TGF-ß-induced upregulation of MMP-2 and MMP-9 depends on p38 MAPK, but not ERK signaling in MCF10A human breast epithelial cells

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Abstract. Transforming growth factor (TGF)-ß has been reported to exert growth inhibitory activity in normal epithelial cells whereas it induces cell proliferation and invasive phenotypes in advanced carcinomas. Our previous study showed that MCF10A, a spontaneously immortalized 'normal' breast epithelial cell line, is resistant to TGF-ß-induced growth inhibition, suggesting that conversion of TGF-ß growth inhibitory signaling into an oncogenic pathway may occur at the early stage of tumor development/progression. To address this issue, we investigated the TGF-ß signaling pathway and its role in phenotypic transformation of MCF10A cells. TGF-ß treatment induced changes in the MCF10A cell morphology from cuboidal to an elongated spindle-like shape, accompanied with down-regulation of epithelial cell marker E-cadherin. TGF-ß treatment was sufficient to induce migrative and invasive phenotypes in these cells, an important phenotypic conversion during tumor progression. We also showed that TGF-ß treatment rapidly activated ERK-1/2 and p38 MAPK leading to upregulation of matrix metalloproteinase (MMP)-2 and MMP-9. Using chemical inhibitors and dominant negative mutants of MAPKs, we provide evidence that while both p38 MAPK and ERKs are required for TGF-ß-induced MCF10A cell migration and invasion, TGF-ß-induced MMP-2 and MMP-9 expression depends on p38 MAPK signaling, but is independent of ERK activity. This study demonstrates the roles of TGF-ß signaling pathways for induction of oncogenic signaling in preneoplastic human breast epithelial cells and will deepen our understanding of TGF-ß signaling in the progress of breast cancer.

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Introduction

Transforming growth factor (TGF)-ß has emerged as a potent inhibitor of the progression of normal epithelial cells and endothelial cells by growth arrest in the cell cycle (1,2). It has been shown that in early tumor stages, transformed epithelial cells are still sensitive to TGF-ß-mediated growth arrest (3). In later stage of tumorigenesis, however, it contributes to tumor progression (4,5) by inducing an epithelial-to-mesenchymal transition (EMT), cell invasion and migration of epithelial tumor cells (6,7) partly through stimulating type IV collagenases, 72 kDa matrix metalloproteinase (MMP)-2 and/or 92 kDa MMP-9 (8), which play a critical role in tumor invasion and metastasis formation (9,10).

TGF-ß binding triggers activation of cell surface receptors, TGF-ß types II and I which in turn phosphorylates Smad2 and Smad3 (11). TGF-ß can also activate mitogen-activated protein kinases (MAPKs), JNK (12), ERK (13) and p38 MAPK (14). Involvement of the p38 MAPK pathway in TGF-ß-stimulated EMT, cell migration and gene expression has been implicated in various cell systems (14-16).

We have previously shown that MCF10A, a spontaneously immortalized breast epithelial cell line which shows normal cell characteristics (17), is resistant to TGF-ß-induced growth inhibition (18). To examine if the growth inhibitory signaling of TGF-ß may be converted to an oncogenic pathway at the early stage of tumor progression, we investigated the TGF-ß signaling pathway and its role in phenotypic transformation of MCF10A cells in this study. Here, we show that TGF-ß induces oncogenic signaling in preneoplastic breast epithelial cells. We provide evidence that both p38 MAPK and ERKs are required for TGF-ß-induced MCF10A cell migration and invasion, whereas TGF-ß-induced MMP-2 and MMP-9 expression depends only on p38 MAPK signaling, but is independent of ERK activity.

Materials and methods

Materials. Dulbecco's modified Eagle's medium (DMEM), penicillin-streptomycin, trypsin-EDTA were purchased from Gibco BRL (Grand Island, NY). TGF-ß, epidermal growth factor (EGF), cholera enterotoxin, amphotericin B, SB203580 and PD98059 were purchased from Sigma-Aldrich (St. Louis, MO).

Cell lines and culture condition. The development and characterization of the MCF10A cells has been previously described (17). Cells were cultured in DMEM/F12 supplemented with 5% horse serum, 0.5 µg/ml hydrocortisone, 10 µg/ml insulin, 20 ng/ml EGF, 0.1 µg/ml cholera enterotoxin, 100 units/ml penicillin-streptomycin, 2 mM L-glutamine and 0.5 µg/ml amphotericin B.

Transfection. Transfection was performed using Lipofectamine reagent (Life Technologies Inc., Rockville, MD) following the manufacturer's instruction. The dominant negative (DN) construct of p38 (19) was kindly provided by Dr Young-Joon Surh (Seoul National University, Seoul, Korea). DN MEK-1 (20) and DN JNK-1 (21) were kindly provided by Dr Yong J. Lee (University of Pittsburgh, Pittsburgh, PA).

Immunoblot analysis. Equal amounts of protein extracts in SDS-lysis buffer were subjected to 12% SDS-PAGE analysis and electrophoretically transferred to nitrocellulose membrane. Anti-phosphorylated Smad2, anti-JNK, anti-phosphorylated JNK, anti-ERK-1,2, anti-phosphorylated ERK-1,2, anti-p38 and anti-phosphorylated p38 antibodies were purchased from Cell Signaling Tech (Beverly, MA). MMP-2, MMP-9, E-cadherin and anti-Smad2 antibodies were from Santa Cruz Biotech (Santa Cruz, CA). ß-actin antibody was from Sigma-Aldrich (St. Louis, MO). Enhanced chemiluminescence (ECL, Amersham-Pharmacia, Buckinghamshire, UK) system was used for detection. Relative band intensities were determined by quantitation of each band with an Image Analyzer (Vilber Lourmat, France).

In vitro invasion assay. *In vitro* invasion assay was performed using a 24-well Transwell unit with polycarbonate filters (Corning Costar, Cambridge, MA) as previously described (22). The lower side of the filter was coated with type I collagen, and the upper side was coated with Matrigel (Collaborative Research, Lexington, KY). Lower compartment was filled with serum-free media containing 0.1% BSA. Cells were placed in the upper part of the Transwell, incubated for 17 h, fixed with methanol and stained with hematoxylin for 10 min followed briefly by eosin. The invasive phenotypes were determined by counting the cells that migrated to the lower side of the filter with microscopy at x400. Thirteen fields were counted for each filter and each sample was assayed in triplicate.

In vitro migration assay using Transwell. *In vitro* migration assay was performed using a 24-well Transwell unit with polycarbonate filters as previously described (23). Experimental procedures were the same as the *in vitro* invasion assay described above except that the filter was not coated with Matrigel for the migration assay.

Gelatin zymography. Cells were cultured in serum-free DMEM/F12 medium with TGF-ß for 48 h. Conditioned medium was collected and centrifuged at 3,000 rpm for 10 min to remove cell debris. The protein concentration was measured using BCA protein assay reagents (Pierce, Rockford, IL). Gelatinolytic activity of the conditioned medium was determined by gelatin zymogram assay as previously described (22,23). Areas of gelatinase activity were detected as clear bands against the blue-stained gelatin background.

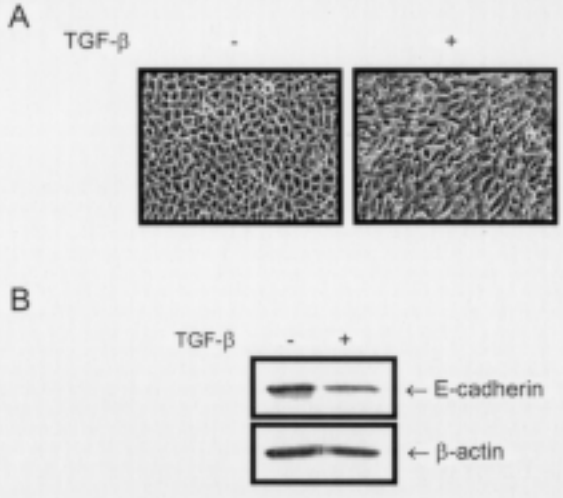

Figure 1. TGF-ß induces morphological alteration in MCF10A cells. (A), Cells were treated with 10 ng/ml TGF-ß for 48 h. Morphological changes by TGF-ß treatment were examined by light microscopy (x100). (B), Cells were treated with 10 ng/ml TGF-ß for 48 h. The levels of E-cadherin were determined by immunoblot analysis. ß-actin was used as a loading control.

Luciferase/ß-galactosidase assays. Cells were seeded in a 6-well plate at $1x10^6$ cells/well and transiently transfected with 4 µg MMP-2 promoter-luciferase construct and MMP-9 promoter-luciferase construct (kindly provided by Dr Benveniste at University of Alabama, Birmingham, AL) and 0.13 µg ß-galactosidase expression plasmid pMDV-lacZ. The transfectants were treated with TGF-ß for 24 h. The luciferase activity in 1 µg of cell lysate was normalized to ß-galactosidase activity and total protein determined by BCA protein assay kit (Pierce, Rockford, IL). Luciferase and ß-galactosidase activities were assayed using luciferase assay kit (Promega, Medison, WI) and Galacto-Light Kit (Tropix Inc, Bedford, MA) and measured with a Luminometer (Tuner Designs, Sunnyvale, CA).

Results

TGF-ß induces EMT, invasion and migration in MCF10A cells. TGF-ß has been shown to induce EMT, cell invasion and migration of epithelial tumor cells (6,7). We investigated if TGF-ß induced these phenotypes in 'normal' MCF10A cells. MCF10A human breast epithelial cells typically have cuboidal shape and adhere tightly to plastic. Treatment of 10 ng/ml TGF-ß for 48 h induced changes in the cell morphology from cuboidal to an elongated spindle-like shape, a typical morphology of mesenchymal cells (Fig. 1A). In order to prove that this morphological change involved an EMT, we detected E-cadherin, an epithelial cell marker (24), in MCF10A cells treated with TGF-ß. As shown in Fig. 1B, E-cadherin level was decreased by TGF-ß treatment, indicating the TGF-ß-induced loss of epithelial cell character.

Since EMT appears to be associated with an increase in cell motility and invasiveness (25), we speculated whether TGF-ß would contribute to tumor progression by stimulating cell invasiveness and migration in MCF10A cells. *In vitro*

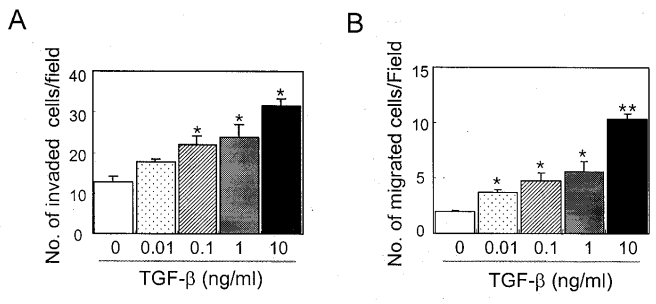

Figure 2. TGF-ß induces invasion and migration in MCF10A cells. TGF-ß-mediated MCF10A cell invasion (A) and migration (B) were measured by *in vitro* invasion assay and *in vitro* migration assay, respectively, as described in Materials and methods. Cells were incubated for 17 h and treated with various concentrations of TGF-ß. The number of invaded cells per field was counted (x400) in thirteen fields. The results represent means ± SE of triplicates. *,**Statistically different from control at $p<0.05$ and $p<0.01$, respectively.

invasion assay and *in vitro* migration assay were performed in MCF10A cells, which originally showed non-invasive and non-migrative phenotypes (22,23) in response to TGF-ß. As shown in Fig. 2A, treatment of TGF-ß rendered invasive phenotype to MCF10A cells in a dose-dependent manner. TGF-ß induced a significant increase in the number of invaded cells at concentrations of 0.1 ng/ml and higher. Motility of MCF10A cells was also dose-dependently induced by TGF-ß as evidenced by *in vitro* migration assay through Transwell chamber (Fig. 2B). The data demonstrate the stimulatory effect of TGF-ß on MCF10A cell invasion and migration.

TGF-ß induces MMP-2 and MMP-9 upregulation, which involves transcriptional activation. Invasive phenotype is often associated with increased expression of MMP-2 and/or MMP-9 (26). Previous studies have shown upregulation of MMP-2 (27) and MMP-9 (28,29) by TGF-ß. We examined the effect of TGF-ß on MMP-2 and MMP-9 expression in MCF10A cells. As shown in Fig. 3A, TGF-ß markedly induced gelatinolytic activities of secreted MMP-2 (72 kDa) and MMP-9 (92 kDa) in a dose-dependent manner. Active form of MMP-2 (62 kDa) was detected in MCF10A cells treated with TGF-ß, indicating that TGF-ß treatment induced not only MMP-2 expression but also activation of proMMP-2 (72 kDa).

To investigate direct gene transcription as a potential mechanism for the TGF-ß-stimulated MMP-2 and MMP-9 activities observed, promoter assay was performed. The MCF10A cells were transfected with luciferase reporter plasmid constructs under the transcriptional control of the wild-type MMP-2 (30) or MMP-9 (31) promoters. As shown in Fig. 3B, a prominent activation of the MMP-2 promoter was detected by treatment of TGF-ß for 24 h. Over 3-fold induction was observed in the MMP-2 promoter activity by treatment of 1 and 10 ng/ml TGF-ß. MMP-9 promoter activity was also increased by TGF-ß, but to a lesser extent compared to MMP-2. A significant induction of MMP-9 promoter activity was observed in the cells treated with the highest concentration (10 ng/ml) of TGF-ß. These results demonstrate that TGF-ß-induced MMP-2 and MMP-9 activities result from upregulation of MMP-2 and MMP-9 expression at the transcriptional level.

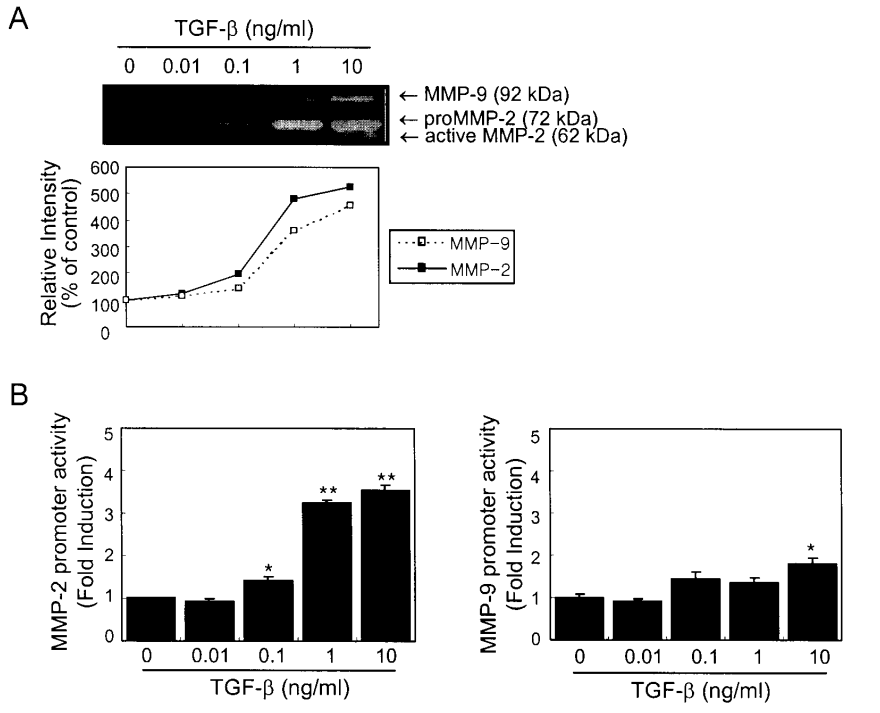

Figure 3. TGF-ß upregulates MMP-2 and MMP-9 in MCF10A cells. (A), Gelatin zymogram assay was performed on the conditioned media of MCF10A cells treated with various concentrations of TGF-ß for 48 h. Relative band intensities were determined by Image analyzer. (B), A luciferase assay was performed to detect promoter activities of MMP-2 and MMP-9 in TGF-ß-treated MCF10A cells. The luciferase activity in 1 µg of cell lysate was normalized to ß-galactosidase activity. The results represent means ± SE of triplicates. *,**Statistically different from control at $p<0.05$ and $p<0.01$, respectively.

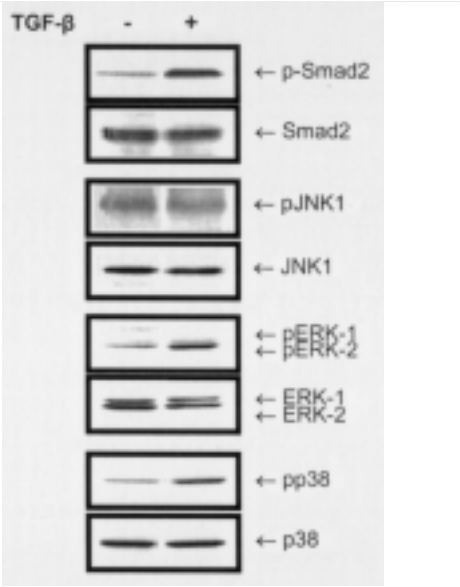

Figure 4. TGF-ß activates Smad2, ERK-1/2 and p38 MAPK in MCF10A cells. The levels of activated Smad2, JNK-1, ERK-1/2 and p38 MAPK in MCF10A cells treated with 10 ng/ml TGF-ß for 1 h were determined by immunoblot analysis using phospho-specific antibodies (p-Smad2, pJNK-1, pERK-1/2 and pp38) and the antibodies which detect total forms of these MAPKs.

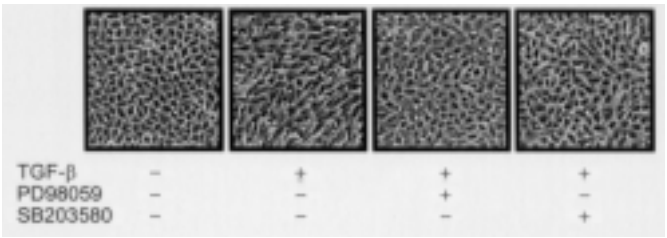

Figure 5. TGF-ß-induced EMT involves the activation of ERK and p38 MAPK pathways. MCF10A cells were treated with 10 ng/ml TGF-ß for 48 h in the absence or presence of 2.5 μM PD98059 or 5 μM SB203580. Morphological alterations by TGF-ß treatment were examined by light microscopy (x100).

TGF-ß activates Smad2, ERK-1/2 and p38 MAPK in MCF10A cells. We evaluated whether Smad2, a direct signaling molecule of TGF-ß, was activated in MCF10A cells following treatment with 10 ng/ml TGF-ß. The concentration was chosen because morphological alteration and a marked induction of invasion, migration and MMP-2/-9 upregulation were observed by treatment of 10 ng/ml TGF-ß in MCF10A cells. As shown in Fig. 4, the phosphorylated level of Smad2 was increased by treatment with 10 ng/ml TGF-ß for 1 h in MCF10A cells.

In addition to the Smad-mediated TGF-ß signaling pathway, mounting evidence suggests that TGF-ß may signal through other pathways such as MAPKs (12-16). We examined the activation of MAPK family members in response to TGF-ß in MCF10A cells. Whereas JNK-1 was not activated by TGF-ß, both ERK-1/2 and p38 MAPK were effectively activated by treatment of 10 ng/ml TGF-ß in 1 h (Fig. 4). The response was rapid, suggesting that it might not be through an indirect activation dependent on Smad signaling.

Activation of ERK and p38 MAPK pathways is required for TGF-ß-induced EMT, invasion and migration. To examine the potential involvement of ERK and p38 MAPK signaling pathways in TGF-ß-induced EMT, we pretreated the MCF10A cells with PD98059 or SB203580, specific inhibitors of ERK and p38 MAPK pathways, respectively. TGF-ß-induced EMT was markedly abolished in the cells pretreated with 2.5 μM PD98059 and 5 μM SB203580 for 48 h (Fig. 5), demonstrating that TGF-ß-induced EMT in MCF10A cells is dependent on the activation of ERK and p38 MAPK pathways.

In order to assess the functional significance of TGF-ß-activated ERKs and p38 MAPK in invasion, we asked if interfering with ERK and p38 MAPK activation by chemical inhibitors diminished TGF-ß-mediated invasiveness *in vitro*. TGF-ß-induced invasive phenotype was almost completely blocked by pretreatment of cells with 25 μM PD98059 or 50 μM SB203580, indicating that the induction of invasion by TGF-ß was dependent on ERKs and p38 MAPK pathways (Fig. 6A). To further investigate the role of ERKs in TGF-ß-induced cellular invasion, we transfected the MCF10A cells with a DN MEK-1 construct (20) in which both of the phosphorylated motifs, Ser^{218} and Ser^{222}, were mutated to Ala. A significant reduction of the number of invaded cells was observed in DN MEK-1 transfectants treated with TGF-ß compared to the TGF-ß-treated control cells. Similarly, TGF-ß-induced invasive phenotype was prominently inhibited in the cells transfected with DN p38 in which the dual phosphorylated motif, Thr-Gly-Tyr, was mutated to Ala-Gly-Phe (19). We then examined the role for JNK-1 in TGF-ß-induced MCF10A cell invasive phenotype using a DN mutant of JNK-1 where Thr^{183} and Tyr^{185} were mutated to Ala and Phe, respectively (21). Interfering with JNK-1 activation by DN JNK-1 transfection did not affect invasiveness, indicating that the TGF-ß-induced invasion was independent of JNK-1 pathway. Transfection efficiency was about 85% as evidenced by cotransfection with green fluorescence protein (data not shown). The results demonstrate that TGF-ß efficiently induces invasive phenotype of MCF10A cells in which the activation of ERK and p38 MAPK pathways are required.

Since migrative capacity is a prerequisite for cell invasion through the basement membrane, we asked whether TGF-ß promoted not only invasive phenotype but also cell motility in MCF10A cells. Interfering with ERK and p38 MAPK pathways by PD98059 and SB203580, respectively, prominently blocked the migrative property of MCF10A cells induced by TGF-ß as evidenced by Transwell migration assay (Fig. 6B). Requirement of the activation of ERKs and p38 MAPK pathways in the TGF-ß-induced migration was confirmed by transfecting the MCF10A cells with the DN mutant constructs of ERKs and p38 MAPK while the DN JNK-1 transfectant cells did not exert any significant reduction of the number of migrated cells. These findings indicate critical roles for ERKs and p38 pathways in TGF-ß-induced invasive phenotype and migration in MCF10A human breast epithelial cells.

TGF-ß-mediated upregulation of MMP-2 and MMP-9 is dependent on p38 MAPK activity, but not on ERK activity. Since the present study shows that ERKs and p38 MAPK are

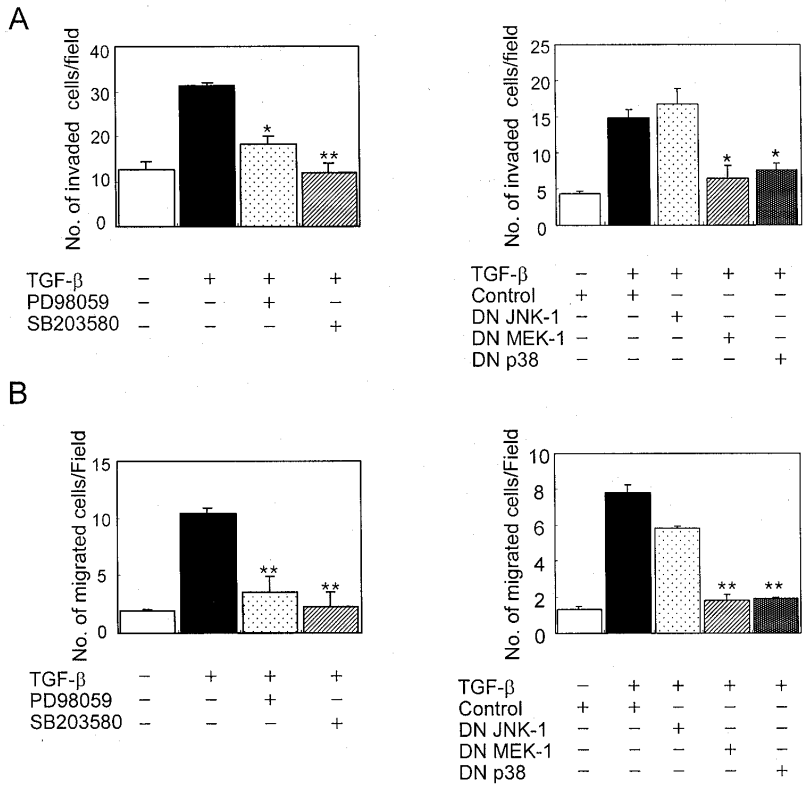

Figure 6. Activation of ERKs and p38 MAPK is required for TGF-ß-induced invasion and migration. (A), MCF10A cells were treated with TGF-ß (10 ng/ml) and subjected to *in vitro* invasion assay in the absence or presence of 25 μM PD98059 or 50 μM SB203580 (left). Transiently transfected cells with control vector, DN JNK-1, DN MEK-1 and DN p38 MAPK constructs were treated with 10 ng/ml TGF-ß and subjected to *in vitro* invasion assay (right). (B), Migratory properties of the MCF10A cells were determined by *in vitro* migration assay, which is identical except that the Transwell was not coated with Matrigel. The number of invaded or migrated cells per field was counted (x400) in thirteen fields. The results represent means ± SE of triplicates. *,**Statistically different from the cells treated with TGF-ß only at $p<0.05$ and $p<0.01$, respectively.

critical for TGF-ß-mediated invasion, we asked whether ERKs and/or p38 MAPK mediate TGF-ß-induced upregulation of MMP-2 and MMP-9. As shown in Fig. 7A, upregulatory effect of TGF-ß (10 ng/ml) on enzymatic activities and expression levels of these MAPKs was inhibited by blocking p38 MAPK pathway by SB203580 in a dose-dependent manner. Treatment of 50 μM SB203580 almost entirely abolished the TGF-ß-induced upregulation of secreted enzymatic activities (Fig. 7A, left) and expression levels (Fig. 7A, right) of MMP-2 and MMP-9 to a comparable extent. In contrast, inhibition of ERKs pathway by PD98059 did not significantly inhibit the TGF-ß-stimulated enhancement of activities and expressions of MMP-2 and MMP-9 (Fig. 7B). In order to ensure that TGF-ß-activated ERK-1/2 was abolished by treatment of PD98059, immunoblot analysis was performed in the cells treated with TGF-ß (10 ng/ml) in the absence or presence of PD98059. As shown in Fig. 7C, the elevated levels of phosphorylated ERK-1/2 were decreased by this compound in a dose-dependent manner indicating that PD98059 efficiently blocked the TGF-ß-activated ERKs pathway. The results demonstrate that TGF-ß-mediated upregulation of MMP-2 and MMP-9 in MCF10A cells is dependent on the activation of p38 MAPK signaling pathway but not that of ERK pathway.

Discussion

TGF-ß has long been considered as both positive and negative effector on tumorigenesis of mammary cells, acting early as a tumor suppressor but later as a stimulator of tumor invasion (32,33). The present study demonstrates that TGF-ß treatment induces a phenotypic transformation in MCF10A 'normal' human breast epithelial cells, which are resistant to TGF-ß-induced growth inhibition. Although the MCF10A cells have gone through crisis upon immortalization and thus may have some altered phenotypes, mounting evidence shows that they have 'normal' cell characteristics (17,22,23). In agreement with our observation, recent studies show that TGF-ß induces EMT and cell migration in normal mouse mammary epithelial cells (34-36). These findings suggest that TGF-ß treatment may induce an oncogenic signaling pathway at the early stage of tumor progression in breast epithelial cells.

It has been reported that TGF-ß-induced EMT was not blocked by inhibitors of JNK (34) or ERK pathways (35) in NMuMG mouse mammary epithelial cells while the involvement of the p38 MAPK pathway in TGF-ß-stimulated EMT has been implicated (14-16). We show that the morphological alteration of MCF10A cells induced by TGF-ß involves both p38 MAPK and ERK signaling pathways, suggesting a differential role of signaling molecules in TGF-ß-mediated phenotypic change in different cell system.

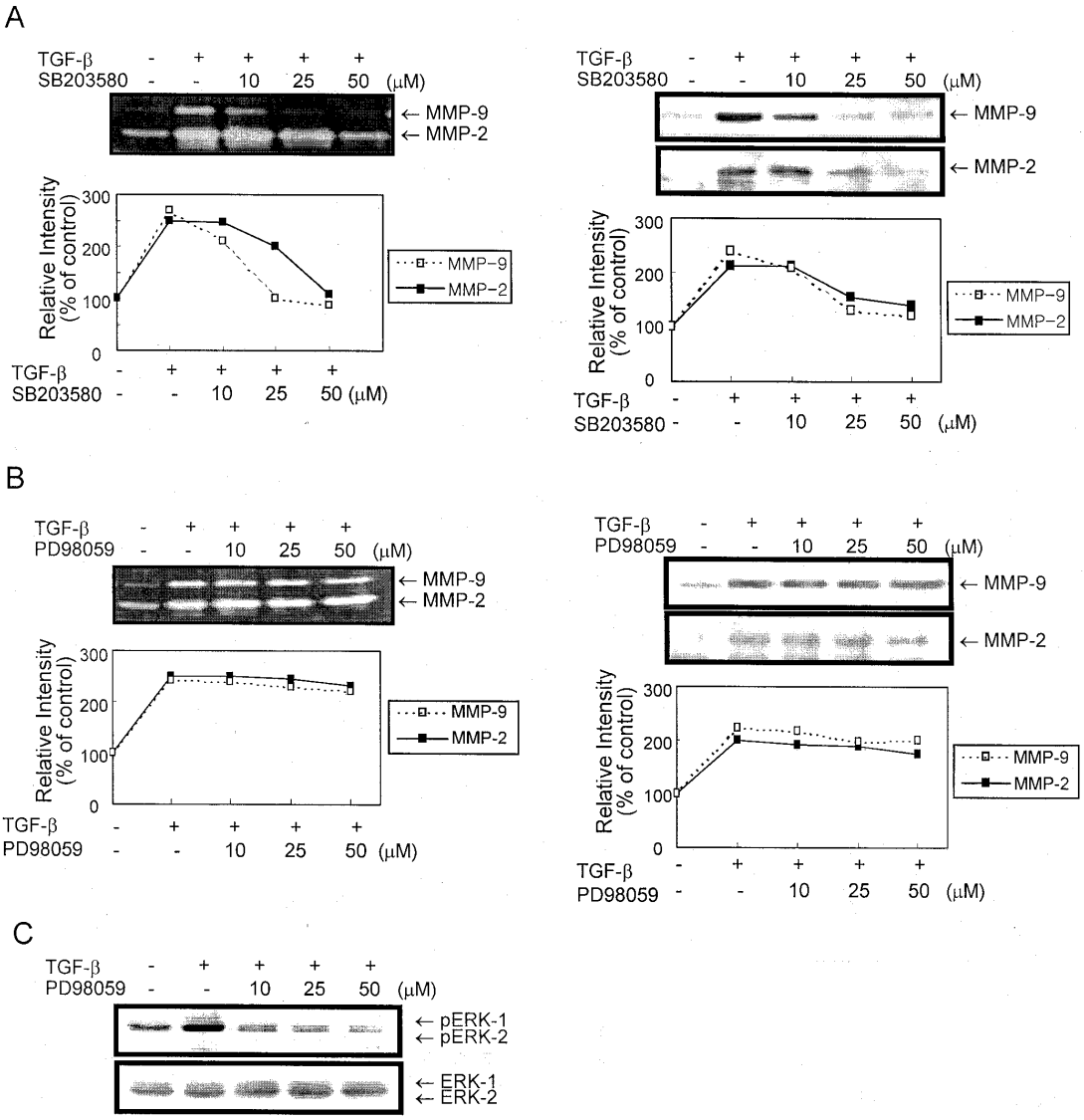

Figure 7. TGF-ß-mediated upregulation of MMP-2 and MMP-9 is dependent on p38 MAPK activity. (A), Cells were incubated with 10 ng/ml TGF-ß and various concentrations of SB203580. Gelatinolytic activities and the expression levels of secreted MMP-2 and MMP-9 were determined by gelatin zymogram assay (left) and immunoblot analysis (right), respectively. Relative band intensities were determined using Image analyzer. (B), Cells were treated with 10 ng/ml TGF-ß and various concentrations of PD98059. (C), Immunoblot analysis of the cells (B) was performed to confirm that phosphorylated ERK levels were decreased by treatment of PD98059.

TGF-ß regulates MMP expression in a cell type-specific manner. Enhanced expression of MMP-2 but not MMP-9 by TGF-ß was reported in pancreatic cancer cells (27), whereas TGF-ß was shown to induce MMP-9 in transformed keratinocytes (28,29). Our data demonstrate that TGF-ß induces upregulation of MMP-2 and MMP-9 through transcriptional activation in MCF10A human breast epithelial cells. In human prostate cancer cells, however, TGF-ß was shown to induce MMP-2 activity by a non-transcriptional pathway, and delayed decay of secreted enzyme activity (37). It should be noted that while TGF-ß induced gelatinolytic activities of MMP-2 and MMP-9 at comparable levels (Fig. 3A), it enhanced MMP-2 promoter activity more efficiently than MMP-9 (Fig. 3B). TGF-ß (10 ng/ml) induced MMP-2 promoter activity 3.5-fold whereas MMP-9 promoter activity was induced 1.8-fold by the same treatment (Fig. 3B). The results suggest that in addition to the transcriptional activation, there might be other mechanism(s) for the regulation of MMP-9 expression by TGF-ß in MCF10A cells, including increased mRNA stability as previously shown in human prostate cancer cells (37).

Our data clearly show that the activation of p38 MAPK pathway, but not that of ERKs pathway, is required for TGF-ß-induced upregulation of MMP-2 and MMP-9 in MCF10A human breast epithelial cells. When the ERK signaling pathway was blocked by PD98059, TGF-ß-mediated induction of EMT, invasion and migration were significantly inhibited (Figs. 5 and 6) although MMP-2 and MMP-9 activities remained intact (Fig. 7). These results suggest that cell invasion and migration are a complex process requiring not only extracellular matrix (ECM)-degrading proteinase activities through MMP-2 and MMP-9 (9,26,38) but also activation of intracellular signaling pathways exerted by ERKs (13) and p38 MAPK (14-16). This observation may be cell type-specific since it has been shown that blocking ERK pathway by PD98059 potently suppressed TGF-ß-induced MMP-9

expression in transformed keratinocytes (29). Additional investigations will be necessary to elucidate the role of p38 MAPK in TGF-ß-activated transcription factors interacting with MMP-2 and/or MMP-9 promoters in MCF10A human breast epithelial cells. MMPs can trigger the morphological transformation of epithelial cells (39,40). Critical role of p38 MAPK- and MMP-dependent pathway in TGF-ß-induced osteoblast elongation was demonstrated (41). Contrary to our observation, expression of MMP-13, but not that of MMP-2, was induced by TGF-ß in this osteoblast cell system.

Signaling responses induced by TGF-ß are mediated by Smad proteins (42), but certain evidence has suggested that activation of p38 MAPK by TGF-ß is independent of Smads (43). A recent finding demonstrates that TGF-ß activates the p38 MAPK pathway, leading to Smad3 phosphorylation, together with TGF-ß receptor type I-dependent phosphorylation of Smad2 in hepatic stellate cells during chronic liver injury (44). Our study shows that not only Smad2 but also p38 MAPK and ERKs are activated upon TGF-ß in MCF10A cells. The stimulatory effects of TGF-ß on p38 MAPK and ERKs were exerted in 1 h. The rapid activation of these MAPKs suggests that it might be through a direct post-translational modification rather than an indirect effect of TGF-ß by Smad-dependent transcriptional regulation. We are currently investigating the potential involvement of TGF-ß receptors and Smads in TGF-ß-mediated activation of p38 MAPK and ERKs in MCF10A cells. The results from these studies will provide insights into the molecular mechanisms for TGF-ß signaling in breast cancer development.

Acknowledgements

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