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A Specific Inhibitor of TGF-β Receptor Kinase, SB-431542, as a Potent Antitumor Agent for Human Cancers[1]

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Abstract

Small molecule inhibitors of signaling pathways have proven to be extremely useful for the development of therapeutic strategies for human cancers. Blocking the tumor-promoting effects of transforming growth factor-β (TGF-β) in advanced stage carcinogenesis provides a potentially interesting drug target for therapeutic intervention. Although very few TGF-β receptor kinase inhibitors (TRKI) are now emerging in preclinical studies, nothing is known about how these inhibitors might regulate the tumor-suppressive or tumor-promoting effects of TGF-β, or when these inhibitors might be useful for treatment during cancer progression. We have investigated the potential of TRKI in new therapeutic approaches in preclinical models. Here, we demonstrate that the TRKI, SB-431542, inhibits TGF-β–induced transcription, gene expression, apoptosis, and growth suppression. We have observed that SB-431542 attenuates the tumor-promoting effects of TGF-β, including TGF-β–induced EMT, cell motility, migration and invasion, and vascular endothelial growth factor secretion in human cancer cell lines. Interestingly, SB-431542 induces anchorage independent growth of cells that are growth-inhibited by TGF-β, whereas it reduces colony formation by cells that are growth-promoted by TGF-β. However, SB-431542 has no effect on a cell line that failed to respond to TGF-β. This represents a novel potential application of these inhibitors as therapeutic agents for human cancers with the goal of blocking tumor invasion, angiogenesis, and metastasis, when tumors are refractory to TGF-β–induced tumor-suppressor functions but responsive to tumor-promoting effects of TGF-β.

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Introduction

Small molecule inhibitors are very useful in understanding the role of individual signaling pathways involved in different biologic processes. These molecules have the potential to be useful for therapeutic applications [1]. There is compelling evidence indicating that TGF-β has complex roles in tumor suppression and progression that are context-dependent and stage-dependent. TGF-β plays a tumor-suppressive role by its ability to maintain tissue architecture, inhibit growth, induce apoptosis, and inhibit genomic instability in nontransformed cells or tissues. Several lines of evidence suggest that carcinoma cells frequently lose antiproliferative response to TGF-β and increase the production of one or more of the TGF-β isoforms. High levels of TGF-β can promote tumor growth in an autocrine and/or paracrine manner through the suppression of immunosurveillance, stimulation of connective tissue formation and angiogenesis, and changes that favor invasion and metastasis. Blocking the tumor-promoting effects of TGF-β by small molecule inhibitors provides an excellent therapeutic opportunity to improve the treatment of cancer. The most desirable approach for developing new therapeutic strategy by targeting TGF-β signaling would be to retain TGF-β–induced tumor suppression function, but to block TGF-β signaling in advanced invasive and metastatic cancers.

The transforming growth factor-β (TGF-β) family of polypeptides regulates a wide variety of biologic functions including cell proliferation, differentiation, matrix formation, and apoptosis [2,3]. The multifunctional effects of TGF-β are elicited through oligomeric complex formation between the type I and type II serine–threonine kinase receptors. TGF-β initiates signals by binding to the type II receptor (TβRII) and stabilizes the heteromeric complex with the type I receptor (TβRI) and, as a result, TβRI is transphosphorylated and activated by TβRII. The activated TβRI then propagates the signals through interaction with, and phosphorylation of, receptor-associated Smads [4]. The Smad proteins are divided into three distinct classes based on their structure and function in signaling by TGF-β family members. Receptor-regulated Smads (R-Smad) are phosphorylated on two serine residues at the C-terminus and thus activated in a ligand-specific manner. Smad2 and

Abbreviations: TGF-β1, transforming growth factor-β1; EMT, epithelial to mesenchymal transition; VEGF; vascular endothelial growth factor

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Smad3 mediate signaling by TGF-β and activin, whereas Smad1, Smad5, and presumably Smad8 are known to be involved in BMP signaling. Smad4 functions as a common mediator of TGF-β, activin, and BMP signaling pathways [2,3]. On phosphorylation by type I receptors, R-Smads form heteromeric complexes with Smad4 and translocate to the nucleus where they modulate the transcription of TGF-β target genes [4–7]. A distinct class of distantly related Smads, including Smad6 and Smad7, has been identified as consisting of inhibitors of these signaling pathways, and these inhibitors function by interfering with the activation of R-Smads. TGF-β–mediated Smad signaling has been shown to be required for the antiproliferative activity of TGF-β, and components in this signaling pathway are frequently inactivated by mutation or silenced in several human cancers [8]. In addition to activating the Smad pathway, TGF-β can also induce other signaling pathways like p38MAPK, ERK, PI3K, JNK, or Rho, which may be important for pro-oncogenic activities with low levels of input signal. Many TGF-β–inducible pro-oncogenic pathways are either independent of Smads, or require cooperation between the Smad and alternative pathways under transforming conditions [9,10]. The possibility of blocking tumor-permissive effects of TGF-β signaling, which involves multiple autocrine and paracrine mechanisms, provides an attractive target for therapeutic intervention. Although pan-TGF-β neutralizing antibodies and TβRII-Fc fusion protein have been used in preclinical studies, they did not show much promise as anticancer drugs due to complex roles of TGF-β signaling [11]. Interestingly, studies with soluble TβRII-Fc did not report any tumor-promoting actions or inflammatory responses *in vivo*, whereas inhibition of TGF-β signaling by this soluble antagonist decreased invasion, migration, and cell survival [12,13]. Small molecule inhibitors of TGF-β function could have potential regarding drug specificity and efficacy in therapeutic interventions. In this regard, a potent inhibitor of TβRI kinase activity (SB-431542) [14], which has no direct effect on the components of the ERK, JNK, or p38 MAP kinase pathways or components of the signaling pathways activated by serum, has recently been developed. SB-431542 is a specific inhibitor of TGF-β superfamily type I receptors ALK4 (activin receptor–like kinase 4), ALK5, and ALK7 [15,16]. However, it is not yet known how these inhibitors might regulate the tumor-suppressive or tumor-promoting effects of TGF-β, or under what circumstances these inhibitors might be useful for treatment during cancer progression. In the present study, we demonstrate that SB-431542 inhibits TGF-β–induced apoptosis and growth suppression in several cell types. SB-431542 efficiently blocks the tumor-promoting effects of TGF-β including cell motility, migration, invasion, and vascular endothelial growth factor (VEGF) secretion in human cancer cell lines. SB-431542 increases the anchorage-independent growth of lung adenocarcinoma cells (A549) that are responsive to TGF-β–induced growth inhibition. Interestingly, this inhibitor reduces colony formation by late-stage colon cancer cells (HT29), whose growth is otherwise promoted by TGF-β. SB-431542 has no effect on colony formation by VMRC-LCD cells that are unresponsive to TGF-β. Hence, inhibition of TGF-β receptor kinase activity may be a new avenue for potential therapy in human cancers that are resistant to TGF-β tumor-suppressor function.

Materials and Methods

Cell Lines and Cultures

Human colon cancer–derived nontumorigenic FET cells (a kind gift from Dr. Brattain), mink lung epithelial cells (Mv1Lu), human kidney cells (293T), human hepatoma cells (HepG2), mouse mammary epithelial cells (NMuMG), human breast cancer cells (MDA-MB 231), pancreatic adenocarcinoma cells (PANC-1), human keratinocyte cells (HaCaT), and rat intestinal epithelial cells (RIE) were maintained in DMEM containing 10% fetal bovine serum (FBS) supplemented with 50 U/ml penicillin and 50 μg/ml streptomycin solution. Stable RIE-iRas cells were cultured and maintained as before [17]. Human lung adenocarcinoma cells (A549), TβRII null human lung cancer cells (VMRC-LCD), and human colon adenocarcinoma cells (HT29) were maintained in RPMI with 10% FBS supplemented with penicillin/streptomycin and L-glutamate. All cell lines were cultured and maintained at 37°C in a humidified incubator in the presence of 5% CO_2.

Reagent

The TGF-β receptor kinase inhibitor, SB-431542, was kindly provided by Dr. Nicholas J. Laping from GlaxoSmith-Kline (King of Prussia, PA). SB-431542 was also purchased from Tocris Cookson, Inc. (Ellisville, MO).

Transcriptional Response Assay

FET cells were transiently transfected with CMV-βgal, and p3TP-Lux or (CAGA)9MLP-Luc reporter plasmids. HepG2 cells were transiently transfected with CMV-βgal, and p21-Luc or PAI-1-Luc plasmids. Transfected cells were incubated in 0.2% FBS with 5 ng/ml TGF-β1 in the presence of SB-431542 for 22 hours. Cell lysates were used to measure both luciferase and β-gal activities, and the normalized luciferase activity was presented.

Immunoprecipitation and Western Blot Analyses

293T, FET, and A549 cells were serum-starved for 2 hours and treated with 12.5 ng/ml TGF-β1 in the presence of SB-431542 for 1 hour. Cells were solubilized in lysis buffer. An equal amount of each protein lysate was incubated with both anti-Smad2 and anti-Smad3 polyclonal antibodies for 2 hours at 4°C, followed by incubation with 20 μl of protein G-Sepharose beads (Sigma Biochemicals, St. Louis, MO) for 1 hour. The immune complexes were analyzed by Western blot analysis with mouse anti-Smad4 antibody. Protein lysates were also analyzed by Western blot analysis with anti–phospho-Smad2 (Upstate, Inc., Lake Placid, NY), anti-Smad2 (Zymed Laboratories, Inc., San Francisco, CA), anti-Smad3 (Zymed Laboratories, Inc.), anti-Smad4 (Santa Cruz Biotechnology,

Inc.), and mouse anti–β-actin (Sigma Biochemicals, St. Louis, MO) antibodies. For other Western blots, extracts were prepared from RIE, RIE-iRas, Mv1Lu, and NMuMG cells treated with TGF-β1 in the presence of SB-431542 as indicated in figure legends.

Nuclear and Cytoplasmic Extracts

Subcellular localizations of endogenous Smad proteins were analyzed by subcellular fractionation. A549 cells were treated with TGF-β in the presence of SB-431542 for either 30 or 90 minutes, and nuclear and cytoplasmic fractions were made according to the manufacturer's protocol (Pierce, Rockford, IL). An equal amount of cytosolic or nuclear extracts (20 μg/lane) was separated in SDS-PAGE and analyzed by Western blot analysis using antibodies against Smad2, Smad3, and Smad4. Complete fractionations of cytoplasmic and nuclear proteins were verified by Western blot analysis with antibodies against Rho-GDI and PARP (Santa Cruz Biotechnology, Inc.).

[^{3}H]Thymidine Incorporation Assay

FET and MDA-MB-231 cells were treated with TGF-β in the presence of SB-431542 for 26 hours in 10% FBS containing DMEM medium. An amount of 4 μCi/well [^{3}H]thymidine (NEN, Boston, MA) was added in each well for an additional 2 hours. Cells were then fixed in 10% cold trichloroacetic acid (TCA), washed, and lysed in 0.2 N NaOH. Radioactivity incorporated into TCA-insoluble [^{3}H]thymidine was measured by scintillation counting and then presented.

Cell Counting Assay

Mv1Lu, MDA-MB-231, A549, VMRC-LCD, and HT29 cells were seeded into 12-well plates. Cells were then treated with TGF-β in the presence of SB-431542 (10 μM) for a total of 6 days. TGF-β and/or SB-431542 containing media was replaced every other day. Cells were counted each day and the average cell numbers from triplicate measurements were plotted.

Flow Cytometric Analysis

Cells from each pool of FET, RIE, and Mv1Lu were seeded into 60-mm plates and allowed to attach for 24 hours. Cells were then treated with 5 ng/ml TGF-β1 in the presence of SB-431542 in 10% FBS-containing medium for 35 hours. Cells were harvested, washed, and suspended in 200 μl of cold phosphate-buffered saline (PBS). One milliliter of propidium iodide cocktail [containing 50 μg/ml propidium iodide (Sigma Biochemicals), 1 mg/ml sodium citrate, 5 μl/ml Triton X-100, and 5 μg/ml RNase A in PBS] was added to each cell suspension and incubated on ice for 30 minutes. Cells were analyzed for red fluorescence through a 620-nm LP filter. Red fluorescence was used as a marker of DNA content and cell cycle status. The percentage of cells present in different phases of the cell cycle was measured and analyzed.

DNA Laddering

HepG2 cells were serum-starved for 24 hours and treated with 5 ng/ml TGF-β1 in the presence of SB-431542 (2 μM) for 46 hours. Cells (floating and adherent) were collected and lysed. RNase A (5 μg/ml) was added to the clear lysate and incubated at 56°C for 2 hours. Proteinase K (200 μg/ml) was then added and incubated at 50°C for an additional 2 hours. Treated samples were extracted twice with TE-saturated phenol/chloroform. DNA was precipitated with cold ethanol, washed, suspended in 20 μl of TE buffer, and analyzed by agarose gel electrophoresis.

Cell Death ELISA

HepG2 cells (20,000 per well) were seeded into 12-well plates and allowed to attach for 20 hours. Cells were serum-starved for 20 hours and then treated with 5 ng/ml TGF-β1 in the presence of SB-431542 for 24 hours. Cells (floating and adherent) were lysed in 200 μl of lysis buffer. Each lysate (10 μl) was used for ELISA (Roche Molecular Biochemicals, Indianapolis, IN).

Immunofluorescent Detection

Immunofluorescent staining was performed according to published method [18]. Briefly, NMuMG and PANC-1 cells were grown into chamber slides, and treated with 5 ng/ml TGF-β1 in the presence of SB-431542 (10 μM) for 24 hours for NMuMG and 60 hours for PANC-1 cells. Cells were fixed in ice-cold 100% methanol and then permeabilized in PBS containing 0.2% Triton X-100 for 10 minutes. Nonspecific sites were blocked with 3% BSA for 1 hour at room temperature. Cells were incubated with E-cadherin (1:1000) and ZO1 (1:300) primary antibodies for 2 hours at room temperature. After washing, cells were incubated for 1 hour with secondary antibody conjugated with Cy3 (Sigma Biochemicals) at room temperature. Cells were washed with PBS and visualized by fluorescence microscope. The intracellular localization of endogenous Smad proteins was studied using HaCaT cells as described above. Nuclei were stained with DAPI (Vector Laboratories, Burlingame, CA).

Cell Migration Assay

A549 cells were seeded in the upper chamber of 8-μM pore transwells coated with collagen and conditioned medium was added as a chemoattractant in the lower chamber. Cells were incubated with 5 ng/ml TGF-β1 in the presence of SB-431542 in 4% FBS media for 5 hours. Cells that migrated through the pores of the filter were stained and counted from six random fields of view at 400× magnification, and the data were expressed as the average number of cells per field of view.

Cell Invasion Assay

For invasion through collagen layer, the upper surface of 8-μM pore transwell chamber was overlaid with 100 μg of collagen dissolved in 100 μl of PBS, and dried overnight. For invasion through Matrigel (BD Biosciences, San Diego, CA) layer, 40 μl (~100 μg) of diluted Matrigel solution was overlaid on the upper surface of the abovementioned transwell chambers. The Matrigel was allowed to gel by incubating the plates for at least 2 hours at 37°C. Thereafter, A549 (30,000) cells in a volume of 100 μl of RPMI media containing

0.2% BSA were added to the upper chamber of each well. Four percent FBS containing RPMI media with 5 ng/ml TGF-β1 in the presence of SB-431542 was used as a chemoattractant in the lower chamber as mentioned above, and then incubated at 37°C for 21 hours. Cells that invaded through the filter were stained and counted from six random fields of view at ×200 magnification and the data were expressed as the average number of cells per field of view.

Wound Healing Assay

MDA-MB-231 cells (2×10^5) were seeded into 12-well plates and allowed to attach for 20 hours. Cells were then serum-starved for 32 hours prior to wounding. Plastic tips were used to make wounding across the cell monolayer. Wounded cells were treated with 3 ng/ml TGF-β1 in the presence of SB-431542 for 30 hours. Phase contrast pictures were taken by an inverted microscope using a magnification of ×200.

ELISA for VEGF and TGF-β1

A total of 100,000 cells from each pool of A549 and HT29 were seeded into each well of 12-well plate. Cells were cultured in media containing 0.2% FBS for 18 hours, and then treated with 5 ng/ml TGF-β1 in the presence of SB-431542 (10 μM) in 0.5 ml of media for 24 hours. One hundred microliters of each supernatant media was used for VEGF assay according to the manufacturer's instruction (R&D Systems, Minneapolis, MN). For TGF-β1 ELISA, 100,000 cells from each pool of A549, VMRC-LCD, and HT29 were seeded into each well of 12-well plates and serum-starved for 20 hours. Cells were then treated with SB-431542 in 0.5 ml of serum-free RPMI media for 24 hours. One hundred microliters of each supernatant media was activated and used for TGF-β1 assay according to the manufacturer's instruction (R&D Systems).

Soft Agarose Assay

A total of 5×10^3 cells from each pool of A549 and VMRC-LCD, and 10×10^3 cells from HT29 were suspended in 1 ml of 0.4% sea plaque agarose containing 10% FBS medium and then plated on the top of 1 ml of semisolidified 0.8% agarose in the same medium in 35-mm plates. Plates were incubated for 2 weeks at 37°C in the presence of 5% CO_2 in a humidified incubator. SB-431542 (10 μM) was added on the top agar layer every fourth day. Colonies grown on soft agarose were counted by automated colony counter, and the pictures of colonies on soft agarose were taken.

Results

SB-431542 Prevents TGF-β–Induced Complex Formation between Smad2/3 and Smad4, and Blocks Nuclear Translocation of Smad2 and Smad3

To determine the potential role of SB-431542 on downstream TGF-β signaling, we tested its ability in blocking functional complex formation between Smad2/3 and Smad4 *in vivo*. Lysates from 293T, FET, and A549 cells were subjected to anti-Smad2/3 immunoprecipitation followed by immunoblotting with anti-Smad4 antibodies. TGF-β1 induced the complex formation between Smad2/3 and Smad4 in all cell types. Treatment of cells with SB-431542 blocked TGF-β–induced complex formation in a dose-dependent manner (Figure 1*A*, *top panel*). The reduced Smad complex formation by the inhibitor was due to blockade of TGF-β–induced phosphorylation and activation of Smad2/3, as shown in Figure 1*A*, *second panel*. To determine the effect of the inhibitor on TGF-β–mediated nuclear translocation of Smad proteins, we analyzed the cytoplasmic (C) and nuclear (N) fractions after treating A549 cells with TGF-β and/or SB-431542. TGF-β induced nuclear translocation of Smad2, Smad3, and Smad4 within 30 minutes, and this was blocked by SB-431542. SB-431542 alone did not show any effect on the localization of Smad proteins (Figure 1*B*). Complete separation of cytoplasmic and nuclear proteins was tested by Western blot analysis for RhoGDI and PARP, respectively. We also found similar results in Mv1Lu, RIE, and NIH-3T3 cell lines (data not shown). To further determine the subcellular localization of endogenous Smad2 and Smad3, HaCaT cells were treated with TGF-β in the presence of SB-431542 for 1 hour and analyzed by immunofluorescence using anti-Smad2 and anti-Smad3 antibodies. It is clear that, after TGF-β stimulation, Smad2 and Smad3 translocate to the nucleus, as shown in Figure 1*C*. SB-431542 blocked TGF-β–mediated nuclear transport of Smad2 and Smad3 and, as a result, Smad2 and Smad3 remained predominantly in the cytoplasm (Figure 1*C*). These results demonstrate that SB-431542 blocks TGF-β–induced complex formation between Smad2/3 and Smad4, and the nuclear translocation of Smad2 and Smad3.

SB-431542 Blocks TGF-β–Induced Transcriptional Responses

To test whether the inhibition of Smad complex formation affects downstream transcriptional responses mediated by TGF-β, we performed transient transfection assays using TGF-β–responsive reporters p3TP-Lux and (CAGA)9-MLP-Luc. TGF-β strongly induced reporter activities in both FET (Figure 2*A*) and Mv1Lu cells (data not shown). SB-431542 blocked these TGF-β1–mediated transcriptional responses in a dose-dependent manner. To examine whether SB-431542 has a similar effect on natural promoters, we performed transient transfection assays with two TGF-β–sensitive gene promoter reporters, p21 Cip1 and PAI-1. SB-431542 blocked TGF-β–induced p21 Cip1 and PAI-1 promoter activation (Figure 2, *B* and *C*). These results suggest that SB-431542 inhibits the activation of Smad pathway and blocks TGF-β–induced transcriptional responses.

SB-431542 is a Potent Inhibitor of TGF-β–Regulated Protein Expression

To investigate the effect of SB-431542 on endogenous protein expression induced by TGF-β, we treated Mv1Lu and RIE cells with TGF-β and/or SB-431542 and tested the expressions of p21 Cip1, PAI-1, and fibronectin using

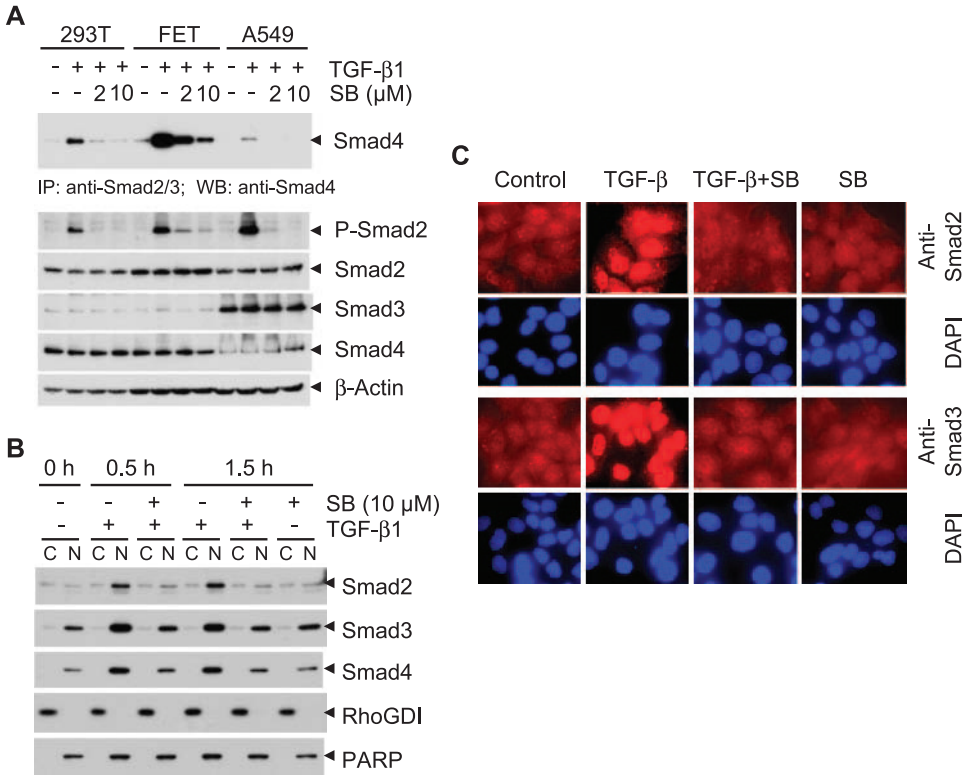

Figure 1. *Effect of SB-431542 in blocking TGF-β signaling. (A) Immunoprecipitation. 293T, FET, and A549 cells were treated with 12.5 ng/ml TGF-β1 in the presence of SB-431542 (SB) for 1 hour. Cell lysates were subjected to immunoprecipitation with anti-Smad2 and anti-Smad3 polyclonal antibodies, and the immunoprecipitates were analyzed by Western blot analysis with anti-Smad4 antibody (top). Cell lysates were subjected to Western blot analysis with anti–phospho Smad2, anti-Smad2, anti-Smad3, and anti-Smad4 antibodies (bottom section). An equal amount of protein loading was verified by Western blot analysis with anti–β-actin monoclonal antibody. Each experiment was repeated four times. (B) Nuclear translocation of Smad proteins was analyzed by separating cytoplasmic (C) and nuclear (N) fractions after treating A549 cells with TGF-β and/or SB-431542 for either 30 or 90 minutes. Smad proteins were detected by Western blot analysis using antibodies against Smad2, Smad3, and Smad4. Complete fractionations of cytoplasmic and nuclear proteins were verified by Western blot analysis with antibodies against Rho-GDI and PARP. (C) Blockade of TGF-β–induced nuclear import of Smad2 and Smad3 by SB-431542 in HaCaT cells as detected by immunofluorescence. Cells were serum-starved and treated with TGF-β in the presence of SB-431542 for 1 hour and processed for immunofluorescence using either anti-Smad2 or anti-Smad3 polyclonal antibodies. Fluorescence was visualized by antirabbit antibody conjugated to Cy3 using a Zeiss Axioplan fluorescence microscope. Nuclei of the same cells were stained with DAPI. Each experiment was repeated four times.*

Western blot analyses, as shown in Figure 3, *A* and *B*. TGF-β induced the expression of PAI-1 protein in both cell types, and SB-431542 inhibited the induction of PAI-1 by TGF-β in a dose-dependent manner (Figure 3, *A* and *B*). SB-431542 also inhibited TGF-β–mediated upregulation of fibronectin and p21 Cip1 (Figure 3, *A* and *B*) and decreased down to basal level. These data, in combination with the results discussed above, indicate that SB-431542 is a potent inhibitor of TGF-β–induced transcription and gene regulation.

Effect of SB-431542 on Tumor-Suppressor Functions of TGF-β

One of the most important biologic effects of TGF-β is its ability to inhibit proliferation of many cell types, including most epithelial cells [19]. However, under transforming conditions, the growth of tumor cells is occasionally stimulated by TGF-β. We examined whether SB-431542 inhibits TGF-β–induced growth arrest by [^{3}H]thymidine incorporation assay (Figure 4*A*). TGF-β alone shows a strong inhibition of [^{3}H]thymidine incorporation in FET cells by 91% at a concentration of 1.0 ng/ml. SB-431542 inhibited this TGF-β–induced inhibition of [^{3}H]thymidine incorporation from 91% to 40% at 2 μM concentration (Figure 4*A*). We then examined the ability of SB-431542 to block the TGF-β–mediated inhibition of cell proliferation by cell counting over the duration of culture for 6 days (Figure 4*B*). TGF-β efficiently suppressed the growth of Mv1Lu cells, whereas SB-431542 significantly blocked TGF-β–induced growth inhibition (Figure 4*B*). To examine whether SB-431542 promotes cells from G0–G1 phase to S phase by blocking TGF-β–induced G1 arrest, we performed flow cytometric analyses by propidium iodide staining of FET, RIE, and Mv1Lu cells. TGF-β1 strongly arrested cells in the G0–G1 phase of the cell cycle with corresponding decrease of cells in the S phase. SB-431542 significantly suppressed TGF-β–induced G1 arrest and induced the accumulation of more cells in the S phase of the cell cycle (Figure 4*C*).

Cell proliferation and programmed cell death are tightly regulated in normal cells, and disruption of this balance may eventually lead to the development of tumors. TGF-β acts as a potent inducer of apoptosis, and this TGF-β–induced apoptosis is cell type–specific. To further characterize whether SB-431542 is able to inhibit TGF-β–induced apoptosis, we performed a quantitative cell death ELISA assay

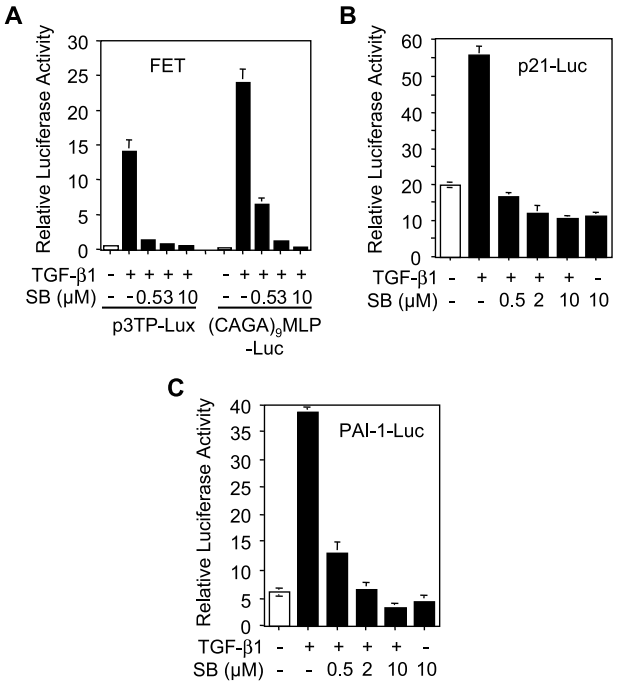

Figure 2. *Effect of SB-431542 in blocking TGF-β–induced reporter gene activation. (A) Reporter assay. FET cells were transiently transfected with CMV-β-gal and p3TP-Lux or (CAGA)9MLP-Luc reporter plasmids. Cells were then treated with 5 ng/ml TGF-β1 in the presence of SB-431542 (0.5, 3, and 10 μM) for 22 hours. Luciferase activity was normalized to β-gal activity, and the relative luciferase activity was expressed as the mean ± SD of triplicate measurements. (B and C) Reporter assay. HepG2 cells were transiently transfected with CMV-β-gal and p21-Luc (B) or PAI-1-Luc (C) plasmids. Cells were treated with 5 ng/ml TGF-β1 in the presence of SB-431542 (0.5, 2, and 10 μM) for 22 hours. Cell lysates were analyzed for both luciferase and β-gal activities as mentioned above. Each experiment was repeated four times.*

as well as qualitative DNA laddering with HepG2 cells. This cell line is known to be responsive to TGF-β–induced apoptosis [20]. TGF-β induced apoptosis in these cells by four-fold (Figure 4*D*). At 2 μM concentration, SB-431542 completely blocked TGF-β1–induced apoptosis. We also found that TGF-β induced a significant amount of DNA fragmentation in HepG2 cells, which was efficiently abrogated by SB-431542 (Figure 4*E*). Taken together, these data suggest that treatment with SB-431542 inhibits TGF-β–induced tumor-suppressor functions including regulation of growth and apoptosis.

SB-431542 Inhibits TGF-β–Induced EMT in NMuMG and PANC-1 Cells

The increased expression and activation of TGF-β by tumor cells profoundly induce the progression of epithelial tumors to the metastatic stage by altered cellular plasticity, loss of cell–cell contacts, increased cell migration and invasion, and degradation of the extracellular matrix. Epithelial to mesenchymal transition (EMT) in epithelial cells is characterized by the acquisition of spindle morphology and increased motility with the loss of tight and adherens junctions. To examine the effect of SB-431542 on TGF-β–induced EMT, we used mouse mammary epithelial cells (NMuMG). TGF-β treatment changes the cell morphology from cuboidal to an elongated spindle-like shape within 24 hours (Figure 5*A*, *top panel*), consistent with previous studies [18]. SB-431542 inhibited TGF-β–induced EMT and delocalization of E-cadherin and ZO1 proteins from cellular junctions (Figure 5*A*). We then verified whether disruption of cell–cell adhesions was due to the altered expression of junctional proteins. No detectable changes in the expression of E-cadherin and β-catenin were observed by either TGF-β or the inhibitor (Figure 5*B*). Interestingly, the expression of ZO1 was downregulated by TGF-β, which was restored by SB-431542 in a dose-dependent manner (Figure 5*B*). We observed a similar SB-431542–mediated blockade of EMT and delocalization of E-cadherin in pancreatic adenocarcinoma cells (PANC-1) induced by TGF-β (Figure 5*C*). TGF-β induced EMT in PANC-1 cells as described previously [21]. We further determined the expression of junctional E-cadherin protein, and we found that E-cadherin is downregulated by TGF-β in PANC-1 cells. Under similar conditions, we did not observe any change in the level of ZO1 (data not shown). Interestingly, SB-431542 treatment completely restored TGF-β–induced downregulation of E-cadherin in PANC-1 cells (Figure 5*D*). These data suggest that TGF-β–mediated EMT, together with the delocalization of junctional molecules, can be blocked by SB-431542.

SB-431542 Blocks TGF-β–Induced Tumor Cell Migration and Invasion

TGF-β is known to stimulate chemotaxis and migration of tumor cells [22,23], although not all tumor cell lines are responsive to TGF-β–induced migration and invasion. In an attempt to study the effect of SB-431542 on TGF-β–mediated cell migration and invasion, we observed that TGF-β stimulated the chemotactic migration of lung adenocarcinoma (A549) cells by three-fold through a polycarbonate membrane (Figure 6*A*). SB-431542 completely blocked TGF-β–induced migration of A549 cells. TGF-β enhanced the invasion of A549 cells by three-fold through the collagen membrane (Figure 6*B*), whereas a six-fold increase in invasion of A549 cells was observed through Matrigel barrier (Figure 6*C*). However, SB-431542 strongly attenuated TGF-β–mediated

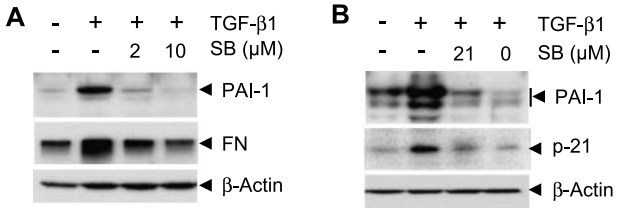

Figure 3. *SB-431542 blocks TGF-β–induced PAI-1, fibronectin (FN), and p21 CIP1 expression. (A) Mv1Lu cells were serum-starved for 16 hours and treated with 5 ng/ml TGF-β1 in the presence of SB-431542 for 6 hours. Cell lysates were analyzed by Western blot analysis with anti–PAI-1 and anti-FN antibodies. (B) RIE cells were treated as above and cell lysates were analyzed by Western blot analysis with anti–PAI-1 (Torrey Pines Biolabs, Inc., Houston, TX) and anti-p21 (Santa Cruz Biotechnology, Inc.) antibodies. An equal amount of protein loading was verified by Western blot analysis with anti–β-actin antibody. Each experiment was repeated three times.*

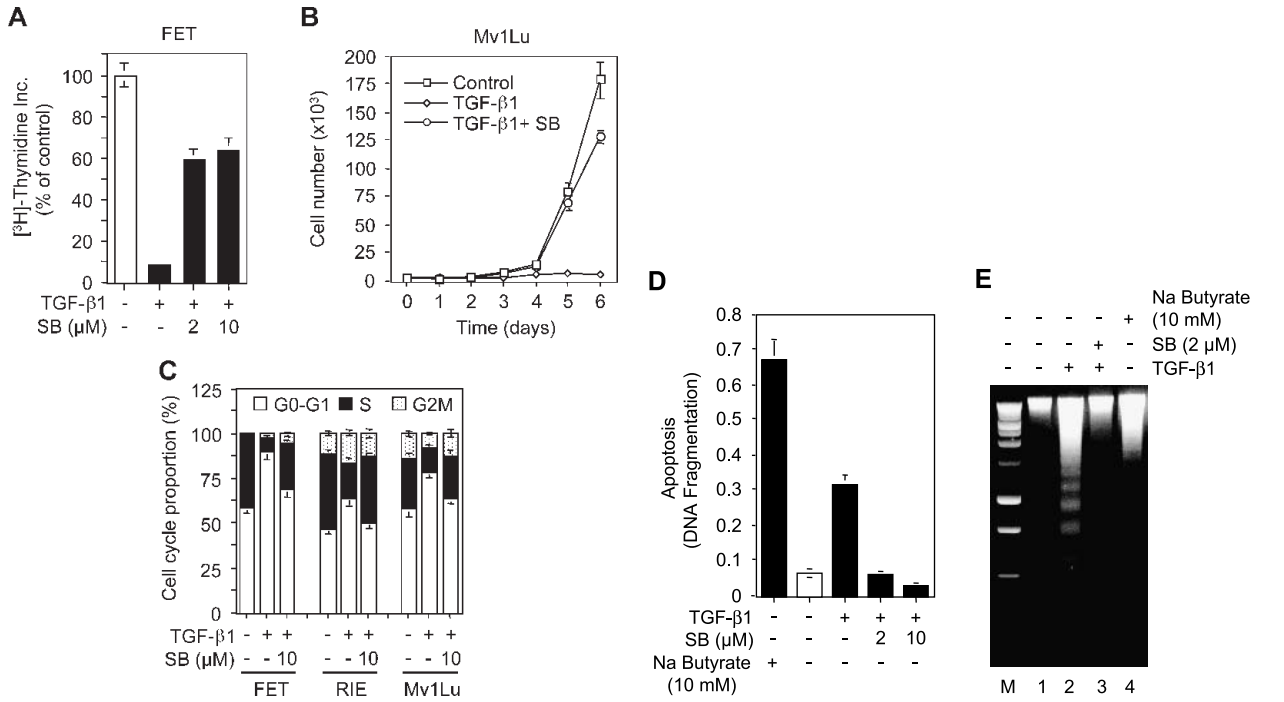

Figure 4. *SB-431542 inhibits TGF-β–induced growth suppression and apoptosis. (A) [³H]thymidine incorporation assay. FET cells were treated with TGF-β1 in the presence of SB-431542 for 26 hours and then treated for an additional 2 hours with [³H]thymidine. Cells were fixed in TCA, washed, and lysed in 0.2 N NaOH. Radioactivity incorporated into TCA-insoluble [³H]thymidine was measured by scintillation counting. Individual data points are the mean ± SD of triplicate determinations. (B) Cell counting assay. Mv1Lu cells were cultured in DMEM containing 10% FBS and treated with 5 ng/ml TGF-β1 in the presence of SB-431542 (10 μM) for a total of 6 days. Cells were counted every day and the cell numbers are plotted. Individual data points are the mean ± SD of triplicate determinations. (C) FACScan analysis. FET, RIE, and Mv1Lu cells were treated with 5 ng/ml TGF-β1 in the presence of SB-431542 for 35 hours. Cells were collected and evaluated for DNA content by flow cytometric analyses. Results are expressed as the mean ± SD of three replicate values of the percentage of cells in different phases of the cell cycle. (D) Quantitative cell death ELISA. HepG2 cells were treated with 5 ng/ml TGF-β1 in the presence of SB-431542 for 24 hours. Cell lysates were analyzed by cell death ELISA as described in the Materials and Methods section. An individual data point is a representative of the mean ± SD of three individual measurements. Each experiment is repeated three times. (E) DNA laddering. HepG2 cells were treated with 5 ng/ml TGF-β1 in the presence of SB-431542 (2 μM) in serum-free medium for 46 hours. Cells were lysed and fragmented DNA was isolated from the cell lysates. Each DNA was loaded onto 1.6% agarose gel and visualized by staining with ethidium bromide.*

invasion in a dose-dependent manner. At 10 μM concentration, it blocked both basal and TGF-β–induced invasion in these cells. We further examined the role of SB-431542 in blocking TGF-β–induced cell migration by a wound healing experiment using the breast carcinoma cell line, MDA-MB-231. This cell line is responsive to TGF-β in wound healing experiments as shown before [24]. TGF-β accelerated wound closure, whereas SB-431542 inhibited TGF-β–induced cell motility and, as a result, the wound remained opened (Figure 6*D*). To verify whether the wound closure in MDA-MB-231 cells is due to TGF-β–induced proliferation or TGF-β–induced migration, we performed cell counting and thymidine incorporation assays, as shown in Figure 6*E*. Our results show that MDA-MB-231 cells are growth-inhibited by about 50% in cell counting experiments (Figure 6*E*, *left*) as reported previously. Thymidine incorporation in these cells was inhibited by TGF-β (Figure 6*E*, *right*). Therefore, proliferation is not involved in the migration of MDA-MB-231 cells induced by TGF-β.

TGF-β is known to induce tumor-promoting effects under transforming conditions, sometimes in cooperation with oncogenic Ras. Numerous studies have suggested that the induction in COX-2 level in human cancers provides growth and survival advantage, and increases invasiveness and angiogenesis. TGF-β synergistically enhances the expression of COX-2 in conditionally Ha-Ras–transformed rat intestinal epithelial cells (RIE-iRas) reported previously [17]. To examine whether SB-431542 can block TGF-β and Ha-Ras–induced COX-2 expression, we treated RIE-iRas cells with IPTG and/or TGF-β in the presence or absence of SB-431542 for 24 hours. IPTG induced activated Ras expression (Figure 6*F*, *second panel*). Activated Ras and TGF-β either independently or synergistically induced COX-2 expression in this cells (Figure 6*F*, *top panel*). SB-431542 blocked TGF-β–induced COX-2 expression in RIE-iRas cells, although IPTG-induced Ha-Ras–mediated COX-2 expression remained unchanged (Figure 6*F*), suggesting a role of SB-431542 in an inhibition of TGF-β–induced COX-2 expression in RIE-iRas cells. Taken together, these results show that SB-431542 can block the tumor-promoting effects of TGF-β including cell migration and invasion, and COX-2 expression.

SB-431542 Blocks VEGF Secretion in Human Tumor Cell Lines

VEGF secretion is upregulated in human tumors and its secretion is often elevated by TGF-β. We tested whether SB-431542 has any effect on VEGF production induced by

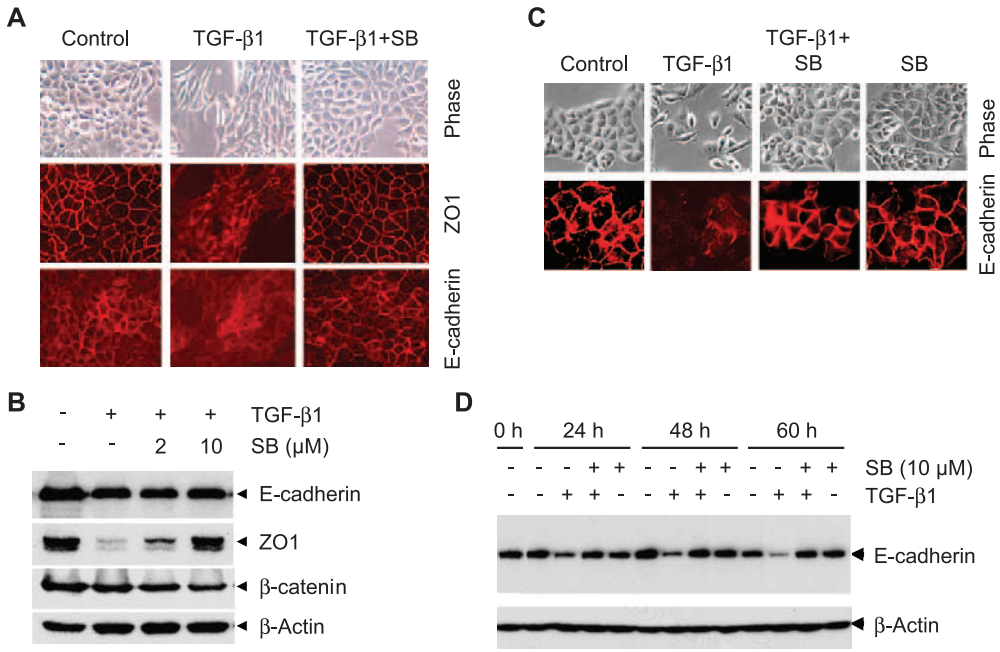

Figure 5. *SB-431542 abrogates TGF-β–induced EMT in both NMuMG and PANC-1 cells. (A) NMuMG cells were treated with 5 ng/ml TGF-β1 in the presence of SB-431542 (10 μM) for 24 hours. Cells were stained with anti–E-cadherin and anti-ZO1 antibodies, and visualized by antimouse antibody conjugated to Cy3. Phase contrast images were recorded at ×200 magnification. (B) Cell lysates were prepared from NMuMG cells as treated above, and were analyzed by Western blot analysis with anti–E-cadherin, anti-ZO1, and anti–β-catenin antibodies. An equal amount of protein loading was verified by Western blot analysis with anti–β-actin antibody. (C) PANC-1 cells were treated with 5 ng/ml TGF-β1 in the presence of SB-431542 (10 μM) for 60 hours as described before, fixed, and incubated with anti–E-cadherin antibody, and visualized by antimouse antibody conjugated to Cy3 as mentioned above. Phase contrast images were recorded at ×200 magnification. (D) PANC-1 cells were treated with TGF-β1 in the presence of SB-431542 for 24, 48, and 60 hours. Cell lysates were analyzed by Western blot analysis with anti–E-cadherin antibody. An equal amount of protein loading was verified by Western blot analysis with anti–β-actin antibody. Each experiment was repeated three times with similar results.*

TGF-β in A549 and HT29 cells. These two cell lines produce good amounts of TGF-β and VEGF. TGF-β induced VEGF secretion by five-fold in A549 cells within 24 hours, and this effect was completely blocked by the inhibitor, as determined by ELISA (Figure 7*A*). A549 cells produced a significant amount of TGF-β as shown in Figure 7*C*. In contrast, in HT29 cells, exogenous TGF-β induced only 1.7-fold VEGF secretion. Interestingly, SB-431542 efficiently blocked the VEGF secretion induced by both exogenous and endogenous TGF-β (Figure 7*B*). We did not observe any significant inhibition of basal VEGF secretion in A549 cells (Figure 7*A*). We observed that HT29 cells secrete a significant level of TGF-β (Figure 7*C*). We further observed that TGF-β secretion in these cells was not significantly blocked by the inhibitor, suggesting that the blockade of VEGF production is due to the blockade of signaling from endogenous TGF-β, and not due to attenuation of the secretion of TGF-β. Interestingly, we did not observe any alteration in VEGF secretion in VMRC-LCD cells by either TGF-β or SB-431542 (data not shown), although TGF-β secretion in these cells is similar to that of A549 and HT29 cells (Figure 7*C*). These findings further indicate the therapeutic efficacies of SB-431542 in blocking the tumor-promoting effects of TGF-β.

Differential Effects of SB-431542 on Anchorage-Independent Growth of Human Cancer Cell Lines

Anchorage-independent growth in semisolid medium is considered to be a useful parameter in assessing the malignancy of human cells. To evaluate the effect of blockade of TGF-β signaling by SB-431542 on anchorage-independent growth, we performed soft agar assays with three cell lines: 1) A549 cell line that is responsive to the growth-inhibitory effect of TGF-βl 2) VMRC-LCD cells that lack TβRII and are not growth-inhibited by TGF-β; and 3) HT29 cells that are growth-promoted by TGF-β. HT29 and VMRC-LCD cells produce bigger colonies than that of A549 cells on soft agarose within 2 weeks (Figure 8, *A*, *C*, and *E*). Interestingly, SB-431542 treatment stimulates colony formation in both number and size by A549 cells, whereas no effect on colony growth was observed in the case of VMRC-LCD cells (Figure 8, *A*, *C*, and *G*) when compared with untreated cells. However, we found that SB-431542 dramatically suppressed the colony growth of HT29 cells (Figure 8, *E* and *G*). In most human tumors, cells first become resistant to TGF-β–induced growth inhibition and, later during cancer progression, high levels of TGF-β can promote tumor growth in an autocrine and/or paracrine manner. We hypothesized that blockade of TGF-β–induced autocrine growth-inhibitory effect in A549 cells might lead to an aggressive tumorigenic phenotype, whereas abrogation of TGF-β–mediated cell proliferation by SB-431542 in HT29 cells might decrease the tumorigenic potential. To confirm this, we tested the effects of TGF-β and the inhibitor on the growth of these three cell lines. We observed that TGF-β inhibited the growth of A549 cells by 40% and SB-431542 blocked this effect induced by both exogenous and endogenous TGF-β (Figure 8*B*).

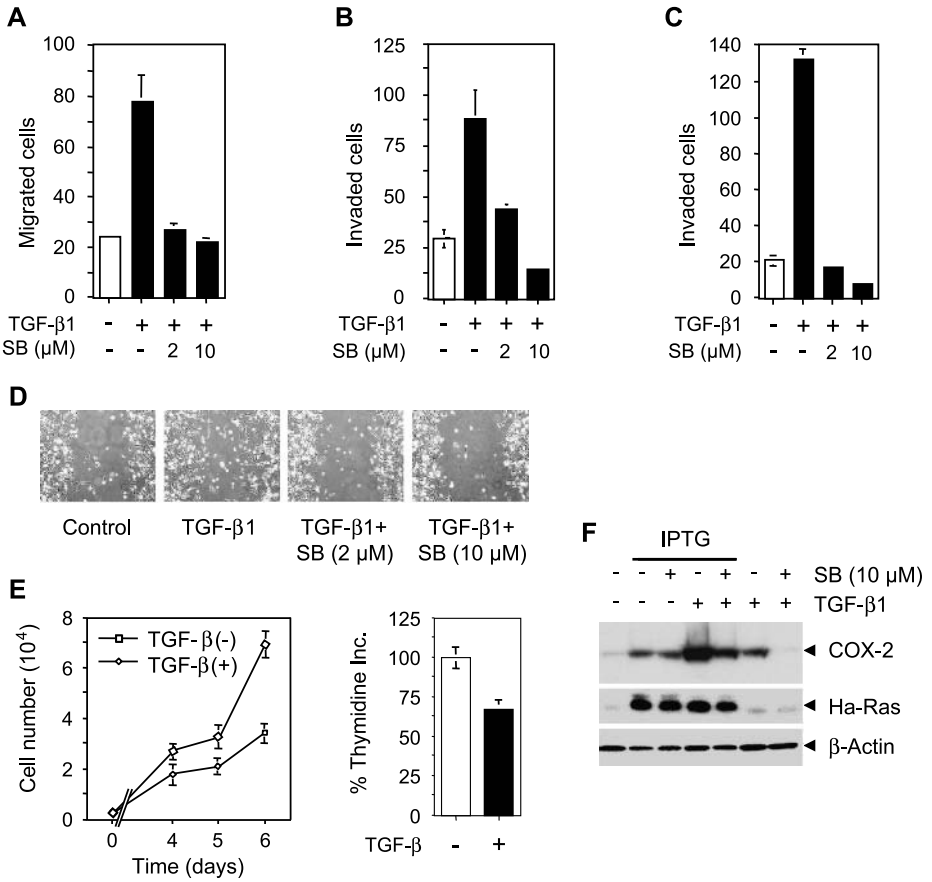

Figure 6. *SB-431542 abrogates TGF-β–induced migration, invasion, wound healing, and COX-2 expression. (A) Migration assay. A549 cells were treated with TGF-β, and SB-431542 cells were allowed to migrate through a 8-μM pore in transwells. Cells that migrated through the pores were stained and counted. The data were represented as the mean ± SD of three independent wells. (B and C) Invasion assays. A549 cells were treated as above and allowed to pass through collagen-coated (B) and Matrigel-coated (C) membrane in transwells. Cells that invaded through the filter were stained and counted. Individual data point is represented as the mean ± SD of three independent wells. (D) Wound healing. Wounded MDA-MB-231 cells were treated with 3 ng/ml TGF-β1 for 30 hours in the presence of SB-431542. Phase contrast images were shown. All of these experiments were repeated four times. (E) Growth inhibition of MDA-MB-231 cells by TGF-β was determined by cell counting (left) and thymidine incorporation (right) assays as described before. (F) Effect of SB-431542 on TGF-β–induced COX-2 expression. Oncogenic Ras-inducible rat intestinal epithelial (RIE-iRas) cells were treated with 5 mM IPTG and/or 5 ng/ml TGF-β1 in the presence of SB-431542 (10 μM) for 24 hours. Cell lysates were analyzed by Western blot analysis with anti–COX-2 (Santa Cruz Biotechnology) and anti–Pan-Ras (Oncogene Research Products) antibodies. An equal amount of protein loading was verified by Western blot analysis with anti–β-actin antibody. This experiment was repeated three times with similar results.*

TGF-β or the inhibitor has no effect on the growth of VMRC-LCD cell lines (Figure 8*D*). Interestingly, TGF-β stimulated the proliferation of HT29 cells. SB-431542 blocked the growth stimulation mediated by both endogenous and exogenous TGF-β as evident from [^{3}H]thymidine incorporation assay (data not shown) and cell counting assay (Figure 8*F*). These data suggest that SB-431542 can enhance the tumorigenicity of A549 cells by blocking the growth-inhibitory effect of TGF-β, whereas it can inhibit the tumorigenicity of HT29 cells by blocking the tumor-promoting function of TGF-β. Therefore, SB-431542 may be useful as a therapeutic agent when tumors lose TGF-β–induced growth suppression and when tumor-promoting effects of TGF-β predominate during cancer progression.

Discussion

Increased production of TGF-β in human tumors is correlated with a poor prognosis [25]. Small molecule inhibitors of TGF-β signaling could be useful for the development of therapeutic strategies for human cancers. In most human tumors, cells first become resistant to TGF-β–induced growth inhibition/apoptosis and, later during cancer progression, high levels of TGF-β can promote tumor growth in an autocrine and/or paracrine manner through the changes that favor invasion and metastasis. The most desirable approach for developing a new therapeutic strategy by targeting TGF-β signaling would be to retain TGF-β–induced tumor-suppressor function, but to block TGF-β signaling in advanced invasive and metastatic cancers. Previous studies with pan-TGF-β–neutralizing antibodies and soluble TβRII-Fc fusion protein suggest that suppression of tumor progression by blocking TGF-β signaling network may provide an attractive target for therapeutic intervention [9]. However, little is known about how the small molecule inhibitors of TGF-β receptor kinase activity might regulate the tumor-suppressive or tumor-promoting effects of TGF-β, and when these inhibitors will be useful for treatment during cancer progression. In the

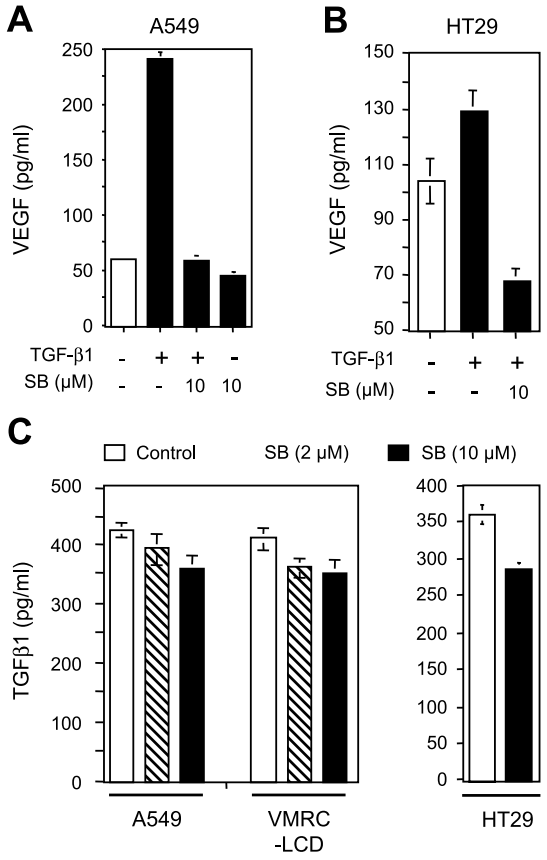

Figure 7. *SB-431542 blocks TGF-β–induced VEGF secretion. A549 (A) and HT29 (B) cells were treated with 5 ng/ml TGF-β1 in the presence of SB-431542 (10 μM) for 24 hours. Supernatant media were analyzed for VEGF secretion by ELISA. (C) ELISA for TGF-β1. A549, VMRC-LCD, and HT29 cells express similar amounts of TGF-β1. Cells were treated with SB-431542 for 24 hours. Supernatant media were activated and used for TGF-β1 assay. Individual data point in either VEGF or TGF-β assay is a representative of the mean ± SD of three individual measurements. Each experiment is repeated at least three times.*

present study, we have demonstrated that SB-431542, a novel ALK5 kinase inhibitor, inhibits the tumor-suppressive function of TGF-β including growth inhibition and apoptosis in several cell types. In contrast, SB-431542 abrogates the pro-oncogenic functions of TGF-β including cell migration, invasion, VEGF secretion, and EMT. We also demonstrate that SB-431542 induces anchorage-independent growth of A549 cells that are growth-inhibited by TGF-β. Interestingly, this inhibitor decreases the tumorigenicity of highly aggressive HT29 cells that are growth-stimulated by TGF-β. However, SB-431542 has no effect on colony formation by VMRC-LCD cells that are not responsive to TGF-β. These studies suggest that small molecule inhibitors of TGF-β receptor kinase activity may be useful in developing a new therapeutic strategy for the treatment of cancers that are resistant to TGF-β–mediated tumor-suppressor function.

A previous study demonstrated that SB-431542 inhibits the activity of ALK5, ALK4, and ALK7, but it has no effect on BMP signaling. However, SB-431542 has no direct effect on ERK, JNK, or p38 MAP kinase pathways [15,16]. Our study with SB-431542 is significant in several ways. We show that this small molecule inhibitor of TGF-β receptor kinase activity blocks both tumor-suppressing function and tumor-promoting activity of TGF-β. Previous studies with soluble Fc:TβRII, used either as an injectable drug [13] or when stably expressed as a transgene [12], antagonize TGF-β signaling and reduce mammary tumor metastasis to the lung. The most encouraging observation in these studies is that soluble Fc:TβRII did not induce tumor-promoting effects by blocking TGF-β–mediated antitumor effects. Consistent with these data, our study indicates that SB-431542 does not completely block the growth-inhibitory effect of TGF-β in normal epithelial cells. We observed that the growth of HT29 cells is stimulated by TGF-β. Interestingly, the growth of HT29 cells induced by both endogenous and exogenous TGF-β was efficiently blocked by SB-431542. This is consistent with previous reports suggesting that SB-421542 inhibits TGF-β–induced proliferation of human glioma cells [26] and human osteosarcoma cells [27]. The expression of TGF-β, particularly TGF-β1, is increased in various tumor types that correlate with the poor patient prognosis [25]. Oft et al. [28] reported that autocrine TGF-β signaling is required for the induction of EMT, invasiveness, and metastasis. We found that TGF-β–induced EMT was blocked by SB-431542 in NMuMG mouse mammary epithelial cells (Figure 5*A*) and in PANC-1 human pancreatic cancer cell line (Figure 5*C*) by reversing delocalization and/or downregulation of junctional proteins. TGF-β downregulates ZO1 in NMuMG cells and E-cadherin in PANC-1 cells as shown in Figure 5, *B* and *D*. As EMT is a frequent event in the late stage of human carcinoma and TGF-β signaling is involved in EMT maintenance, blockade of EMT by the inhibitor increases its effectiveness as anticancer drug. We have shown that SB-431542 blocks the migration and invasion of tumor cells mediated by both endogenous and exogenous TGF-β, suggesting a potential role of the inhibitor in blocking metastasis.

Induction in COX-2 expression in human cancers is known to promote tumor growth, increase tumor cell invasion, and enhance tumor angiogenesis. Increased level of TGF-β in tumors may contribute to upregulation of COX-2 activity. Our study shows that SB-431542 can inhibit TGF-β–induced COX-2 expression, suggesting an effect of the inhibitor in blocking oncogenic functions of COX-2. Tumor angiogenesis is critical for the progressive growth and metastasis of a neoplasm. Increased TGF-β production in the tumor can induce the angiogenic factor, VEGF. Interestingly, the VEGF production in both A549 and HT29 cells is inhibited by SB-431542. These cells express a significant amount of TGF-β; however, this expression is not affected by the inhibitor. Although VMRC-LCD cells produce a similar amount of TGF-β, we did not see any change in the level of VEGF by TGF-β or SB-431542. This is because these cells are not responsive to TGF-β due to the lack of TβRII. These results suggest that the attenuated secretion of VEGF in cancer cell lines is through the blockade of TGF-β signal transduction pathway and not by blocking TGF-β secretion.

In this study, we also provide evidence regarding when these small molecule inhibitors will be useful as therapeutic agents during cancer progression. We have observed

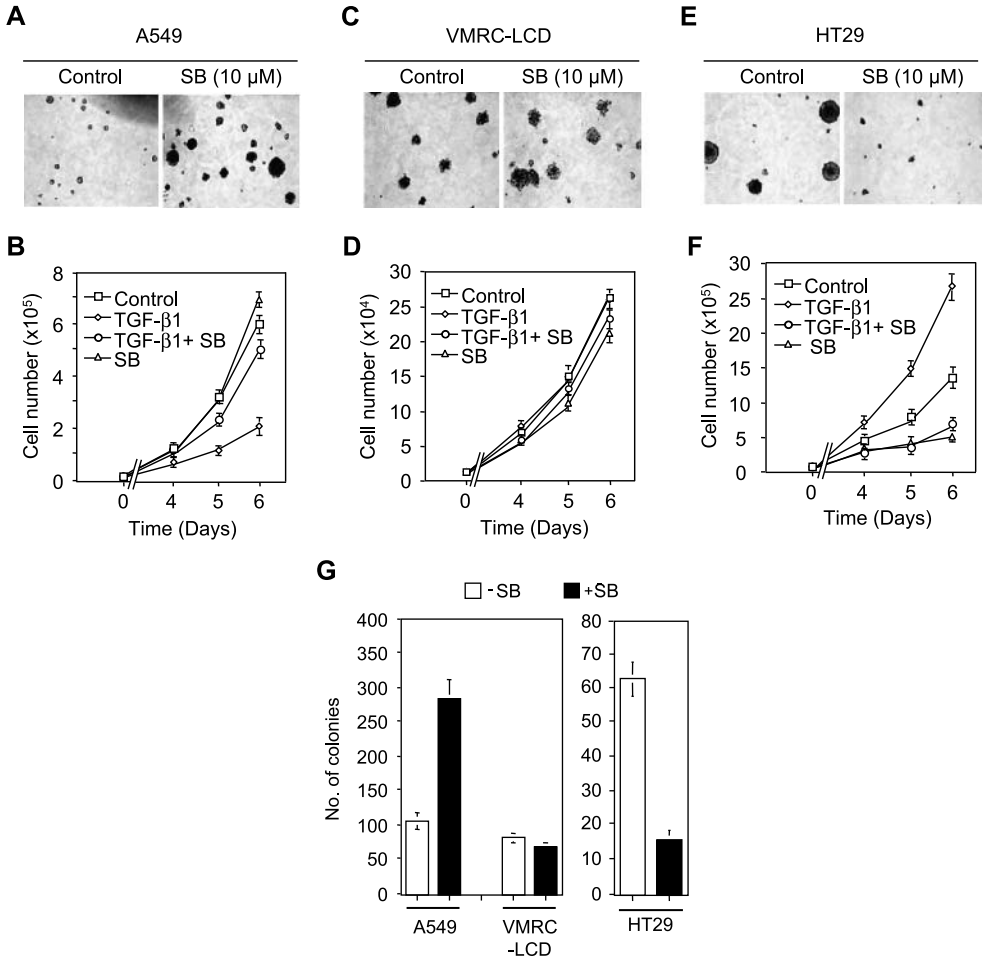

Figure 8. *Differential effects of SB-431542 on anchorage-independent growth of different cell lines. (A, C, and E) Soft agarose assay. A549, VMRC-LCD, and HT29 cells were plated and treated with SB-431542 (10 μM) for 2 weeks. Pictures of colonies grown on soft agarose were shown. (G) Colonies were counted by automated colony counter and the data are represented as the mean ± SD of three individual plates. (B, D, and F) Cell counting assay. A549 (B), VMRC-LCD (D), and HT29 (F) cells were treated with 7 ng/ml TGF-β1 in the presence of SB-431542 (10 μM) for a total of 6 days. Cells were counted and the results are plotted. Individual data points are the mean ± SD of triplicate determinations. Each experiment was repeated three times.*

differential effects of SB-431542 on the tumorigenic potential of different cancer cell lines. SB-431542 induces anchorage-independent growth of A549 cells as evident from both colony number and size in the soft agar assay. In contrast, SB-431542 dramatically suppressed the colony growth of HT29 cells. However, SB-431542 has no effect on colony formation in the case of VMRC-LCD cells that are not responsive to TGF-β due to lack of TβRII expression (Ref. [29] and unpublished data). HT29 cells have functional receptor complex, but lack Smad4. Therefore, these cells do not have a functional Smad pathway that is thought to be important for TGF-β–induced growth inhibition. However, TGF-β still can send signals in these cells through non-Smad pathways including ERK, p38 MAPK, JNK, PI3K, etc., which are supposed to be involved in pro-oncognic functions of TGF-β. In the HT29 cell line SB-431542 can block TGF-β signaling through these non-Smad pathways. In this cell line, SB-431542 inhibits the growth-stimulatory effects of endogenous TGF-β (Figure 8*F*). In contrast, A549 cells have an intact classic TGF-β–mediated growth-inhibitory Smad signaling (Figure 1*A*), which is inhibited by SB-431542. Interestingly, the endogenous TGF-β secreted by A549 cells inhibits growth and SB-431542 increases tumorigenicity by attenuating the growth-inhibitory effects of TGF-β (Figure 8*B*). SB-431542 has been shown to reduce TGF-β–induced inhibition of proliferation of embryonic stem cell–derived endothelial cells [30]. In VMRC-LCD cells, Smad and non-Smad pathways are not activated by TGF-β due to the absence of TβRII.

In conclusion, SB-431542 cannot block the tumorigenic growth of cancer cell lines that are either unresponsive to TGF-β, or are growth-inhibited by TGF-β. Abrogation of TGF-β–induced pro-oncogenic effects by SB-431542 decreases the tumorigenic potential of cancer cells. These inhibitors may also block paracrine pro-oncogenic effects of TGF-β produced by tumors that lack functional TGF-β receptors. Although the loss of TGF-β receptors in solid tumors is a rare event outside the GI tract, TβRII levels are reduced in many advanced human tumors through epigenetic mechanisms [10]. This low level of TβRII activation may facilitate pro-oncogenic effects in the advanced stage of human cancers (Figure 9). Our results from this preclinical

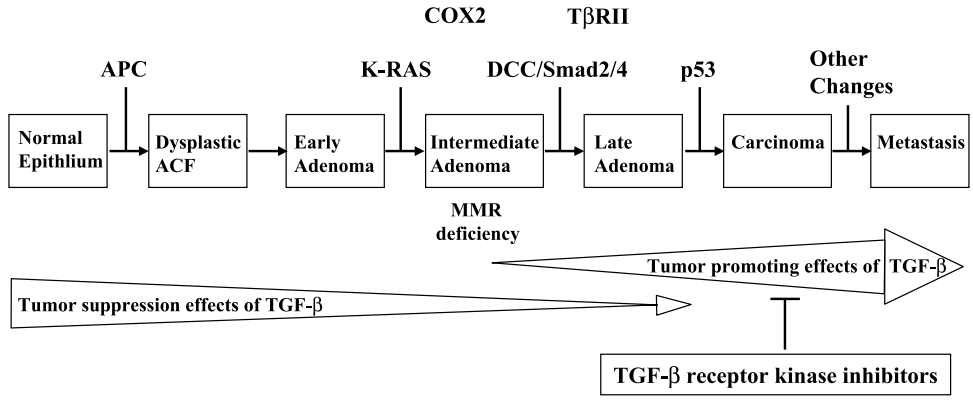

Figure 9. *Blockade of tumor-permissive effects of TGF-β by TRKIs represents a potential therapeutic approach. Genetic changes that are associated with colorectal cancer progression are shown [31] and used as an example. TGF-β has biphasic effects during tumorigenesis. It plays a tumor-suppressive role by its ability to inhibit growth, induce apoptosis, and inhibit genomic instability in normal epithelium and in the early stage of tumor progression. During cancer progression, high levels of TGF-β promote tumor growth in an autocrine and/or paracrine manner by inducing angiogenesis, invasion, and metastasis. The mechanism by which TGF-β promotes tumor progression represents a novel potential application of these inhibitors as therapeutic agents in advanced human cancers.*

study suggest that SB-431542 could be a potential therapeutic agent when tumors are resistant to TGF-β–induced growth suppression and apoptosis, and when tumor-promoting effects of TGF-β predominate during cancer progression (taking colorectal cancer as an example; Figure 9). Because an elevated level of TGF-β has been known to be associated with poor prognosis in a wide variety of human tumors, small molecule inhibitors of TGF-β activity could be useful for a developing therapeutic strategy for treating cancers refractory to classic antitumor agents. This study also suggests that identification of the mechanism underlying divergent responses (pro- and anti-oncogenic) may yield more specific therapeutic targets for novel small molecule inhibitors that would, ideally, preserve tumor-suppressive effects while abrogating the tumor-promoting effects of TGF-β.

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