 (TGF-β type I receptor, TGF-βRI), ALK4 (activin type I receptor), and ALK7 (nodal type I receptor; ref. 28). SB-431542 can inhibit TGF-β–mediated activation of SMAD2 and induction of fibronectin and collagen expression in TGF-β–response cell lines (29). A recent report showed that SB-431542 blocked TGF-β–mediated increase in proliferation in a mesenchymal cell line (30). We now present the first report of an analysis of the potential therapeutic benefit in a cancer that is dependent on TGF-β. Treatment of glioma cultures with SB-431542 blocks activation of the TGF-β pathway and expression of important effectors of the TGF-β–mediated phenotype. SB-431542 inhibits cell proliferation and blocks cell motility, strongly suggesting that small molecule inhibitors of TGF-RI activity may offer novel therapies in the treatment of malignant glioma.

Materials and Methods

Cells and Culture

The human glioma cell line U87MG was purchased from the American Type Culture Collection (Manassas, VA). D. Bigner provided the human glioma cell lines D54MG, D270MG, U373MG, D423MG, and D538MG. The cell lines were maintained in an improved zinc option media with 10% fetal bovine serum and glutamine (Invitrogen, Carlsbad, CA).

TGF-βRI Inhibitor

SB-431542, a TGF-β inhibitor, was generously provided to us from GlaxoSmithKline Pharmaceuticals (King of Prussia, PA). SB-431542 was dissolved in 100% DMSO at a stock concentration of 10 mmol/L. This stock was then diluted to various concentrations in 100% DMSO for subsequent experiments and kept at −80°C. Porcine TGF-β_1 was a kind gift of R&D Systems (Minneapolis, MN).

Thymidine Incorporation Assay

Cells were plated into 12-well plates at a density of 2×10^4 cells/well and treated with DMSO or various concentrations of SB-431542 for 48 hours. The media were generally supplemented with 10% fetal bovine serum. The cells were labeled for 4 hours with 4 μCi of [^{3}H]thymidine, fixed in 10% trichloroacetic acid, and lysed in 0.2 N NaOH. [^{3}H]thymidine incorporation into the DNA was measured with a scintillation counter. Each data point was done in triplicate.

Propidium Iodide Flow Cytometry

Cell lines were plated into 6-cm plates at a density of 1.5×10^5 cells/well and treated with DMSO or SB-431542 for 48 hours. Cells were then trypsinized, washed with PBS, resuspended in 50% fetal bovine serum-50% PBS solution, fixed in 70% ethanol, washed once in PBS, and resuspended in RNase A (100 μg/mL) and propidium iodide (50 μg/mL). Samples were analyzed on a FACScan (Becton Dickinson, Franklin Lakes, NJ) flow cytometer. Each experiment was done in triplicate.

ELISA

Cells were either plated at a density of 1×10^5 in 12-well plates, allowed to attach overnight, serum starved for 48 hours or plated at 2.5×10^4 in 12-well plates, and incubated for 72 hours. Media were either acidified to determine total TGF-β amount or not acidified to determine the active TGF-β secreted by the cells. The media were then placed in the ELISA plates (R&D Systems). The ELISA procedure from the Quantikine manual was followed to perform the TGF-β_1 and TGF-β_2 ELISAs. The procedure used to acidify was the procedure found in the TGF-β_2 ELISA kit. For vascular endothelial growth factor (VEGF) measurements, cells were grown in serum-free media and added to the ELISA plate directly as per manufacturer's directions.

Luciferase Reporter Assays

A total of 0.1×10^6 cells/well was seeded into six-well plates and grown under normal culture conditions for 24 hours. Cells were transfected with a standard Fugene6 transfection protocol according to manufacturer's instructions. Three micrograms of total DNA including 1 μg of the indicated reporter construct (see Fig. 4), 0.2 μg of a cytomegalovirus β-galactosidase (CMV-β-Gal) expression vector, and 1.8 μg of filler vector were transfected into the cells of each well in triplicate for each condition shown. The 4xSBE-Lux reporter construct was made by annealing primers 5′-GATCTAAGTCTAGACGGCAGTCTAGAC-3′ and 5′-GATCGTCTAGACTGCCGTCTAGACTTA-3′ and then concatamerizing them into the *Bgl*II site of pGL2-T+I (31). At 16 hours posttransfection, 100 pmol/L TGF-β, SB-431542, or vehicle and fresh medium was added to each well, and 24 hours later, luciferase activity was assessed from harvested total cell lysates. The determined luciferase activity in each sample was normalized to measured levels of corresponding β-galactosidase expression to account for differences in transfection efficiencies.

Western Analysis

Glioma cells were plated in 10-cm culture plates and grown to 50% to 70% confluency. For SMAD2 immunoblotting with increasing SB-431542, cells were treated overnight with DMSO or SB-431542 and then stimulated with 100 pmol/L TGF-β_1 for 15 minutes. For plasminogen activator inhibitor-1 (PAI-1) immunoblotting, cells were incubated for 24 hours with SB-431542 and 100 pmol/L of TGF-β_1. Plates were washed twice with PBS. Cell lysates were prepared by lysing cells in a lysis buffer containing 50 mmol/L Tris-HCl (pH 7.5), 150 mmol/L NaCl, 0.5% NP40, 50 mmol/L NaF, 1 mmol/L sodium orthovanadate, 0.1 mmol/L sodium molybdate, 1 mmol/L DTT, 1 mmol/L phenylmethylsufonyl fluoride, and protease inhibitors. Protein concentrations were determined, and equal protein amounts of each sample were resolved on 8% acrylamide-0.05% bisacrylamide-SDS gel for SMAD2 and

Phospho-SMAD2 were run on a 10% or 12% acrylamide-0.05% bisacrylamide-SDS gel, while PAI-1 and Tubulin were run on a NuPage Novax 10% Bis-Tris gel. Proteins were then transferred to Immobilon P transfer membrane (Millipore, Billerica, MA). Blocking and antibody incubations were done in 5% dried milk and 0.1% Tween 20 in PBS for 1 hour. Antibodies used for Western blots were as follows: phospho-Smad2 (Cell Signaling, Beverly, MA), Smad2 (Zymed, South San Francisco, CA), ALK5 (Cell Signaling), ALK4 and ALK7 (R&D Systems), anti-PAI-1 (Santa Cruz, CA), Tubulin (Sigma, St. Louis, MO), and horseradish peroxidase-conjugated goat anti-rabbit and goat anti-mouse (Zymed). Western blots were developed with the enhanced chemiluminescence reagent (Pierce, Rockford, IL).

Semi-quantitative Reverse Transcription-PCR

RNA from glioma cell line cultures were collected using a Qiagen RNAeasy kit (Qiagen, Valencia, CA). ALK5 primers were purchased from R&D Systems. Other primers used were: for ALK4 AGCAGAGATATACCAGACGG and GCATTTCCTCAATGGAAGGG, for ALK7 AACAGAAGGAGCATGTTGGG and CTTCCATGCCACACCTCACC, and for glyceraldehyde-3-phosphate dehydrogenase CGGAGTCAACGGATTTGGTCGTAT and AGCCTTCTCCATGGTGGTGAAGAC. Reverse transcription-PCR was done using the Titan One Tube reverse transcription-PCR System (Roche, Indianapolis, IN) as per manufacturer's instructions.

Migration Assay

Prior investigation of the impact of TGF-β on glioma invasion has involved pretreatment of cell lines for greater than 24 hours (32). We have used a similar protocol as follows: Cells were plated in 6-cm plates and when 70% confluent, they were treated with increasing concentration of SB-431542 overnight in serum-free media. Cells were trypsinized and diluted in PBS. The cells were spun down and resuspended in serum-free zinc option media. After 300 μL of Zinc Option with 10% serum was added to the bottom of each chamber, 25×10^3 cells were plated in the membrane chambers in 200 μL of media and treated again with increasing concentrations of SB-431542. After 24 hours, the membranes were fixed in 4% paraformaldahyde overnight and stained in toluidine blue O (0.5%) overnight. All invading cells were then counted manually. Each point was done in duplicate.

Statistical Analysis

Data in all studies were compared by using the nonparametric Wilcoxon's rank-sum test (33).

Results

Human Glioma Cell Lines Express and Respond to TGF-β

We previously characterized the response of multiple human glioma cell lines to exogenous TGF-β and selected several of these lines based on variable proliferative responses and variable expression of key mediators, including PAI-1 and VEGF (ref. 22, Table 1). We further characterized D54MG, U87MG, D270MG, U373MG, D423MG, and D538MG glioma cultures by measuring the secretion of the two most common TGF-β ligands expressed by gliomas, TGF-β_1 and TGF-β_2. In serum-free conditions, every tested cell line expressed significant levels of total TGF-β1 (Fig. 1A). However, only D54MG, U373MG, and D423MG expressed detectable levels of total TGF-β_2 (Fig. 1A). In addition to expressing latent TGF-β that may be activated by conditions in the cellular environment, gliomas can also secrete activated TGF-β ligand. Indeed, all the glioma cell lines tested expressed levels of activated TGF-β1 (Fig. 1B), albeit at lower levels than total TGF-β levels, confirming that gliomas are capable of producing an autocrine loop of TGF-β signaling. Although all the lines produced active TGF-β_1, only D54MG expressed activated TGF-β_2 (Fig. 1B). To determine the ability of TGF-β to activate downstream components of the pathway, we measured activation of the receptor via SMAD2 phosphorylation at baseline and with exogenous TGF-β_1 administration. Despite the secretion of activated TGF-β ligand, basal phosphorylation of SMAD2 was low but was inducible on treatment with exogenous TGF-β_1 (Fig. 2). Thus, the glioma cell lines used for our study produce and respond to TGF-β and provide an adequate model with which to study the inhibition of TGF-β signaling by SB-431542.

SB-431542 Inhibits SMAD Phosphorylation and Nuclear Translocation

After determining the utility of selected glioma cell lines for the study of TGF-β signaling, we evaluated the effects of an inhibitor of TGF-β Type I receptor, SB-431542, on the TGF-β pathway in these cells. Activation of the TGF-β

Table 1. Malignant glioma cell line TGF-β responses and TGF-β pathway component expression

Cell Line	TGF-β Growth Effects	VEGF Induction	PAI-1 Induction	Receptor Binding	Smad2 Protein	Smad3 Protein	Smad4 RNA
D54MG	Inhibitory	++	+++	++	+	+	+
U87MG	Inhibitory	0	++	+++	+	++	++
D423MG	Inhibitory	+	++	+++	++	+++	+
D538MG	No effect	++	+	++	+++	++	++
D270MG	Stimulatory	++	+	+++	++	+	++
U373MG	Stimulatory	++	0	+	++	+	+

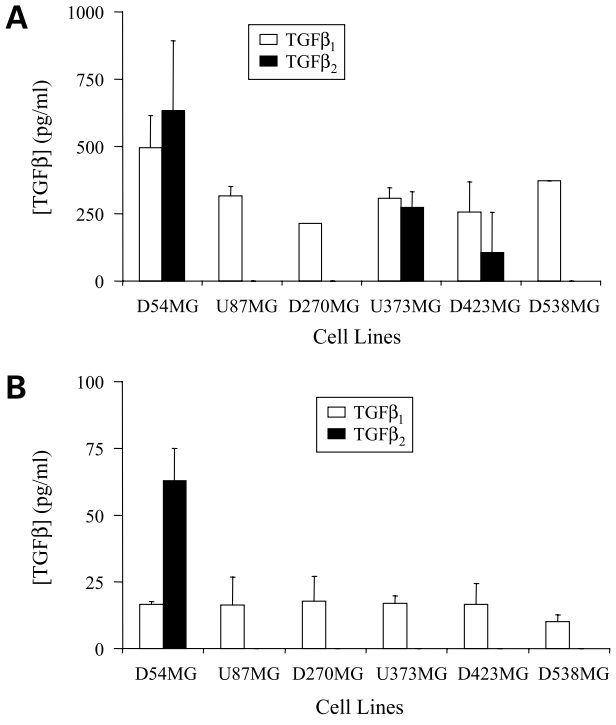

Figure 1. Malignant glioma cell lines secrete both active and latent TGF-β ligands. Human malignant glioma cell lines were grown in serum-free conditions for 48 hours. Conditioned media were assessed for TGF-β levels by ELISA. **A,** total TGF-β_1 and TGF-β_2 levels were measured through acidification to release the latency-associated peptide. All the cell lines secreted TGF-β_1, but only D54MG, U373MG, and D423MG secreted TGF-β_2. **B,** activated TGF-β levels were measured without acidification. All the cell lines secreted activated TGF-β_1, while only D54MG secreted activated TGF-β_2.

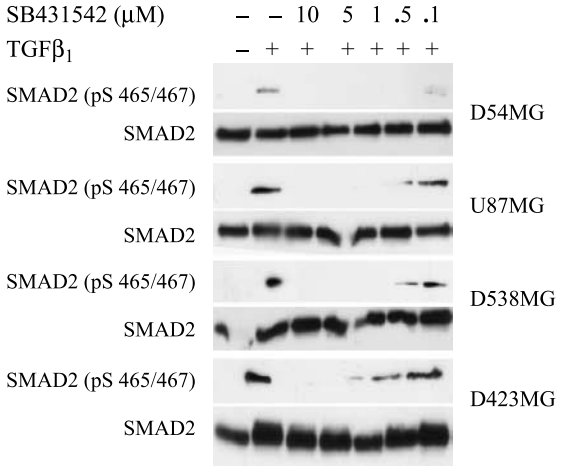

Figure 2. SB-431542 blocks the phosphorylation of SMAD2 in response to TGF-β. Cells were treated for 30 minutes with SB-431542 or DMSO and then stimulated for 1 hour with TGF-β. Whole cell lysates were prepared, resolved by SDS-PAGE, and immunoblotted for phosphorylated SMAD2. Membranes were stripped and reprobed for total SMAD2 to confirm equal protein loading.

receptor complex induces phosphorylation and nuclear translocation of the receptor SMADs. Initial studies determined the ability of increasing concentrations of SB-431542 to prevent the phosphorylation of SMAD2 in response to exogenous TGF-β1. The cell lines displayed significant variability in the concentration required to totally inhibit SMAD2 phosphorylation, ranging from greater than 100 nmol/L to greater than 1 μmol/L (Fig. 2). A positive relationship was noted between the expression levels of SMAD2 and concentration of SB-431542 required to suppress SMAD2 phosphorylation (data not shown). Furthermore, SB-431542 also blocked TGF-β–induced nuclear localization of SMAD3 in both D54MG and U87MG (Fig. 3). Because SB-431542 can act to block the activities of three type I TGF-β receptors (ALK4, ALK5, and ALK5; ref. 28), we investigated the expression of these receptors in each of our cell lines. All of the cell lines expressed the type I TGF-β receptor (ALK5) protein on Western analysis but ALK4 or ALK7 protein was not detectable (data not shown). However, ALK4 but not ALK7 RNA was detected by semi-quantitative reverse transcription-PCR (data not shown). Thus, the effects of SB-431542

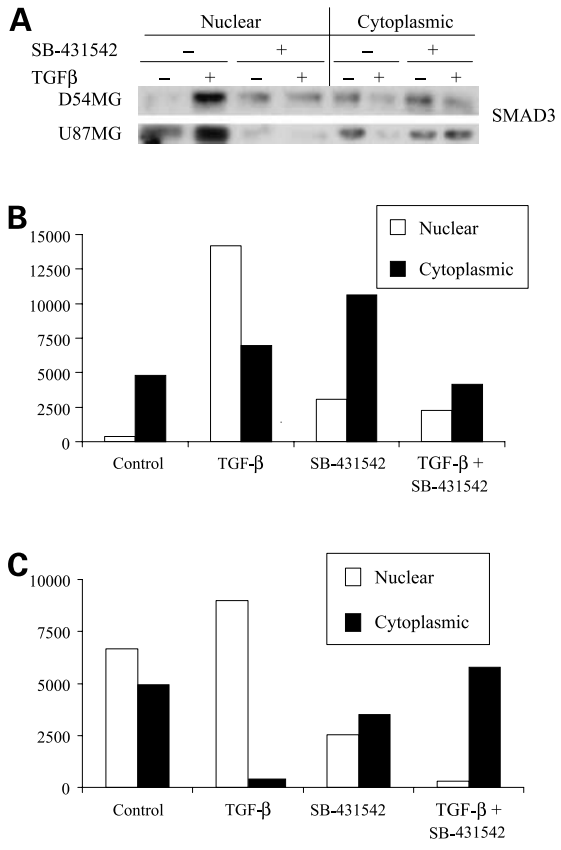

Figure 3. SB-431542 inhibits SMAD3 nuclear localization in response to TGF-β. Cells were treated with SB-431542 for 1 hour and then stimulated with TGF-β for 15 minutes. **A,** nuclear lysates were prepared, resolved by SDS-PAGE, and immunoblotted for SMAD3. The relative intensity of the bands relative to the blank background were imaged and graphed using Image J software (**B,** D54MG; **C,** U87MG).

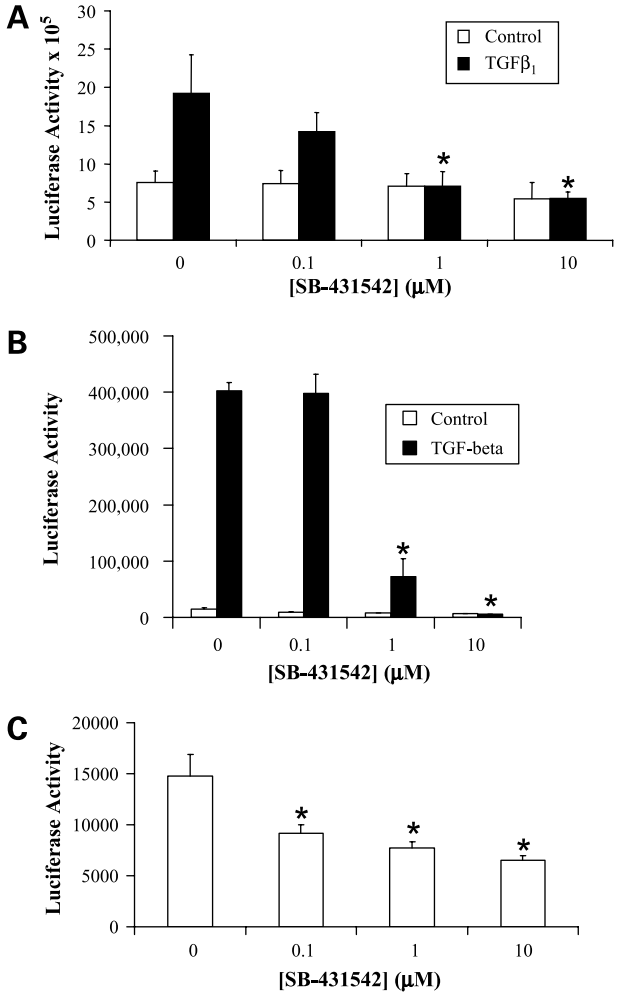

Figure 4. SB-431542 blocks TGF-β–mediated SMAD-dependent transcription. U87MG cultures were transfected with a general TGF-β luciferase promoter construct, 3TP-Lux (**A**) or 4xSBE-Lux (**B**), and treated with increasing concentrations of SB-431542 or DMSO with or without TGF-β for 24 hours. Lysates were prepared and luciferin added. Bioluminescence was measured and corrected for β-galactosidase activity. **C,** basal 4xSBE-luciferase activity is inhibited by SB-431542 concentrations greater than 1 μmol/L. *, $P < 0.05$ relative to TGF-β–treated control.

are likely mediated by ALK5. Taken together, these results suggest that SB-431542 can effectively inhibit TGF-β signal transduction in malignant glioma lines regardless of the resistance of these same lines to TGF-β–mediated growth inhibition.

SB-431542 Inhibits TGF-β–Mediated Transcription of Target Genes

TGF-β mediates many of its effects through the regulation of transcription of critical target genes. We, therefore, examined the ability of SB-431542 treatment to block TGF-β–mediated transcription. Using the TGF-β–responsive luciferase reporter 3TP-lux, we found that SB-431542 inhibited TGF-β–induced transcription in U87MG (Fig. 4) and D54MG (data not shown) in a concentration-dependent fashion. TGF-β regulates the expression of many genes that may contribute to tumor malignancy. Of particular interest in malignant gliomas are those genes regulating cellular motility, invasion, and angiogenesis. PAI-1 is a regulator of the extracellular matrix in tissues by inhibiting urokinase plasminogen activator (uPA) that is overexpressed in malignant gliomas (34, 35). Increased PAI-1 expression in malignant gliomas has been linked to tumor grade and is an independent predictor of poor survival (35). PAI-1 expression regulates glioma invasion (36) and neoangiogenesis (37). PAI-1 is a transcriptional target of TGF-β and SMAD DNA binding elements have been identified in the promoter (38). SB-431542 treatment inhibited both basal PAI-1 expression and PAI-1 levels induced in response to exogenous TGF-β (Fig. 5) in all tested lines, demonstrating that SB-431542 may reduce autocrine TGF-β responses. Like PAI-1, VEGF has been linked to the progression of malignant gliomas and is regulated by TGF-β (34). VEGF is well recognized as a critical mediator of tumor neoangiogenesis (39). SB-431542 specifically blocked TGF-β–induced VEGF expression in a concentration-dependent fashion as measured by ELISA (Fig. 6). Thus, SB-431542 effectively inhibits the expression of key mediators of TGF-β in invasion and neoangiogenesis.

SB-431542 Treatment Blocks *In vitro* Measures of Glioma Malignancy

Gliomas grow through cellular proliferation and invasion into normal tissues. Although insufficient quantities of SB-431542 were available to evaluate the impact of treatment on *in vivo* tumor behavior, we studied the effects of SB-431542 in cell culture assays. The invasive and

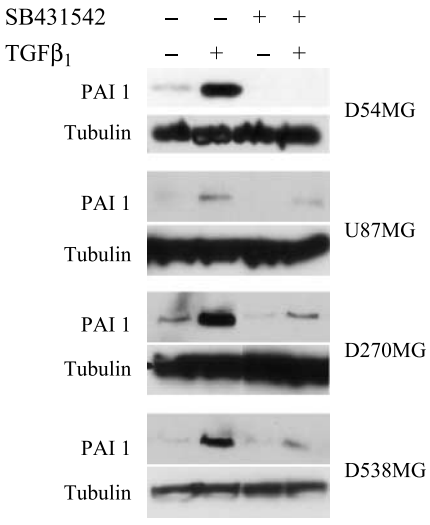

Figure 5. SB-431542 blocks the expression of the TGF-β downstream mediators, PAI-1. Cells were treated with DMSO or SB-431542 in the presence and absence of TGF-β for 24 hours. Whole cell lysates were prepared, resolved by SDS-PAGE, and immunoblotted for PAI-1. Membranes were stripped and reprobed for tubulin to confirm equal protein loading.

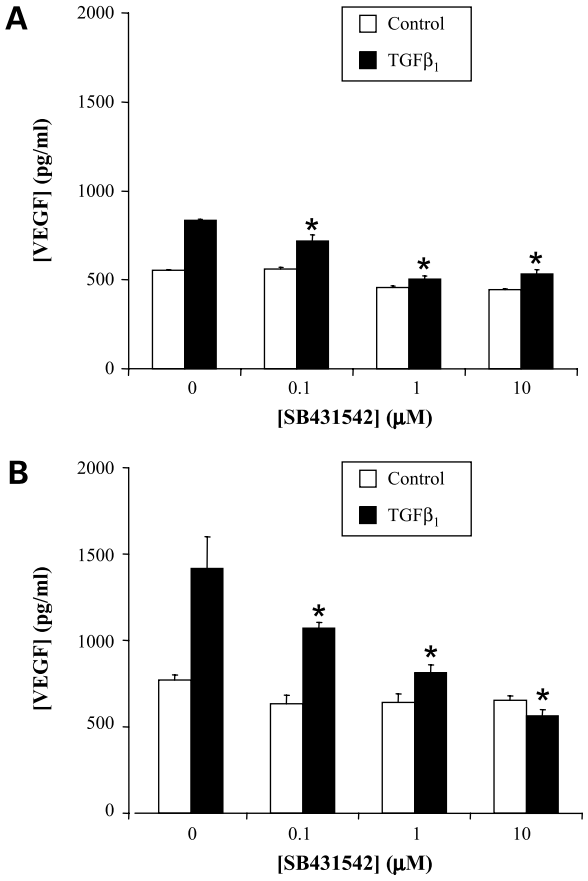

Figure 6. SB-431542 blocks TGF-β–induced VEGF expression. D270MG (**A**) and D423MG (**B**) cultures were indicated either in the absence or presence of 100 pmol/L TGF-β with either DMSO control or increasing concentrations of SB-431542 for 48 hours in serum-free media. VEGF levels were determined by ELISA according to the manufacturer's directions. *, $P < 0.05$ relative to TGF-β–treated control.

angiogenic effects of TGF-β that promote tumorigenesis are often overshadowed by TGF-β's recognized role as a tumor suppressor. TGF-β mediates a strong anti-proliferative response on a variety of non-transformed epithelial cells through the induction of G_1 cell cycle arrest. Therefore, we considered the possibility that an inhibitor of TGF-β signaling, such as SB-431542, could counteract TGF-β–mediated growth inhibition, leading to proliferation. To address this possibility, we analyzed SB-431542 effects on DNA synthesis using thymidine incorporation assays. SB-431542 at both 0.1 and 1 mol/L had a modest effect on DNA synthesis, but at 10 mol/L, there was a consistent 60% to 70% reduction of thymidine incorporation for D54MG, U87MG, and U373MG cells (Fig. 7). No evidence of apoptotic cell death on SB-431542 treatment was detected either by direct observation or on propidium iodide flow cytometric analysis (data not shown). Thus, SB-431542 acts to inhibit the proliferation of the tested malignant glioma lines.

TGF-β induces phenotypic changes in tumor cells to enable cellular invasion, a hallmark of gliomas. Exogenous TGF-β induces cell rounding of both D54MG and U87MG (Fig. 8 and data not shown), a cell morphology associated with propensity toward motility. SB-431542 treatment did not induce morphologic changes, but prevented TGF-β–induced morphologic changes in D54MG and U87MG cultures (Fig. 8 and data not shown). In parallel, inhibiting TGF-βRI signaling with SB-431542 blocked cell migration of D54MG cells in a concentration-dependent fashion (Fig. 9). In summary, SB-431542 potently restrains advantageous tumor cell phenotypes induced by TGF-β.

Discussion

TGF-β has an important role in the development of many different types of tumors, including gliomas, indicating that specific targeting of the TGF-β pathway could be useful for cancer therapy. To date, TGF-β has been targeted in several ways, including antisense oligonucleotides, soluble receptors, and inhibitors of ligand activation. However, blockade of the TGF-β pathway at the type I receptor may offer significant benefit in cancer therapy for several reasons. First, targeting a single ligand may be only partially effective as gliomas secrete multiple ligands. Second, large circulating molecules may block the systemic immunosuppressive effects of TGF-β but will fail to directly modulate TGF-β functions at the sites of the glioma cells—including tumor cell invasion, regulation of angiogenic factor expression, and local immunosuppression (4, 5). Finally, blockade of the activation of latent TGF-β ligand will fail to inhibit the effects of constitutive release of activated ligand. Rather, activation of the TGF-βRI seems to be necessary and sufficient for the induction of most of the effects mediated by TGF-β (13). Thus, the ability to target TGF-βRI with small molecule inhibitors may offer significant benefit in the treatment of malignant gliomas.

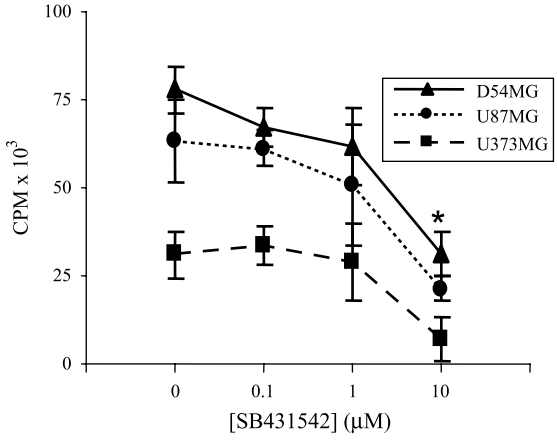

Figure 7. DNA synthesis is blocked by treatment with SB-431542. Both U87MG (**A**) and D54MG (**B**) were treated with increasing concentrations with SB-431542 for 24 hours. Cells were exposed to a 4-hour pulse of [^{3}H]thymidine after which cells were fixed with 10% trichloroacetic acid and lysed with 0.2 N NaOH. *, $P < 0.05$ relative to TGF-β–treated control.

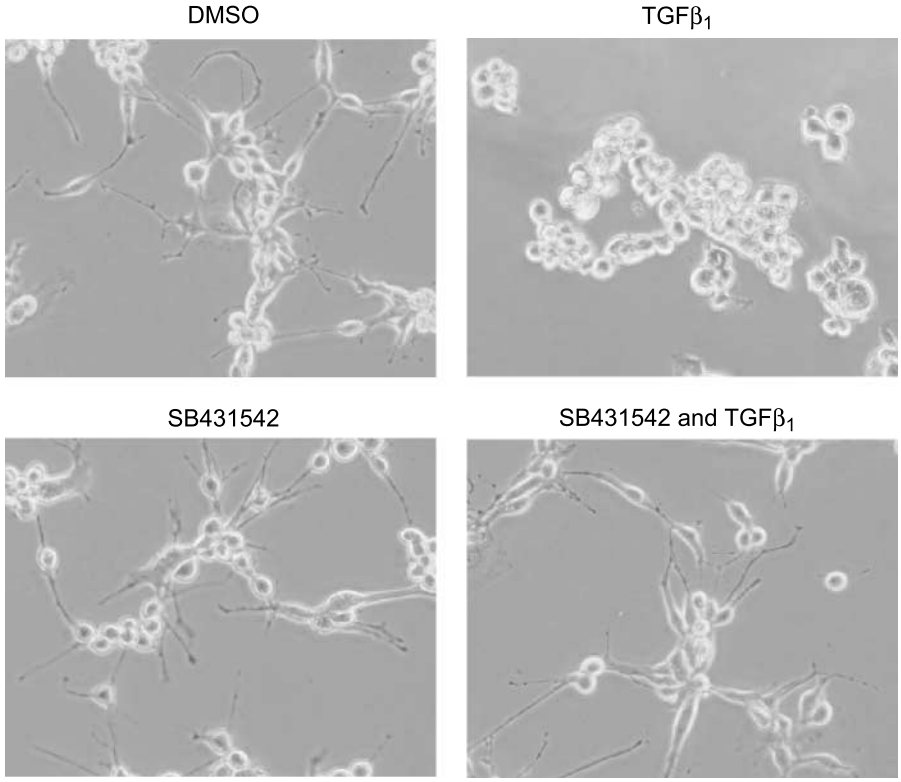

Figure 8. SB-431542 inhibits TGF-β–induced morphologic changes. U8 D54MG cultures were treated with DMSO (control), 100 pmol/L TGF-β alone, 1 mmol/L SB-431542 alone, or 100 pmol/L TGF-β and 1 mmol/L SB-431542 in combination. Representative areas of the culture are presented.

SB-431542 is a novel small molecule that potently inhibits TGF-βRI activity at nanomolar concentrations (28). This compound can block activation of SMAD2 and induction of extracellular matrix components by TGF-β in TGF-β–responsive cells (29). SB-431542 inhibited TGF-β–mediated c-*myc* expression and the proliferation of osteosarcoma cell line that is growth stimulated in response to TGF-β (30). Less clear are the effects of SB-431542 on the TGF-β signaling and phenotypic changes on epithelial cancer cells that have disruption of normal TGF-β responses. In normal mesenchymal cells (like many bone cells), TGF-β may play a growth stimulatory role before cellular transformation.

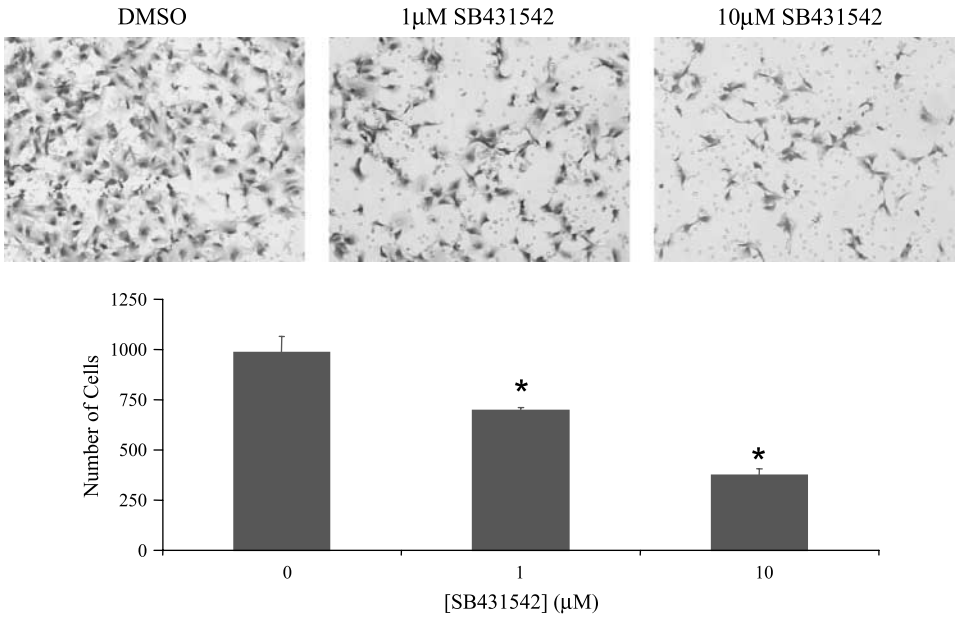

Figure 9. SB-431542 blocks *in vitro* malignant glioma migration. D54MG cells were trypsinized and placed in Transwell plates containing either DMSO or SB431542. Cells were allowed to migrate for 4 hours then were fixed and stained. **A,** cells treated with DMSO underwent rapid migration while the SB-435142–treated cells were inhibited. **B,** the migration of the cells was reduced by 60% to 70%. *, $P < 0.05$ relative to TGF-β–treated control.

In fact, this is responsible for the naming of TGF-β as a transforming growth factor (40). In non-transformed epithelial cells, TGF-β acts in a growth suppressive fashion. In the transition of epithelial cancers into an invasive and metastatic phenotype, a switch in the response to TGF-β occurs such that growth suppressive effects are lost, often through disruption of the normal TGF-β signaling components. We and others have found that malignant gliomas retain expression of TGF-β receptors and the intracellular TGF-β signal transducers, the SMADs (8, 22, 41). We found that TGF-β treatment induced cell cycle arrest of astrocytes, the presumed cells-of-origin of astrocytomas, but malignant glioma cell lines can induce important mediators of the pro-tumorigenic effects of TGF-β but are not growth inhibited (22). Thus, it was unclear what the effects of an inhibitor of TGF-βRI might be in cancers for which anti-TGF-β treatments have shown promise in both preclinical and clinical trials.

We have now shown that SB-431542 blocks TGF-β–mediated signaling in all tested human glioma cell lines with the inhibition of expression of transcriptional targets of TGF-β, including PAI-1 and VEGF. PAI-1 may play critical roles in the phenotype of many cancers, including malignant gliomas. Malignant glioma patients whose tumors have high expression of PAI-1 have a shorter survival than those without PAI-1 expression (35). PAI-1 can stimulate the invasion of tumor cells (42) and neoangiogenesis through regulation of endothelial cell motility (37). Chemotherapy-resistant gliomas express higher levels of PAI-1 (43). In a parallel fashion, VEGF has been linked to malignant behavior of many cancers through induction of neoangiogenesis (39). TGF-β regulates VEGF expression through transcription in association with activator protein 1 (AP-1) family members (44). Anti-VEGF therapies have shown promise in the treatment of malignant gliomas (45). Thus, the ability to block TGF-β–mediated PAI-1 and VEGF expression through SB-431542 treatment may offer significant benefit in the inhibition of tumor invasion and angiogenesis. We have now shown that SB-435142 inhibits tumor cell proliferation and motility in a concentration-dependent fashion with specific reversal of TGF-β–induced morphologic changes. Although insufficient quantities of SB-431542 are available presently for *in vivo* assays, treatment with SB-431542 may offer even greater benefit in control of tumor growth through suppression of TGF-β–mediated angiogenesis and reversal of TGF-β–induced immunosuppression.

Together our work shows that treatment of gliomas with SB-431542 may be a promising new therapy. SB-431542 decreases tumor cell proliferation, extracellular matrix production, and migration. Thus, SB-431542 has the potential to target at least two different phenotypes required for tumor development and progression. Considering the formidable odds against surviving invasive gliomas, we believe our results with SB-431542 offer exciting new hope for patient therapy. Future development of small molecule TGF-βRI inhibitors may offer yet additional promise in combination with immune therapies, traditional cytotoxics, and other signal transduction inhibitors because TGF-β may act to increase tumor resistance to these agents.

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