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Epidermal growth factor up-regulates transforming growth factor-β receptor type II in human dermal fibroblasts via p38 mitogen-activated protein kinase pathway

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Abstract

TGF-β receptors (TβRs) are serine/threonine kinase receptors that bind to TGF-β and propagate intracellular signaling through Smad proteins. TβRs are repressed in some human cancers and expressed at high levels in several fibrotic diseases. We demonstrated that epidermal growth factor (EGF) up-regulates type II TGF-β receptor (TβRII) expression in human dermal fibroblasts. EGF-mediated induction of TβRII expression was inhibited by the treatment of fibroblasts with a specific p38 mitogen-activated protein kinase (MAPK) inhibitor, SB203580, whereas MEK inhibitor PD98059 did not block the up-regulation of TβRII by EGF. EGF induced the TβRII promoter activity, and this induction was significantly blocked by SB203580, but not by PD98059. The overexpression of the dominant negative form of p38α or p38β significantly reduced the induction of TβRII promoter activity by EGF. These results indicate that the EGF-mediated induction of TβRII expression involves the p38 MAPK signaling pathway. The EGF-mediated induction of TβRII expression may participate in a synergistic interplay between EGF and TGF-β signaling pathway.

Keywords: Signal transduction; Growth factors; Protein kinases

Transforming growth factor (TGF)-β is a multifunctional protein that plays an important role in regulating cellular growth, differentiation, adhesion, and apoptosis in many biological systems [1–3]. TGF-β inhibits the growth of most cell types. In addition, TGF-β causes the deposition of extracellular matrix (ECM) by simultaneously stimulating fibroblasts to increase the production of ECM proteins, such as collagen, fibronectin, and proteoglycan, decrease the production of matrix-degrading proteases, increase the production of inhibitors of these proteases, and modulate the expression of integrins [1,3]. TGF-β binds to transmembrane receptors that have intrinsic serine/threonine kinase activity [4]. The type II TGF-β receptor (TβRII) binds TGF-β, and then the type I TGF-β receptor (TβRI) is recruited into the forms of a heteromeric complex. TβRII transphosphorylates the glycine-/serine-rich domain (GS domain) of TβRI kinase [4]. Following the phosphorylation of Smad2 or Smad3 by the activated TβRI, a heteromeric complex is formed with Smad4, resulting in translocation of the complex into the nucleus [5,6]. The complex can act directly as a transcriptional activator and can also indirectly regulate gene transcription by interacting with other transcriptional factors [7–10].

A critical mechanism for regulating the cellular response to cytokines and hormones resides at the level of receptor expression. The modulation of the level of TβRI and TβRII expression plays important roles both in the mechanism of wound healing and in the progression of malignancy. Disorders of TβR expression lead to various

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diseases. For example, the up-regulated expression of TβR has been demonstrated in fibrotic diseases such as systemic sclerosis, localized scleroderma, hepatic fibrosis, idiopathic hypertrophic obstructive cardiomyopathy, and atherosclerosis [11–15]. Up-regulated expression of TβR would result in the deposition of ECM components. In contrast, the reduction of TβR levels contributes to the resistance of tumor cells to TGF-β. Several lines of evidence suggest that the transcriptional repression of the TβR gene may be a major mechanism to inactivate TβR in epithelia compartment of carcinomas or adenomas developing different epithelial lineages [16].

Epidermal growth factor (EGF) is a key regulatory component of cell growth and differentiation in a variety of cell types [17]. In human skin fibroblasts, EGF is both motogenic and mitogenic. EGF signaling occurs predominantly through binding to the EGF receptor and its dimerization partner Erb B2. Autophosphorylation of activated EGF receptors stimulates a number of signal transduction pathways, including the Ras/Raf/MAP kinase (MEK)/MAP kinase (ERK) pathway. It has been increasingly clear that EGF induces the production of diverse mediators (e.g., FGF-binding protein, cyclooxygenase-2) via the p38 mitogen-activated protein kinase (MAPK) [18,19]. p38 MAPK is activated by cytokines and stress [20,21]. Several transcription factors are substrates for p38 MAPK isozymes, including MAP-KAP kinase-2 [22], ATF-1 [23], ATF-2 [24], CHOP/GADD153 [25], MAX [26], and ternary complex factor [27]. A pyridinylimidazole compound, SB203580, is a highly specific inhibitor of p38 MAP kinase [28], and has been reported to inhibit cytokine production either at the translational level [29] or the transcriptional level [30,31].

For regulation of tissue homeostasis, the balance of EGF and TGF-β signaling in human dermal fibroblasts seems to be critical. Furthermore, clarifying the mechanism of the regulation of TβR expression in normal human dermal fibroblasts should be instructive for elucidating the pathogenesis of the progression of fibrotic diseases. In the present study, we examined the regulation of TβR expression in human dermal fibroblasts by EGF. We also investigated the signaling pathway which is involved in the EGF-mediated regulation of TβR expression.

Materials and methods

Reagents. PD98059 and SB203580 were purchased from Calbiochem (La Jolla, CA). Recombinant human EGF was obtained from R&D Systems (Minneapolis, MN). Actinomycin D and cycloheximide were purchased from Sigma. The luciferase assay kit was purchased from Promega (Madison, WI). Antibodies specific for TβRII were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Antibodies specific for p38 MAPK and phospho-p38 MAPK, and the p38 MAP kinase assay kit were from New England Biolabs (Beverly, MA).

Cell cultures. Fibroblasts were obtained by skin biopsy of five healthy donors. All biopsies were obtained with informed consent and institutional approval. Primary explant cultures were established in 25-cm^2 culture flasks in DMEM supplemented with 10% FBS, 2 mM glutamine, and 50 μg/ml gentamicin, as described previously [32–34]. Monolayer cultures were maintained at 37 °C in 5% CO_2 in air. Fibroblasts between the third and sixth subpassages were used for experiments.

Cell lysis and immunoblotting. Fibroblasts were washed with PBS at 4 °C and solubilized in lysis buffer containing 50 mM Tris–HCl, pH 7.4, 150 mM NaCl, 2 mM EDTA, 1% Nonidet P-40, 0.1% SDS, 50 mM sodium fluoride, and 1 mM PMSF, as described previously [35–37]. Proteins were subjected to SDS–polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. Membranes were incubated overnight with anti-TβRII (50 ng/ml) antibodies, washed, and incubated with a secondary antibody against rabbit IgG for 60 min. After washing, visualization was performed by enhanced chemiluminescence (Amersham Pharmacia Biotech).

RNA preparation and Northern blot analysis. Fibroblasts were grown to 80% confluence in DMEM supplemented with 10% FBS, and then incubated for 12 h in serum-free medium, as described previously [36]. Poly(A)$^+$ RNA was extracted from total RNA using an oligotex-dT30 ⟨Super⟩ kit (Takara Shuzo, Otsu, Japan) and analyzed by Northern blotting, as described previously [38,39]. A total of 2 μg of poly(A)$^+$ RNA was subjected to electrophoresis on 1% agarose/formaldehyde gels and blotted onto nylon filters (Roche Diagnostics, Indianapolis, IN). The filters were UV cross-linked, prehybridized, hybridized, and sequentially hybridized with DNA probes for GAPDH, or RNA probes for TβRII. The membrane was then washed and exposed to X-ray film.

Plasmids. TβRII promoter luciferase constructs were kindly provided by Seong-Jin Kim (NCI, Bethesda, MD) [40]. Dominant negative mutant forms of p38α and p38β were kindly provided by Jiahuai Han (The Scripps Research Institute, La Jolla, CA) [25]. Plasmids used in transient transfection assays were purified twice on CsCl gradients, as described previously [33]. At least two different plasmid preparations were used for each experiment.

Transient transfection and luciferase assays. For each transfection, 1 μg of TβRII promoter luciferase construct, 1 μg of β-galactosidase (transfection efficiency control), and 4 μl of FuGENE™ 6 (Roche Diagnostics, Indianapolis, IN) were combined and added to cells. For co-transfections, 1 μg of TβRII promoter luciferase construct, indicated doses of expression vector, 1 μg of β-galactosidase (transfection efficiency control), and FuGENE™ 6 were combined and added to cells. Transfected cells were treated for 18 h with EGF in serum-free DMEM before cell lysis in 50 μl of Reporter Lysis Buffer (Promega). Luciferase activity was normalized by co-transfected β-galactosidase activity to correct for transfection efficiency. All transfections were repeated at least three times.

Assay of p38 MAPK activation. The activation of p38 MAPK was examined using a p38 MAP kinase assay kit according to the manufacturer's instructions, and immunoblotting was performed using antibodies specific for phosphorylated, activated forms of p38 MAPK (Thr180/Tyr182). In both types of experiment, fibroblasts were serum-starved for 18 h and treated with EGF for the indicated times. Then the fibroblasts were washed with ice-cold PBS and lysed. For immunoblotting using antibodies against phospho-p38 MAPK, membranes were incubated with anti-phospho-p38 MAP kinase (Thr180/Tyr182) monoclonal antibody (1:1000 dilution) overnight at 4 °C. As loading controls, immunoblotting was also performed using antibodies against total p38 (1:1000 dilution). For the p38 MAP kinase assay, 200-μl aliquots of the lysates were incubated with immobilized anti-phospho-p38 MAP kinase (Thr180/Tyr182) monoclonal antibody (1:10 dilution) overnight at 4 °C for immunoprecipitation. For kinase assays, the beads were then incubated with 200 μM ATP and 2 μg of activating transcription factor (ATF)-2 fusion protein as a substrate for p38 MAPK. The reaction was terminated by addition of 25 μl of SDS sample buffer. The samples were boiled for 5 min, subjected to SDS–polyacrylamide gel electrophoresis, and transferred to nitrocellulose membranes. Membranes were incubated overnight with anti-phospho-ATF-2 (Thr71) antibody (1:1000 dilution) at 4 °C. The membranes were washed and incubated with a secondary antibody against rabbit IgG for 1 h. After washing, visualization was performed by enhanced chemiluminescence.

Results

TβRII expression in human dermal fibroblasts is up-regulated by EGF

In the present study, we investigated the effects of various cytokines on the expression of TβRII in human dermal fibroblasts. Human dermal fibroblasts were cultured until they were 80% confluent and then incubated for an additional 24 h under conditions of serum starvation [36]. Cells were subsequently incubated in serum-free medium for 24 h in the presence or absence of various cytokines, which was added 24 h prior to protein lysis. Under these experimental conditions, we studied the effects of various cytokines, EGF, platelet-derived growth factor (PDGF)-AB, interferon (IFN)-γ, and tumor necrosis factor (TNF)-α, on the expression of TβRII protein in human dermal fibroblasts. As shown in Fig. 1A, TβRII protein level was not significantly affected by IFN-γ. EGF and PDGF-AB induced TβRII protein level. In contrast, TNF-α decreased the TβRII protein level. Next, we used a TβRII promoter luciferase construct (−1670/+35) to determine the effects of various cytokines on TβRII promoter activity. EGF and PDGF-AB induced TβRII promoter activity, whereas IFN-γ and TNF-α did not change (Fig. 1B). Taken together, these results indicate that EGF can induce TβRII protein level at the transcriptional level.

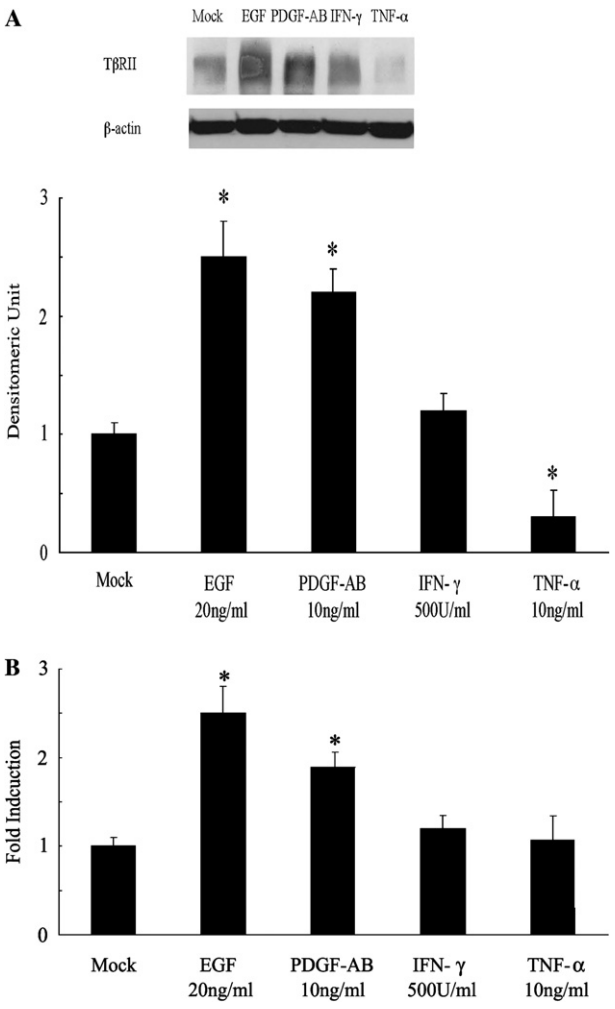

Fig. 1. The effects of various cytokines on TβRII protein level and TβRII promoter activity. Human dermal fibroblasts were serum-starved for 24 h and incubated in the absence or presence of 20 ng/ml EGF, 10 ng/ml PDGF-AB, 500 U/ml IFN-γ, or 10 ng/ml TNF-α for 24 h. (A) Cell lysates (15 μg of protein/sample) were subjected to immunoblotting using anti-TβRII antibodies. Whole cell lysates were also prepared and examined by immunoblotting using anti-β-actin antibodies. TβRII protein levels quantitated by scanning densitometry are shown relative to the level in untreated cells (1.0). (B) Human dermal fibroblasts were transiently transfected with the −1670/+35 TβRII promoter construct. Transfected cells were then treated in the absence or presence of 20 ng/ml EGF, 10 ng/ml PDGF-AB, 500 U/ml IFN-γ, or 10 ng/ml TNF-α for 24 h. TβRII promoter activity was determined by the fold increase in luciferase activity relative to that in untreated cells (1.0). Data are expressed as means ± SE of three independent experiments. $^{*}p < 0.05$ as compared with the value in untreated cells.

SB203580 inhibits EGF-mediated up-regulation of TβRII mRNA and protein

We investigated whether p38 MAPK activation is involved in EGF-mediated TβRII mRNA induction. Pretreatment of cells with SB203580 slightly reduced the basal expression of TβRII mRNA and partially blocked EGF-mediated up-regulation of TβRII mRNA in a dose-dependent manner (Fig. 2A). Moreover, EGF-induced up-regulation of TβRII protein was also partially inhibited by the treatment with SB203580, as determined by immunoblotting (Fig. 2B). MEK inhibitor PD98059 had no inhibitory effect on the EGF-mediated TβRII up-regulation. These results indicate that the activity of p38 MAPK is involved in the EGF-mediated induction of TβRII expression, whereas the EGF-mediated TβRII up-regulation is independent of MEK/ERK signaling pathways.

Contribution of p38 MAPK signaling pathway to TβRII promoter activity

To investigate the role of the p38 MAPK signaling pathway in the transcriptional regulation of TβRII, we examined the TβRII promoter activity in human dermal fibroblasts treated with pharmacological reagents. SB203580 significantly blocked the EGF-mediated TβRII promoter activity, whereas PD98059 did not alter the EGF-mediated induction of TβRII promoter activity (Fig. 3A). This finding suggests the importance of this kinase in mediating the induction of TβRII by EGF. The family of p38 MAPKs contains at least four kinases: p38α, β, γ, and δ. The SB203580 compound efficiently blocks the activities of p38α and β MAPKs [41]. To examine whether p38α, p38β, or both molecules were required for TβRII expression, the dominant negative mutant of p38α or p38β MAPK was transiently transfected into human dermal fibroblasts. The overexpression of the p38α dominant negative mutant in human dermal fibroblasts suppressed the EGF-induced TβRII promoter

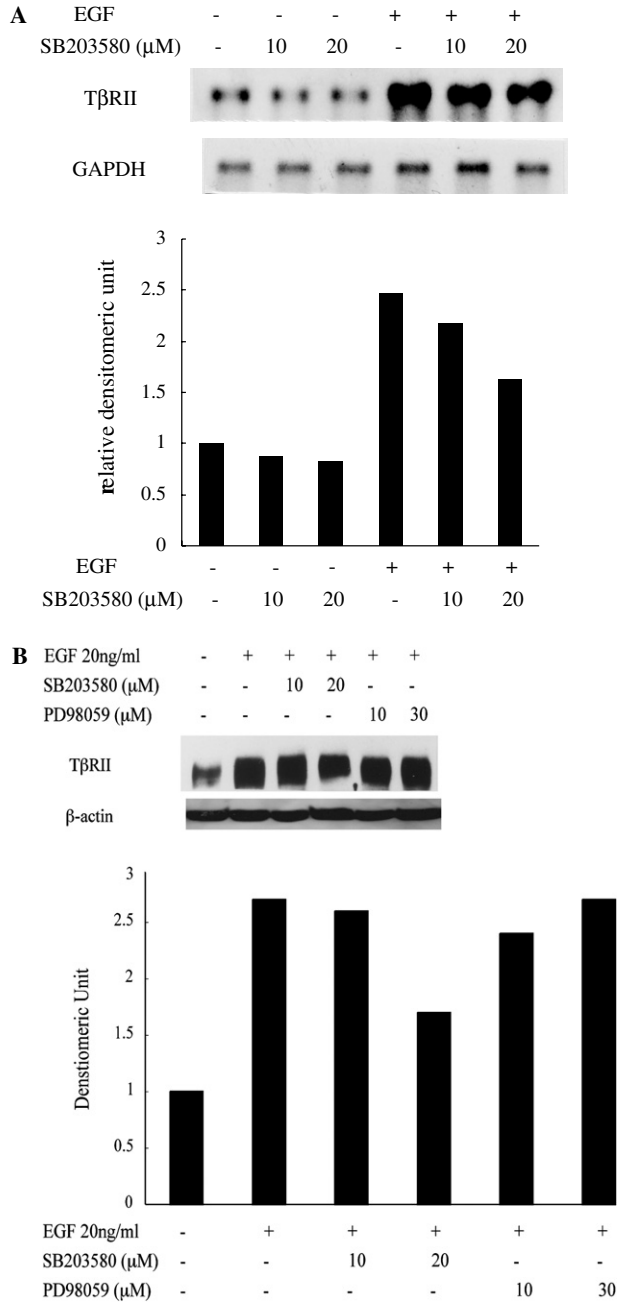

Fig. 2. SB203580 inhibits EGF-mediated up-regulation of TβRII mRNA and protein. (A) Human dermal fibroblasts were serum-starved for 24 h and pre-treated with 10 or 20 μM SB203580 for 1 h prior to the addition of 20 ng/ml EGF for 24 h. Equal loading of poly(A)$^+$ samples (2 μg) was checked by determining the expression of GAPDH as a housekeeping gene. TβRII mRNA levels quantitated by scanning densitometry and corrected for the level of GAPDH in the same samples are shown relative to the level in untreated cells (1.0). (B) Human dermal fibroblasts were serum-starved for 24 h and pre-treated with 10 or 20 μM SB203580, or 10 or 30 μM PD98059, for 30 min prior to the addition of 20 ng/ml EGF for 24 h. Cell lysates (15 μg of protein/sample) were subjected to immunoblotting with anti-TβRII antibodies. Whole cell lysates were also prepared and examined by immunoblotting using anti-β-actin antibodies. TβRII protein level quantitated by scanning densitometry is shown relative to the level in untreated cells (1.0). One experiment representative of three independent experiments is shown.

activity (Fig. 3B). Moreover, the overexpression of the p38β dominant negative mutant in human dermal fibroblasts also abolished the EGF-mediated TβRII promoter activity. These results suggest that both p38α and p38β MAPK signaling pathways participate in the regulation of TβRII promoter activity.

Temporal activation of p38 MAPK following EGF treatment and SB203580 inhibits EGF-induced p38 MAPK activity

We investigated whether EGF treatment induces p38 MAPK phosphorylation. Immunoblotting using anti-phospho-p38 MAPK (Thr 180/Tyr 182) antibody revealed a marked activation (4.9-fold) of p38 MAPK at 0.25 h after treatment with EGF, followed by a rapid decrease in the cellular level of the activated p38 MAPK by 0.5 h (Fig. 4A). Immunoblotting for total p38 protein demonstrated that the amount of p38 did not significantly change in the presence of EGF. The p38 MAP kinase assay showed that EGF stimulation increased the p38 MAP kinase activity. The level of phosphorylated ATF-2 was maximal after 0.25 h (4.2-fold) in the presence of EGF (Fig. 4B). These results suggest that the treatment with EGF results in the activation of p38 MAPK in human dermal fibroblasts. A specific inhibitor of p38 MAPK, SB203580, was used to inhibit the activation of p38 MAPK in human dermal fibroblasts stimulated by EGF. Cells were pre-treated with SB203580 for 1 h prior to the stimulation with EGF, and then the p38 MAPK assay was performed. SB203580 (20 μM) completely inhibited the p38-induced phosphorylation of ATF-2 (Fig. 4B), while MEK inhibitor PD98059 did not affect the p38 MAPK activity.

Discussion

A complex network of cytokines and growth factors orchestrates cell proliferation, differentiation, and wound healing in the skin. Various stimuli activate divergent signaling pathways and induce distinct cellular responses. Among these stimuli, TGF-β signaling plays a critical role in cellular growth and ECM production. The repression of TβR occurs in various types of human cancer cells, and the up-regulation of TβR expression occurs in fibrotic disorders. TβRs expression is regulated by a plethora of external factors, including cytokines and growth factors. It has been shown by Northern blot analysis and binding studies that 1,25-dihydroxyvitamin D3 and prostaglandin E2 down-regulate TβRII expression in human osteoblastic cells and human fibroblasts, respectively [42,43]. However, vitamin D3 has recently been shown to up-regulate the TβRII level in MCF-7 breast cancer cells [44]. These reports indicate that the same factor may induce different results in different cell types. Furthermore, the binding studies revealed down-regulation of TβRI in human monocytes by interferon-γ [45]. In human lung fibroblasts [46] and human corpus carvernosum smooth muscle cells [47], TGF-β1 increases the steady-state level of TβRI mRNA, possibly by increas-

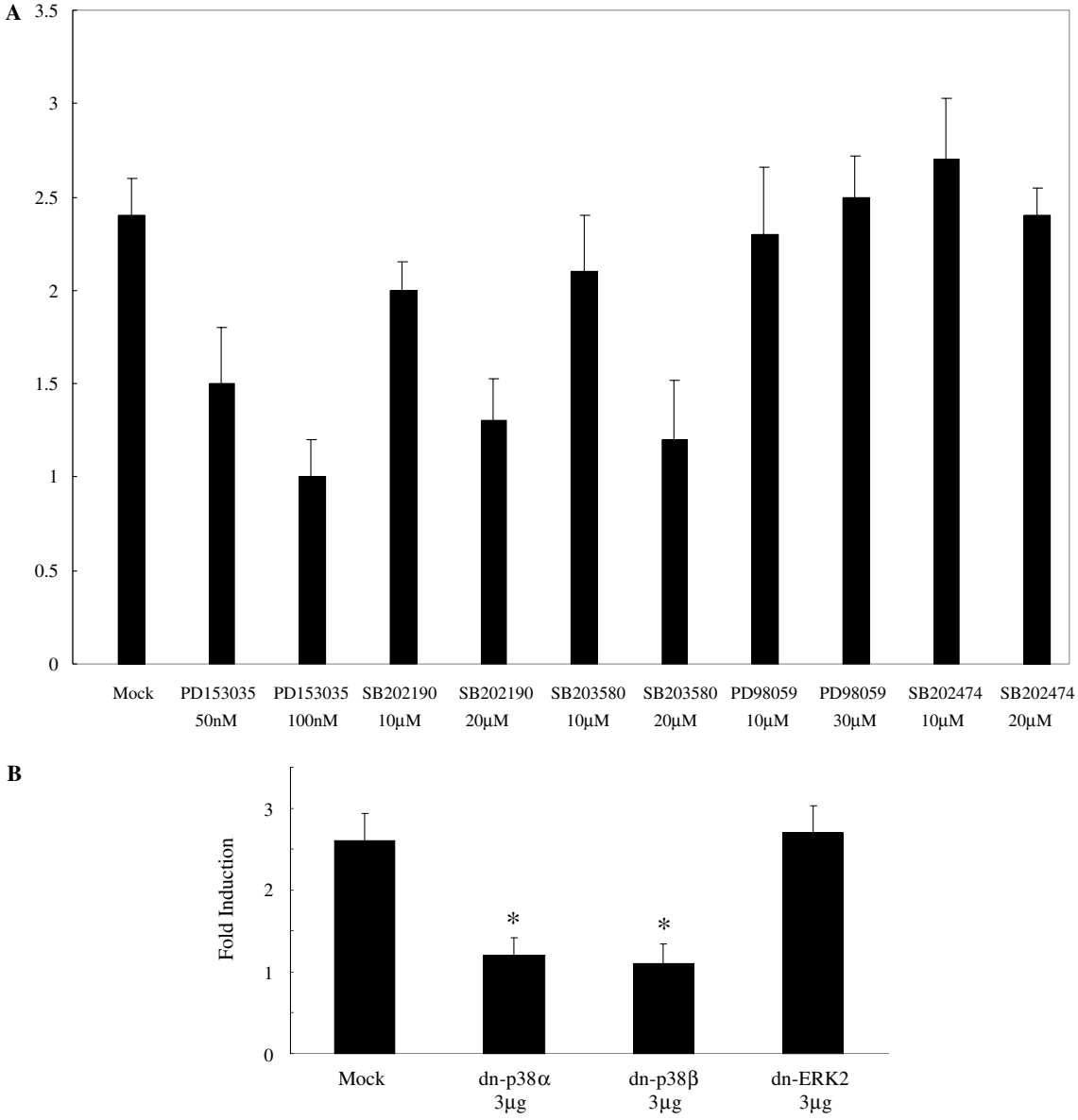

Fig. 3. Contribution of p38MAPK signaling pathway to TβRII promoter activity. (A) SB203580 inhibited the EGF-mediated induction of TβRII promoter activity. Human dermal fibroblasts were transiently transfected with the −1670/+35 TβRII promoter construct along with either vehicle alone (control), 10 or 20 μM SB203580, or 10 or 30 μM PD98059. Transfected cells were then treated with 20 ng/ml EGF for 18 h. The TβRII promoter activity was determined by the fold increase in luciferase activity relative to that in untreated cells (1.0). (B) Effect of dominant negative p38 MAPKs on the EGF-mediated induction of TβRII promoter activity. Human dermal fibroblasts were transiently co-transfected with the −1670/+35 TβRII promoter construct along with either empty vector or with the dominant negative mutant construct for p38α or p38β at the indicated doses. Transfected cells were either untreated or treated with 20 ng/ml EGF for 18 h. TβRII promoter activity was determined by the fold increase in luciferase activity relative to that of the empty vector control in the absence of EGF (1.0). Data are expressed as means ± SE of three independent experiments. $^{*}p < 0.05$ as compared with the value in untreated cells transfected with empty vector.

ing TβRI promoter activity [46]. Recently, it has been reported that, in COLO-357 pancreatic cancer cells, TβRI and TβRII mRNA and protein levels are up-regulated by TGF-β1 [36]. Regarding the effects of integrins, α2β1 integrin interaction with type I collagen down-regulates TβRs [48], whereas α5β1 integrin binding to fibronectin up-regulates TβRII [49]. Although the effects of many cytokines and hormones on the modulation of TβR have been demonstrated, the exact mechanisms involved in growth factor regulation of TβR expression remain unclear.

p38 MAPK is activated in response to a variety of stimuli, including growth factors, cytokines, and environmental stress [50]. Using several different approaches, we showed that p38 MAPK signaling is required for EGF-mediated up-regulation of TβRII. We demonstrated that the EGF-mediated induction of TβRII protein and mRNA levels is inhibited by pyridinyl imidazole compound SB203580, which selectively inhibits p38α and p38β isoforms but has no effect on the activity of other p38 isoforms, JNK, or ERK [28]. We have examined the distinct isozyme-specific

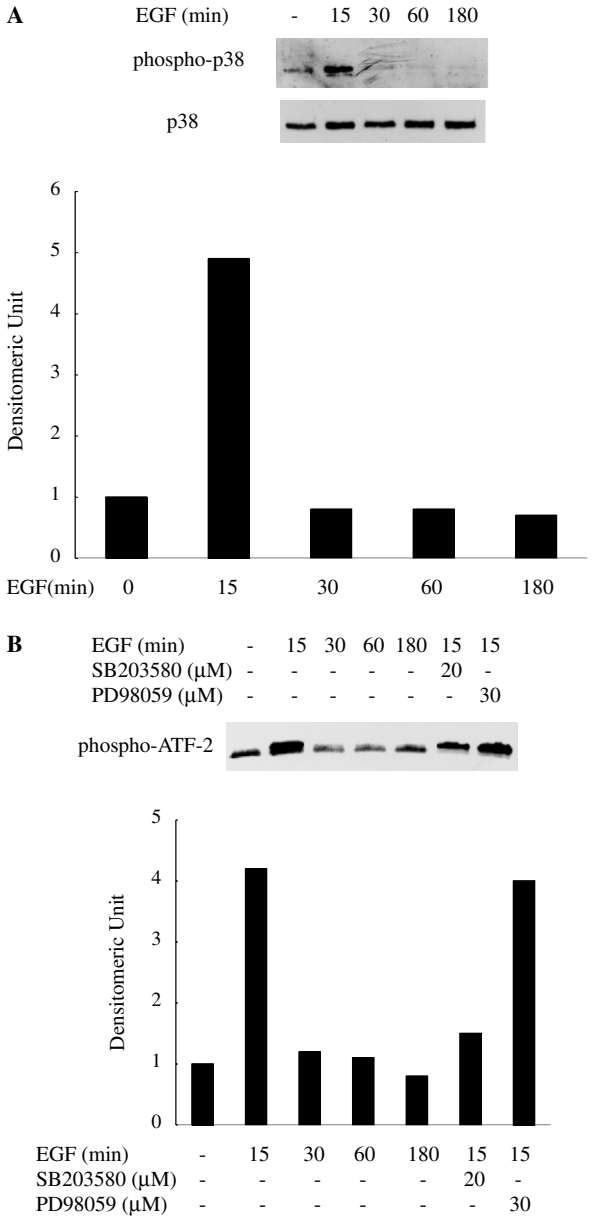

Fig. 4. Activation of p38 MAPK in human dermal fibroblasts by EGF. Human dermal fibroblasts were serum-starved for 24 h and treated with 20 ng/ml EGF for the indicated times. (A) Cell lysates (30 μg of protein/sample) were subjected to immunoblotting with anti-phospho-p38 MAPK antibodies. That the amounts of p38 MAPK proteins were unchanged was confirmed by immunoblotting using anti-p38 MAPK antibodies. The level of activated p38 MAPK quantitated by scanning densitometry and corrected for the level of total p38 MAPK in the same samples is shown relative to the level at 0 h (1.0). (B) To determine the time course of p38 MAP kinase activity, cells were preincubated for 1 h with 20 μM SB203580 or 30 μM PD98059, and then stimulated for 0.25 h with 20 ng/ml EGF. After the treatment, p38 MAPK was collected by immunoprecipitation and subjected to an in vitro kinase assay in the presence of ATF-2. The samples were subjected to immunoblotting with anti-phospho-ATF-2 antibodies. The level of activated ATF-2 quantitated by scanning densitometry is shown relative to the level at 0 h (1.0). One experiment representative of three independent experiments is shown.

effects of p38 MAPK family members, using dominant negative forms of p38α and p38β. In addition, we have examined the effect of EGF on other p38 molecule, p38δ. But the expression of p38δ was not changed after the EGF stimulation (data not shown). Our observation that the EGF-mediated induction of TβRII promoter activity is inhibited by the expression of the dominant negative form of p38α or p38β indicated that both p38 MAPKs may participate in the regulation of TβRII promoter activity. EGF activates other signaling pathways, including MEK/ERK, yet activation of these kinases apparently does not result in TβRII up-regulation, since MEK inhibitor PD98059 did not affect the EGF-mediated TβRII promoter activity or the protein level of TβRII in immunoblotting. These results suggest that the p38 MAPK pathway is critical, and the MEK/ERK pathway does not play a role in the regulation of TβRII expression by EGF in human dermal fibroblasts.

p38 MAPK signaling pathways have been reported to play a role in increasing transcription. Previous reports showed that p38 MAPK regulates transcription through various transcription factors, including CREB, ATF-1, and ATF-2 [20,23,51]. In the present study, an inhibitor of p38 MAPK, SB203580, diminished EGF-mediated induction of TβRII as shown by immunoblotting and Northern blotting analysis. In addition, in the TβRII promoter luciferase assay, SB203580 inhibited EGF induction of the TβRII promoter activity. Kim and his co-workers have demonstrated that ATF-1 controls expression of TβRII [52]. Whether or not ATF-1 plays a role in EGF-mediated up-regulation of TβRII at the level of transcription remains to be elucidated. In future studies, the role of the downstream signaling cascade of p38 MAPK and the crosstalk between the p38 MAPK pathway and other signaling pathway in the EGF-mediated induction of TβRII expression need to be examined.

The loss of sensitivity to TGF-β has been shown to occur in many types of human cancer cells. The loss of expression of TβRII, more frequently than that of TβRI, due to transcriptional repression is a frequent cause of this deficiency [16]. TGF-β signaling is initiated by binding of TGF-β to TβRII, and cancer cells, in which TβRII is repressed, are resistant to TGF-β. Thus, TβRII plays a critical role in receptor activation and subsequent signal propagation, functioning both to bind ligand and to activate TβRI. Additionally, it has been reported that COLO-357 pancreatic cancer cells are relatively sensitive to TGF-β1-mediated growth inhibition since the TGF-β-mediated up-regulation of TβRI and TβRII enhances the expression of cyclin-dependent kinase inhibitors $p15^{Ink4B}$, $p21^{Cip1}$, and $p27^{Kip1}$ [36]. The regulation of TβRs thus seems to be very important for the modulation of tumor growth.

The accumulation of ECM in tissues is the chief pathologic feature of fibrotic disorders. TGF-β signaling has been implicated in the primary pathogenesis of fibrosis [1]. With respect to systemic sclerosis, in which progressive fibrosis in the skin is a major cause of disease, it was reported that scleroderma fibroblasts of the involved area did not secrete increased level of TGF-β1 [53]. The mechanism of tissue fibrosis in such diseases remains to be determined. We have reported the overexpression of TβRI and TβRII

in scleroderma fibroblasts compared to normal human dermal fibroblasts, indicating one possible mechanism of autocrine control of TGF-β activity by the overexpression of TβRI or TβRII [11]. Furthermore, the co-transfection of TβRI and TβRII expression vector and an α2(I) collagen promoter/chloramphenicol acetyltransferase reporter gene showed that increasing the TβR level induced a three to fourfold increase of collagen promoter activity, and this increase was sensitive to anti-TGF-β1 antibody [11]. In idiopathic hypertrophic obstructive cardiomyopathy, which is characterized by regional myocardial hypertrophy with marked cardiomyocyte hypertrophy and a significant increase in extracellular matrix, TβRs are overexpressed on cardiomyocytes and fibroblasts [15]. In addition, with regard to the EGF receptor, it has been demonstrated that a twofold increase in receptor expression led to at least a 10-fold decrease in the concentration of ligand required to induce a biologic response [54]. These findings suggest that the autocrine regulation of TGF-β activity might result from receptor up-regulation rather than an increase of ligand. Recently, adenovirus-mediated local expression of a dominant negative TβRII and infusion of soluble TβRII have been demonstrated to be effective for the prevention of hepatic fibrosis [55,56]. Taken together, these results suggest that, in fibrotic disorders, TGF-β signaling may play a central role and the mechanism of regulation of TβRI and TβRII in such diseases is very critical. Moreover, our results indicate that the p38 MAPK signaling pathway has a significant relation to the modulation of TβRII, and that the blockage of the p38 MAPK signaling pathway may also have therapeutic value.

Several studies have shown synergistic interaction between TGF-β and EGF signaling pathways [19,57]. The combination of TGF-β1 and EGF led to additive stimulation of glycosaminoglycan synthesis in human dermal fibroblasts [57]. Saha et al. demonstrated the collaborative interaction of TGF-β1 and EGF signaling in the induction of cyclooxygenase-2 and prostaglandin production via p38 MAPK. In light of crosstalk between TGF-β1 and EGF, deCaestecher et al. proposed a model for the synergistic action of TGF-β and EGF signaling pathways, in which EGF stimulation results in the phosphorylation of Smad2 on a subset of TGF-β-dependent sites, which is sufficient to induce a weak transcriptional effect. Additionally, several lines of evidence demonstrated that TGF-β1 up-regulates EGF receptor [58,59]. Collectively, our findings about the EGF-mediated induction of TβRII expression might reflect synergistic interplay between TGF-β and EGF signaling pathways.

In conclusion, we showed that EGF up-regulates TβRII expression at the transcriptional level, and we demonstrated for the first time that the p38 MAPK signaling pathway is essential for the EGF-mediated induction of TβRII expression. Identification of p38 MAPK as a critical signaling pathway in the induction of TβRII in normal human dermal fibroblasts suggests p38 MAPK as a target for inhibition of overexpression of TβRII in fibrotic diseases such as systemic sclerosis, and the possible therapeutic value of induction of TβRII in cancer cells that are resistant to TGF-β signaling due to the loss of TβRs.

Acknowledgments

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