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Differential Activation of Smads in HeLa and SiHa Cells That Differ in Their Response to Transforming Growth Factor-β*

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We assessed the responsiveness of six human cervical cancer cell lines to transforming growth factor (TGF)-β with p3TP-lux reporter assay and found that HeLa and SiHa cells were highly responsive to TGF-β. However, when pSBE4-BV/Luc reporter with four Smad binding elements was used, only the SiHa, not the HeLa, cells showed Smad activation. Smad DNA binding activity was relatively more in SiHa than in HeLa cells upon TGF-β treatment, and the active complex contained Smad 2 and Smad 4. In 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assays, HeLa cells treated with 5 ng/ml of TGF-β for 24 h showed proliferation, whereas SiHa cells showed growth inhibition under the same conditions. TGF-β treatment resulted in G_0/G_1 arrest with a reduction in S-phase only in SiHa cells. A chemical inhibitor of Smad activation (SB203580) blocked the growth inhibitory effect of TGF-β in SiHa, whereas the proliferative response in HeLa was unaffected. TGF-β-induced translocation of phospho-Smad 2 was relatively less in HeLa than in SiHa cells. MAPK activation occurred within 5 min and persisted up to 15 min upon TGF-β treatment in HeLa but was negligible in SiHa cells. TGF-β activated JNK in HeLa, but SiHa cells showed a down-regulation of its activity. When an inhibitor of MAPK (U0126) was used, the TGF-β-mediated proliferative response in HeLa cells was completely abolished. SB203580 did not affect MAPK activation induced by TGF-β in HeLa cells. We report for the first time an activation, presumably independent of Smad activation, of TGF-β-dependent MAPK within 5 min of treatment that resulted in cell cycle progression in a cervical adenocarcinoma cell line, HeLa.

The transforming growth factor-β (TGF-β)[1] family includes a large number of structurally related polypeptide growth factors, TGF-βs, activins, and bone morphogenetic proteins, that are important in homeostasis and repair of virtually all tissues (1). TGF-βs were identified as regulators of mesenchymal growth and differentiation, cell cycle arrest in epithelial and hematopoetic cells, extracellular matrix production, and immunosuppression (2). TGF-β induces the assembly of heteromeric complexes of transmembrane serine/threonine kinase receptors, transforming growth factor-β receptor I (TβRI), and transforming growth factor-β receptor II (TβRII), and typically TβRII phosphorylates TβRI (3). Activated TβRI phosphorylates Smad 2 and/or Smad 3, and a heterotrimeric complex is formed with Smad 4 that translocates into the nucleus, binds with a consensus sequence called Smad binding element (SBE), and directly or indirectly (by interacting with other transcription factors) regulates gene transcription (4, 5). Other signaling pathways involving mitogen-activated protein kinase (MAPK) (6), TAK-1 (7), p38 MAPK (8), and Jun N-terminal kinase (JNK) (6) are also known to be involved in TGF-β signaling. Targets for the activated MAP kinases are transcription factors that include cAMP-response element-binding protein and Elk-1. p38 activates ATF-2 (9). JNK phosphorylates c-Jun, a member of the AP-1 family of transcription factors that can bind to and activate transcription from AP-1 or phorbol ester-responsive sites. Many TGF-β-regulated gene promoters also contain AP-1 sites (7), suggesting AP-1/Smad cooperation.

In many epithelial cell types, TGF-β inhibits growth and/or induces apoptosis, but these cells may also lose their sensitivity and responsiveness to TGF-β because of loss of TGF-β or because of functional components of the TGF-β pathway that promote the development of cancer (10). Defective TGF-β signaling because of mutations in *Smads* or TGF-β receptors is reported in certain human cancers (11–14). Deletion or inactivation of *Smad 2* in colorectal cancer (15) and head and neck squamous cell carcinoma (11) and of *Smad 4* in pancreatic cancers has been reported (16). Loss of responsiveness to TGF-β1 and intragenic deletions of TβRII were reported in cervical cancer cell lines (17–19), and the results are contradictory with reference to the involvement of defects in the receptors or distal components of the TGF-β signaling pathway (17–20). Recently we have reported loss of expression and mutations of *Smad 2* and *Smad 4* in human cervical cancer (21). The present study was undertaken to understand the responsiveness of human cervical cancer cells to TGF-β. The results reveal variations among these cells and suggest that TGF-β enhances a proliferative pathway in HeLa but a growth inhibitory pathway in SiHa cells under the same conditions. The proliferative pathway in HeLa involves the activation of MAPKs, and this MAPK activation probably does not depend on Smad activation.

EXPERIMENTAL PROCEDURES

Chemicals, Reagents, and Antibodies—Dulbecco's modified Eagle's medium and LipofectAMINE reagents were purchased from Invitrogen. [γ-^{32}P]ATP and [^{3}H]thymidine were procured from the Bhabha Atomic Research Center, Mumbai, India. T4-polynucleotide kinase (M0201S) was from New England Biolabs, Inc. Goat polyclonal antibody to Smad 2 (sc-6200), rabbit polyclonal antibody to Smad 4 (sc-7154), oligonucleo-

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[1] The abbreviations used are: TGF-β, transforming growth factor-β; EMSA, electrophoretic mobility shift assay; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; SBE, Smad binding element; TβR, transforming growth factor-β receptor.

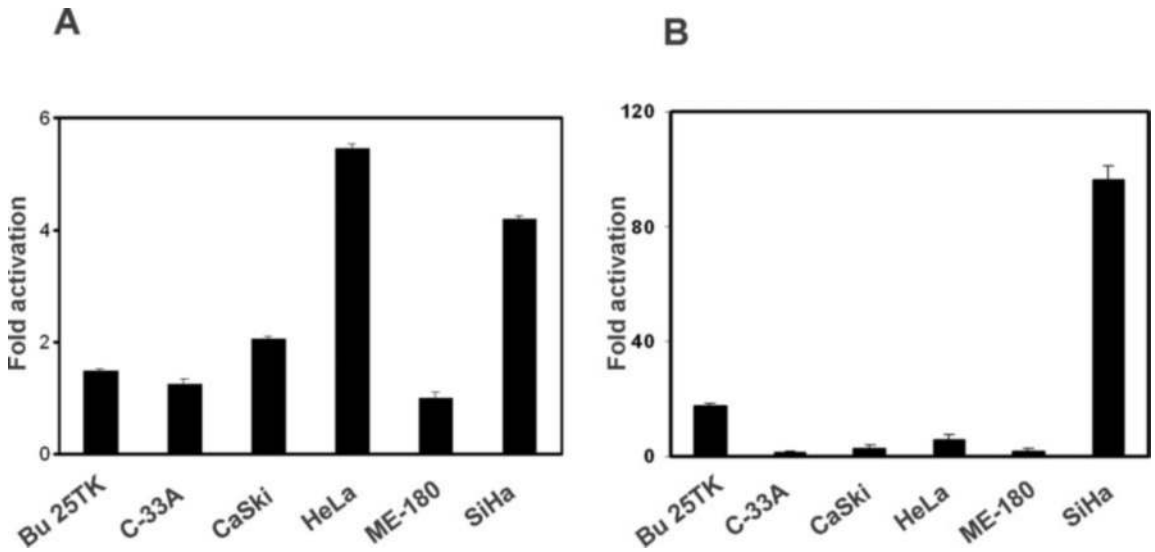

FIG. 1. **Changes in TGF-β sensitivity and Smad activation induced by TGF-β in cervical cancer cells.** *A*, human cervical cancer cells were transfected with p3TP-lux and pRL/CMV constructs and treated with or without 5 ng/ml human recombinant TGF-β for 16 h. Luciferase activity was then measured as described under "Experimental Procedures." The activity obtained for the reporter gene was normalized using the *Renilla* luciferase activity of pRL/CMV. The values are expressed as -fold activation over those of untreated cells. The *error bars* indicate the S.D. of four values. Similar results were obtained when the experiment was repeated once again. *B*, human cervical cancer cells were transfected with pSBE4-BV/Luc and pRL/CMV constructs and treated with or without 5 ng/ml human recombinant TGF-β for 16 h. Luciferase activity was calculated as in *panel A*. The *error bars* indicate the S.D. of four values. Similar results were obtained when the experiment was repeated once again.

tide probes of Smads (sc-2603) and AP-1 (sc-2501), and chemiluminescence luminol reagent (sc 2048) were bought from Santa Cruz Biotechnology. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), peroxidase- and rhodamine-conjugated secondary antibodies, mouse monoclonal antibody against β-actin (A5441), human recombinant epidermal growth factor (E9644), human recombinant TGF-β1 (T7039), and propidium iodide were obtained from Sigma. Rabbit polyclonal antibody to phospho-p42/44 MAPK (9910) was obtained from Cell Signaling Technology. Anti-phospho-specific Smad 2 (566415) and U0126 were procured from Calbiochem, and SB203580 was purchased from Alexis Corporation. The p3TP-lux construct was a generous gift from Dr. P. Kondaiah, IISc, Bangalore, and pSBE4-BV/Luc was a generous gift from Dr. Bert Vogelstein, The Johns Hopkins Oncology Center Molecular Genetics Laboratory. The plasmid pRL/CMV, the dual luciferase assay kit (E 1910), and RNase A were purchased from Promega.

Cell Culture and Luciferase Assay—Human cervical cancer cell lines were grown as monolayer cultures in Dulbecco's modified Eagle's medium (Invitrogen) containing 10% fetal bovine serum (Sigma) and antibiotics (100 units/ml penicillin and 100 μg/ml streptomycin) in a humidified atmosphere of 5% CO_2 at 37 °C. Cells (3×10^4) were transfected with the reporter plasmid and the pRL/CMV plasmid in 100:1 ratio using LipofectAMINE according to the manufacturer's protocol (Invitrogen). After overnight transfection, the cells were allowed to recover for 10 h in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum and were treated with or without TGF-β (5 ng/ml) in serum-free medium for 16 h. Luciferase assay was performed using the dual luciferase assay reagent (E-1910) following the manufacturer's protocol (Promega). Bioluminescence was measured using a Turner Designs luminometer (TD-20/20) from Promega.

Electrophoretic Mobility Shift Assay (EMSA)—Nuclear extracts for EMSA were prepared essentially as reported earlier (22). Briefly, the binding reaction mixture (containing 0.5 ng of oligonucleotide labeled with [γ-^{32}P]ATP using T4-polynucleotide kinase, 10 μg of nuclear extracts in 10 mM Tris (pH 7.5) buffer, 50 mM NaCl, 1 mM dithiothreitol, 1 mM EDTA, 5% glycerol, and 1 μg of poly(dI-dC) was incubated for 1 h on ice, and appropriate antibodies were included before incubation for supershift assays. DNA-protein complexes were resolved by electrophoresis through 4% PAGE containing 2.5% glycerol with 0.25× Tris borate ethylenediamine tetraacetic acid. The gels were dried and exposed on a phosphorimaging screen (Bio-Rad) for 1 h for visualization.

MTT Assay—Cells were seeded in 96-well plates (5000 cells/well) and treated with or without TGF-β in serum-free medium. MTT was added (25 μl of 5 mg/ml stock) and incubated for 2 h. The formazan crystals formed were solubilized in MTT lysis solution (20% SDS in 50% formamide) for 4 h, and the absorbance was measured in a 96-well plate reader (Bio-Rad) at 570 nm.

Cell Cycle Analysis—Cells were seeded (1×10^6 cells) in 60-m Petri plates, treated with or without TGF-β for 24 h, and trypsinized and washed in phosphate-buffered saline. The cells were fixed in 70% ethanol at −20 °C overnight. The cells were washed with phosphate-buffered saline containing 0.2% Tween 20, suspended in phosphate-buffered saline containing 25 μg/ml of RNase, and incubated at 37 °C for 1 h. The cells were stained with 25 μg/ml of propidium iodide for 15 min. The cell suspensions were then analyzed in a fluorescence-activated cell sorter analyzer. Only the single cells were included in the analysis.

Immunofluorescence—Monolayer cells were cultured on coverslips and treated with or without TGF-β for 24 h. Monolayer was fixed with 4% buffered paraformaldehyde, permeabilized with 0.2% Triton X 100, and then treated with anti-phospho-Smad 2 for 4 h at 37 °C. Rhodamine-conjugated secondary antibody was treated for 45 min, and the cells were visualized under UV light.

Western Blotting—Western blotting was performed as described (23) using an antibody to phospho-p42/44 MAPK; p42 MAPK antibody was used as the control. Appropriate peroxidase-conjugated secondary antibodies were used, and the blot was developed using chemiluminescence luminol reagent according to the manufacturer's protocol (Santa Cruz Biotechnology).

RESULTS AND DISCUSSION

TGF-β responsiveness of human cervical cancer cell lines was assessed with a reporter assay using p3TP-lux containing multiple response elements from the promoter of plasminogen activator inhibitor, which include the SBE and three phorbol ester-responsive elements (24) that together mediate optimal activation by TGF-β (25, 26). Upon treatment with TGF-β, the response (expressed as -fold activation of luciferase over untreated control) was high in HeLa (5.5-fold) followed by SiHa (4.2-fold), whereas Bu 25TK, C-33A, Ca Ski, and ME-180 cells were relatively less responsive to TGF-β (Fig. 1*A*). When a Smad 4-activated transcriptional reporter with four SBEs (pSBE4-BV/Luc) was used to check the involvement of Smads alone in TGF-β responsiveness, it was very high in SiHa cells, whereas others, including HeLa, were relatively less responsive (Fig. 1*B*). Thus, both SiHa and HeLa cells were sensitive to the p3TP-lux reporter assay, whereas SiHa, but not HeLa, cells were sensitive to TGF-β with pSBE4-BV/Luc. Loss of responsiveness to TGF-β in some of the human cervical cancer cell lines observed in the present study is consistent with earlier

reports in pancreatic (27), head and neck (11), and colon cancers (28), but it appears to be a rare event in esophageal squamous cell carcinoma (29). The loss of sensitivity to TGF-β observed in Bu25TK, C-33A, CaSki, and ME-180 may be because of the loss of function of any of the components of the TGF-β pathway; in addition, intragenic deletions of TβRII were reported in ME-180 and C-33A (17, 18). In agreement with our results, TGF-β has been shown to induce the expression of the plasminogen activator inhibitor gene in SiHa and HeLa cells (17). However, in another study, SiHa cells were reported to be resistant to TGF-β because of a mutation in *Smad 4* (19), although we could not detect any mutations in *Smad 2* or *Smad 4* in SiHa cells (21). Actual reasons for these contradictory results in SiHa cells are not clear, but accumulation of genetic variations within a cell line acquiring new characteristics is known to occur and the responsiveness to TGF-β depends on its concentration (30, 31).

To analyze the involvement of Smad activation in TGF-β response, DNA binding ability of Smads in the presence and absence of TGF-β was then examined in human cervical cancer cells by EMSA using ^{32}P-labeled SBE. TGF-β enhanced the DNA binding activity of Smads more prominently in SiHa than Ca Ski, Bu 25TK, and HeLa, whereas no activation could be observed in C-33A and ME-180 (Fig. 2*A*). To know whether the active complexes that bound SBE actually contained Smads, nuclear extracts from SiHa cells treated with TGF-β (that showed maximum DNA binding activity) were incubated with antibodies to Smad 2 or Smad 4 while carrying out EMSA. Both the antibodies shifted the active complex (supershift), slowing down its migration in the gel, suggesting that it contains Smad 2 and Smad 4 (Fig. 2*B*). Together these results show that human cervical cancer cells vary in their response to TGF-β, indicating the existence of defective TGF-β signaling in some cervical cancer cells. These results also suggest that even though HeLa cells respond to TGF-β, the activation of Smads is minimal in these cells. It is possible that the induction observed with p3TP-lux reporter construct by TGF-β in HeLa cells may be independent of the activation of Smads. The negligible stimulation of pSBE4-BV/Luc that contains four SBEs by TGF-β in HeLa cells suggests that because Smads are either inadequate or not essential for TGF-β response, additional factors are necessary. It is also to be noted that the DNA binding activity of Smads in HeLa upon TGF-β treatment is relatively less than that of SiHa cells.

Because HeLa and SiHa cells differed in their response to TGF-β when assessed by EMSA and two different reporter assays, it was decided to analyze their growth response to TGF-β (5 or 10 ng/ml) by MTT assay for different time intervals (24, 48, and 72 h). In HeLa cells at 24 h, 5 ng/ml of TGF-β induced proliferation (110 ± 2.1%), but at 10 ng/ml it inhibited cell growth (95 ± 2.1%). The viability decreased at other time points although more effectively at 48 than 72 h with both the concentrations of TGF-β (Fig. 3*A*). TGF-β decreased the viability in SiHa cells at all the time periods of the study, but the effect was maximum at 24 h for both the concentrations (85 ± 2.5 and 84 ± 0.5% over control for 5 and 10 ng/ml, respectively) (Fig. 3*A*). To get a better picture of TGF-β-mediated changes in growth, the changes in cell cycle stages were analyzed with and without TGF-β; to understand the involvement of Smads, a chemical inhibitor of Smads (SB203580) was used (32). As shown in Fig. 3*B*, 68.2% of HeLa cells were at G_0/G_1 -phase, 13.3% at S-phase, and 18.4% at G_2/M-phase, but only 56% of TGF-β-treated HeLa cells were at G_0/G_1-phase. Treatment with SB203580 did not have much effect either alone or if given before TGF-β treatment. In the case of untreated SiHa cells, 72.6% were at G_0/G_1-phase, 9.8% at S-phase, and 12.8% at

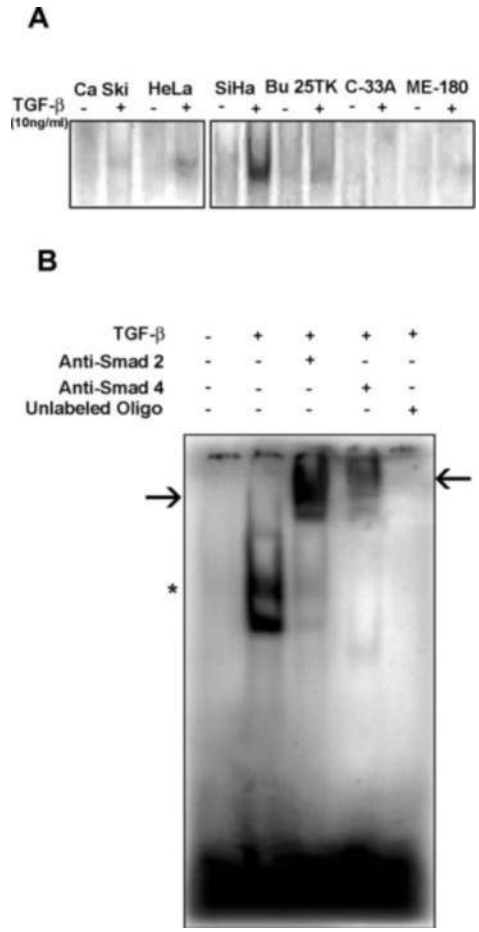

FIG. 2. **Changes in the DNA binding ability of Smads induced by TGF-β in human cervical cancer cells and the nature of the active Smad DNA binding complex in SiHa cells.** *A*, human cervical cancer cells were treated with (+) or without (−) 10 ng/ml TGF-β for 1 h, and EMSA was carried out as described under "Experimental Procedures." *B*, supershift assay showing nuclear extracts from SiHa cells (15 μg) that were treated with or without TGF-β (10 ng/ml) for 1 h and incubated with the indicated antibody (2 μg) or unlabeled oligo. EMSA was performed as above. The position of the active band is indicated with an *asterisk*. *Arrows* indicate the supershift. Results shown were confirmed in two other independent experiments.

G_2/M-phase, and treatment with TGF-β induced a G_0/G_1 arrest in SiHa cells (85%) and subsequent reduction in S-phase (4.9%) (Fig. 3*B*). As in HeLa, SB203580 treatment alone did not cause any significant change in cell cycle phases of SiHa cells, but pretreatment with SB203580 almost reversed TGF-β-induced changes in G_0/G_1- (75.5%) and S-phase (8.5%) (Fig. 3*B*). These results indicate that TGF-β-induced growth responses differ between SiHa and HeLa cells and that TGF-β-induced response in HeLa could not be blocked by SB203580, whereas the same compound blocked the growth arrest induced by TGF-β in SiHa cells. To confirm the role of Smads in these differential TGF-β-induced responses, localization of phospho-Smad 2 was analyzed by immunofluorescence with or without TGF-β treatment or SB203580 pretreatment. The untreated and SB203580-treated SiHa cells showed 16 and 12.8% nuclear positivity, respectively. In the SB203580-treated cells, accumulation on the nuclear membrane was observed in several cells, indicating a blockade of nuclear translocation. When the cells were treated with TGF-β, the intensity as well as the nuclear positivity for phospho-Smad 2 increased (68.6%); the nuclear localization was blocked when the cells were treated with TGF-β after SB203580 treatment (17%) (Fig. 3*C*). HeLa cells showed basal nuclear positivity for phospho-Smad 2 (18%), and

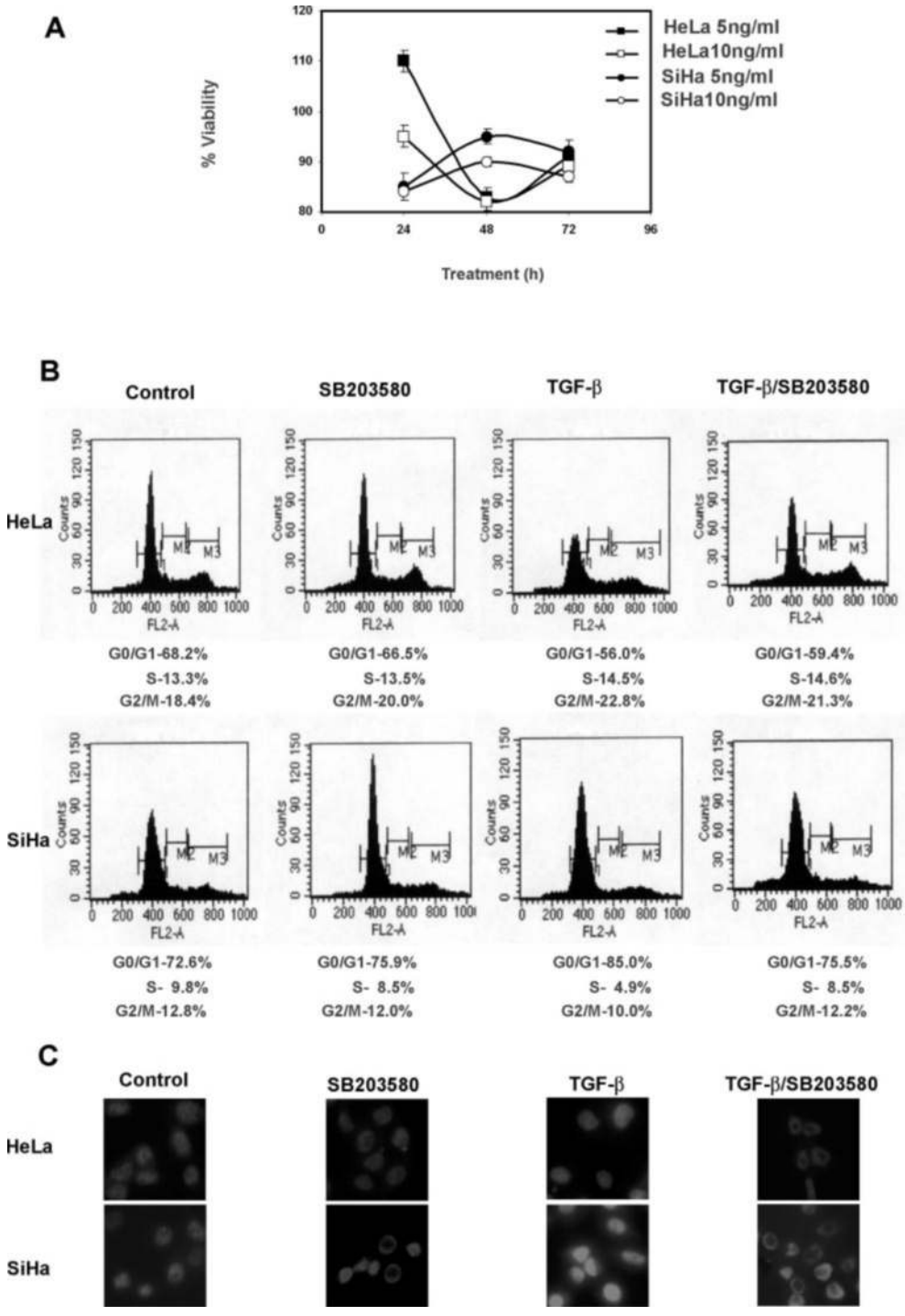

FIG. 3. **Changes in cell viability induced by TGF-β and variations in cell cycle and phosphorylated Smad 2 in HeLa and SiHa cells induced by TGF-β or SB203580 or a combination of both.** *A*, cells were treated with (5 or 10 ng/ml) or without the indicated concentrations of TGF-β for the time intervals shown, and MTT assay was performed as described under "Experimental Procedures." The percentage of viability over the control is shown. The *error bars* indicate the S.D. of six values, and the results were confirmed in another independent experiment. *B*, to analyze cell cycle changes, the cells were treated with 5 ng/ml TGF-β for 24 h (with 1 h of pretreatment of 1 μM SB203580 wherever indicated), fixed with ethanol, stained with propidium iodide, and analyzed for cell cycle as described under "Experimental Procedures." M1, M2, and M3 indicate the cells in G_0/G_1-, S-, and G_2/M-phases, respectively. *C*, the nuclear accumulation of phospho-Smad-2 was analyzed by immunofluorescence. The cells were treated with 5 ng/ml TGF-β for 24 h (with 1 h of pretreatment of 1 μM SB203580 wherever indicated), and immunofluorescence was done as described under "Experimental Procedures."

this was unaffected in SB203580-treated cells. Upon TGF-β treatment, the nuclear accumulation increased to 30.3%. SB203580 treatment could also inhibit the TGF-β-induced nuclear translocation (14%) of Smads in HeLa cells (Fig. 3*C*). Thus, in SiHa cells TGF-β-mediated growth inhibition depends on the nuclear accumulation of activated Smads. In HeLa cells the cell cycle progression induced by TGF-β is presumably independent of Smad activation because the use of SB203580 did not alter the TGF-β-induced decrease in G_0/G_1-phase cells, although the marginal Smad activation induced by TGF-β was completely blocked by this compound. Even though a small fraction of the HeLa cells showed a nuclear accumulation of activated Smads, the effects exerted by TGF-β on cell cycle do not seem to depend on this activation of Smads as the inhibi-

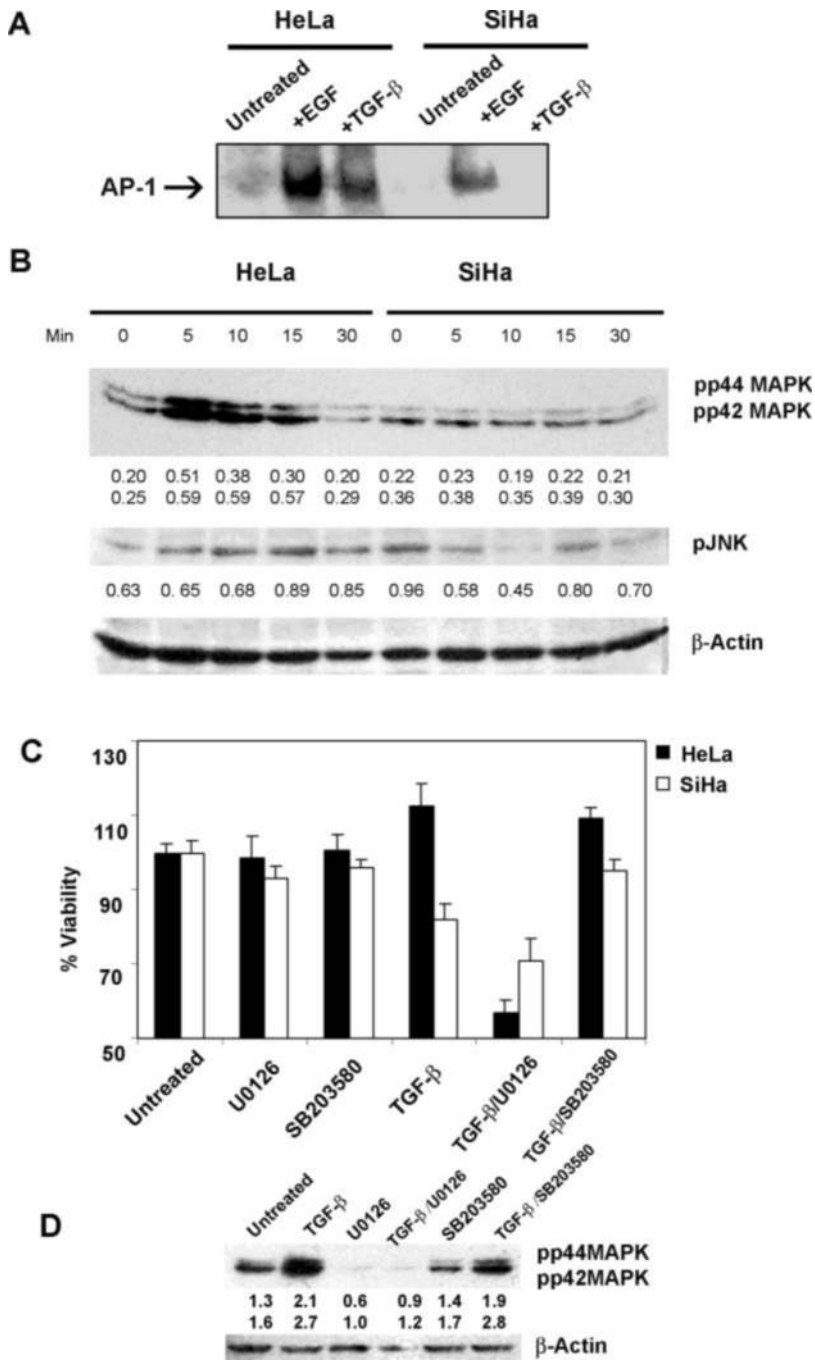

FIG. 4. **TGF-β-mediated activation of AP-1, MAPK, and JNK in HeLa and SiHa cells.** *A*, cells were treated with 5 ng/ml of TGF-β for 1 h or 5 ng/ml of EGF for 30 min, and EMSA was carried out using the AP1 consensus probe as described under "Experimental Procedures." *B*, the cells were treated with 5 ng/ml of TGF-β for different time intervals as indicated. Western blotting was done using anti-phospho-p42/p44 MAPK as described under "Experimental Procedures." The membrane was reprobed with anti-phospho-JNK antibody and then with anti-β-actin antibody. Densitometric analysis was done using the normalized values for β-actin as shown. *C*, the cells were treated with TGF-β for 24 h. U0126 (10 μM) and SB203580 were added and incubated for 1 h either alone or before the addition of TGF-β as indicated. MTT assay was performed as described earlier. *D*, HeLa cells were treated with TGF-β for 15 min with pretreatment of U0126 or SB203580 wherever indicated. Western blotting was performed with anti-phospho-p42/44 MAPK antibody, and the membrane was reprobed with anti-β-actin. Densitometric units normalized for β-actin are shown.

tion of Smads with SB 203580 did not alter the decrease in the percentage of cells at G_0/G_1. Thus, in HeLa cells at least in the initial stages, a TGF-β-dependent pathway is activated that probably does not require Smad activation. Without a functional *Smad 4* gene, VACO-235, a colon cancer cell line, exhibited TGF-β-mediated growth inhibition as well as activation of p3TP-lux reporter, but not pSBE4-BV/Luc (33), suggesting a possible Smad-independent pathway in this cell line. Growth inhibitory responses to TGF-β appear to be transactivated, at least in part, by a Smad 4-independent pathway (34–36). TGF-β insensitivity may also involve novel mechanisms independent of Smads (33, 37).

MAPK pathway is the major proliferative pathway operational in most cells, resulting in the activation of AP-1. The results from EMSA indicate that EGF, used as a positive control, induced DNA binding activity of AP-1 in HeLa and SiHa cells, whereas TGF-β enhanced it in HeLa cells, but not SiHa cells (Fig. 4*A*). Western blot for phospho-p42/p44 MAPK shows that activation occurred within 5 min in HeLa cells and persisted up to 15 min upon TGF-β treatment, whereas the activation in SiHa cells was negligible (Fig. 4*B*). Changes in JNK activation were also analyzed by Western blot using phospho-JNK antibody. After 10 min of TGF-β activation, there was up-regulation of phospho-JNK in HeLa, but SiHa cells showed a down-regulation of its activity at the same time. These results suggest that TGF-β activates MAPK pathway and DNA binding activity of AP-1 more effectively in HeLa, but not SiHa, cells. Cell viability was determined to confirm the role of MAPKs and Smads in TGF-β response with and without specific blockers of p42/44MAPK (U0126) (38) and Smads (SB203580) (Fig. 4*C*). These compounds alone did not change the cell viability compared with untreated cells, and at 24 h of TGF-β treatment, cell viability increased in HeLa and decreased in SiHa cells as in Fig. 2*A*. U0126 significantly blocked

the proliferative response in HeLa cells and enhanced the growth suppression in SiHa cells, whereas SB203580 could block the growth suppression induced by TGF-β in SiHa cells but did not affect the proliferative response in HeLa cells (Fig. 4*C*). The level of activation of p42/44 MAPK has been assayed by Western blot in HeLa cells. The results show that TGF-β induced the activation of both p42 and p44 MAPKs and this activation was blocked by U0126 treatment, but not by SB203580 (Fig. 4*D*). Thus, the response to TGF-β differs considerably in two cervical cancer cell lines, HeLa and SiHa. It appears that the proliferative response is mediated by the activation of MAPKs probably without the involvement of Smad activation, whereas the growth inhibition observed in SiHa cells is dependent on Smad activation. It is known that cyclin D1 expression and cell cycle progression are regulated positively by the classical MAPK pathway (9). Activation of AP-1 and Ras/MAPK pathway has been shown to induce proliferation in many cell types (39); the activation peaks within 30 min when it induces proliferation (40, 41), which is similar to the results of the present study. MAPKs are also activated by different apoptotic stimulants, but MAPK activation is rather a late event (after 4 h) (42, 43). Synergistic activation of Smads and AP-1 complex by TGF-β usually results in apoptosis (44), and cross-talk between the TGF-β-activated MAPKs is important for apoptosis in the FaO rat hepatoma cell line (45). However, these TGF-β-induced activations of MAPK pathway involved in apoptosis are rather late events (4–16 h) (46, 47). TGF-β-induced activation of p21, a cdk inhibitor necessary for growth inhibition, is dependent on MAPK pathway that depends upon Smad activation (48). Therefore, the existing evidence indicates that in TGF-β-induced apoptosis or growth inhibition, MAPKs are activated in a delayed manner dependent on Smad activation. However, in the present study a TGF-β-induced activation of AP1 complex, p42/44 MAPK, and JNK, but not p38MAPK (data not shown), is observed in HeLa cells that is unaffected by the extent of Smad activation. Although transient and sustained activation of MAPKs resulting in differential response in many systems is known (49), we report for the first time that TGF-β-dependent activation of MAPKs in a cervical adenocarcinoma cell line, HeLa, results in cell cycle progression and cell proliferation and that these events appear to be independent of the extent of Smad activation. Further studies are needed to reveal the existence (if any) of Smad-independent pathways in human cervical cancer cells.

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