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TGF-β-induced transcriptional activation of MMP-2 is mediated by activating transcription factor (ATF)2 in human breast epithelial cells

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Abstract

We have previously shown that transforming growth factor (TGF)-β up-regulates matrix metalloproteinase (MMP)-2 leading to the induction of oncogenic signaling in preneoplastic MCF10A human breast epithelial cells. The present study investigated the mechanism of transcriptional regulation of MMP-2 by TGF-β in MCF10A cells. By using 5′ deletion constructs of MMP-2 promoter, we demonstrated that binding sites for p53, S1, AP-1 and Sp1, and to a lesser extent CREB, GCN-His and PEA3, were potential *cis*-acting elements for TGF-β-induced transcriptional activation of MMP-2 in MCF10A cells. Since activating transcription factor (ATF)2 was shown to mediate the TGF-β-induced cellular responses, we examined the involvement of ATF2 in TGF-β-activated MMP-2 gene transcription. TGF-β increased DNA binding activity of AP-1 in which ATF2 was involved as evidenced by electrophoretic mobility shift assay. TGF-β induced phosphorylation of ATF2 through p38 MAPK signaling. A dominant-negative (DN) ATF2 significantly inhibited the TGF-β-induced up-regulation of MMP-2, but not that of MMP-9, suggesting that ATF2 may be a transcription factor responsible for transcriptional activation of MMP-2 gene by TGF-β. Invasive and migratory phenotypes induced by TGF-β were significantly inhibited by DN ATF2, indicating a critical role of ATF2 in TGF-β-induced oncogenic progression of MCF10A cells. Taken together, this study demonstrates that ATF2 mediates the TGF-β-induced MMP-2 transcriptional activation, elucidating a molecular mechanism for the malignant progression of human breast epithelial cells exerted by TGF-β.

Keywords: TGF-β; MMP; p38 MAPK; ATF2; AP-1; Breast epithelial cells

1. Introduction

Expression of matrix metalloproteinase (MMP)-2 and MMP-9 has been demonstrated in a variety of human tumors and a major impact of these enzymes on the acquisition of a malignant phenotype during multiple stages of tumor progression has been emphasized [1–3]. Transforming growth factor (TGF)-β is a multifunctional cytokine with a wide range of physiological as well as pathological effects [4,5]. TGF-β contributes to tumor progression by inducing an epithelial-to-mesenchymal

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transition (EMT), cell invasion and migration of epithelial tumor cells partly through stimulating MMP-2 and/or MMP-9 [6,7]. Our previous studies showed that TGF-β induced up-regulation of MMP-2 and MMP-9 leading to the invasive and migratory phenotypes in MCF10A human breast epithelial cells [8] and H-Ras-activated MCF10A cells [9].

Cellular responses induced by TGF-β are mediated by Smad proteins [10]. In addition to the classical Smad pathway, TGF-β receptor activation stimulates the mitogen-activated protein kinase (MAPK) pathway [11]. TGF-β receptor-activated p38 MAPK has been shown to mediate Smad-independent TGF-β responses [12]. We have previously reported that TGF-β up-regulates MMP-2 *via* p38 MAPK pathway in MCF10A cells [8].

Studies on the transcriptional regulation by TGF-β have shown that many promoters of TGF-β-regulated genes such as PAI-1 [13], TIMP-1 [14], MMP-1 [15] and α2(I) collagen [16] contain AP-1 sites while others such as fibronectin [17], cyclin Dl [18] and cyclin A [19] contain CREB binding sites. Activating transcription factor (ATF)2, a member of CREB/ATF family of transcription factors, is a nuclear target of the TGF-β signaling [20,21]. Recently, we have shown that ATF2 is a key transcription factor for the transcriptional activation of MMP-2 gene in p38 MAPK-activated MCF10A cells [22].

These previous reports allowed us to hypothesize that ATF2 might play an important role in the TGF-β-induced transcriptional activation of MMP-2 in MCF10A cells. Here we show that ATF2 activation by p38 MAPK signaling mediates MMP-2 transcriptional activation by TGF-β in MCF10A cells. We also show that ATF2 is required for TGF-β-induced invasive and migratory phenotypes, revealing ATF2 as a potential transcription factor responsible for TGF-β-induced MMP-2 up-regulation leading to malignant progression of human breast epithelial cells.

2. Materials and methods

2.1. Materials

Dulbecco's modified Eagle's medium (DMEM), L-glutamine, penicillin–streptomycin and trypsin–EDTA were purchased from Gibco BRL (Grand Island, NY). TGF-β, epidermal growth factor (EGF), cholera enterotoxin, amphotericin B, hydrocortisone and SB203580 were purchased from Sigma–Aldrich (St. Louis, MI).

2.2. Cell culture

Development and characterization of MCF10A cell line have been previously described [23]. 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.

2.3. Transfection

Transfection was performed using Lipofectamine^{2000} (Invitrogen, Carlsbad, CA) following the manufacturer's instruction. A dominant-negative (DN) p38 [24] was kindly provided by Dr. Y.-J. Surh (Seoul National University, Seoul, Korea). A DN ATF2 (A-ATF2) construct [25] was a kind gift from Dr. C. Vinson at National Cancer Institute, National Institute of Health (Rockville, MD).

2.4. Synthesis of small interfering RNA (siRNA) targeting MMP-2

An siRNA for MMP-2 was synthesized from Invitrogen (Carlsbad, CA). Specific oligonucleotide sequence targeting MMP-2 was 5′-AAUACCAUCGAGACC AUGCGG-3′ (sense; [26]). Stealth RNAi (Invitrogen) was used as a negative control. Cells cultured in a 6-well plate were transfected with 25 pmol of siRNA in 250 μl of OPTI-MEM and 250μl of Lipofectamine^{2000}-OPTI-MEM mixture following the manufacturer's instruction.

2.5. Immunoblot analysis

Immunoblot analysis was performed as previously described [27]. Anti-ATF2 and anti-phospho-ATF2 antibodies were purchased from Cell Signaling Tech (Beverly, MA). MMP-2 and MMP-9 antibodies were purchased from R&D systems (Minneapolis, MN) and Santa Cruz Biotech (Santa Cruz, CA), respectively.

2.6. Gelatin zymography

Conditioned medium was collected and centrifuged at 3000 rpm for 10 min to remove cell debris. Gelatinolytic activity of the conditioned medium was determined by gelatin zymogram assay as previously described [28]. Areas of gelatinase activity were detected as clear bands against the blue-stained gelatin background.

2.7. In vitro invasion assay and transwell migration assay

In vitro invasion assay and transwell migration assay were performed using 24-well transwell units as previously described [27,28].

2.8. Luciferase reporter assay

Cells were seeded in a 6-well plate at 1×10^6 cells/well and transiently transfected with 4 μg promoter construct and 0.13 μg β-galactosidase expression plasmid pMDV-lacZ. The luciferase activity in 1 μg of cell lysate was normalized to β-galactosidase activity. Luciferase and β-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). For MMP-2 and MMP-9 promoter assays, full-length human MMP-2 [29] and a series of MMP-2 deletion constructs [30] and MMP-9 [31] promoter-luciferase construct were kindly provided by Dr. Benveniste (University of Alabama, Birmingham, AL). For AP-1 transcriptional activity assay, the AP-1-driven plasmid pAP-1-Luc construct, which encodes luciferase under the control of heptamer AP-1 enhancer elements, seven tandem repeats of the AP-1 DNA binding sequence TGACTAA was purchased from Stratagene (La Jolla, CA).

2.9. Electrophoretic mobility shift assay (EMSA)

EMSA was performed using nuclear extracts from untreated cells or cells treated with 10 ng/ml TGF-β for 1 h. The following oligonucleotides were used as probes: the AP-1 oligonucleotide (5′-CGCTTGATGACTCA GCCGGAA-3′) and Sp1 oligonucleotide (5′-ATTCG ATCGGGGCGGGGCGAG-3′) were purchased from Santa Cruz Biotech (Santa Cruz, CA). DNA binding reaction was carried out as previously described [32]. For competition experiments, nuclear extracts were incubated with 50-fold, 100-fold and 200-fold molar excesses of unlabeled AP-1 or Sp1 oligonucleotide at 25 °C for 30 min before addition of the labeled probe. For immunoinhibition analysis, antibody directed against ATF2 was incubated with nuclear extract at 37 °C for 30 min in binding buffer, followed by an additional incubation for 30 min at room temperature with labeled oligonucleotide as previously described [22]. The samples were subsequently subjected to electrophoresis on a polyacrylamide gel, dried, and visualized by autoradiography.

3. Results

3.1. MMP-2 up-regulation is critical to the TGF-β-induced invasive and migratory phenotypes

Our previous report has shown that TGF-β induces MMP-2 expression, invasion and migration of MCF10A cells [8]. We first investigated the contribution of MMP-2 up-regulation to the induction of invasive and migratory phenotypes using an siRNA targeting MMP-2. TGF-β-induced MMP-2 expression was markedly inhibited by siRNA for MMP-2 as evidenced by immunoblot analysis (Fig. 1a). Inhibition of MMP-2 expression by siRNA caused a significant reduction of invasion and migration of MCF10A cells treated with TGF-β (Fig. 1b). The results demonstrate that MMP-2 up-regulation plays an essential role in TGF-β-induced invasive and migratory phenotypes.

3.2. TGF-β induces transcriptional activation of MMP-2 through p38 MAPK signaling

We have previously shown that TGF-β-induced MMP-2 up-regulation at the level of protein expression and enzymatic activity depends on p38 MAPK signaling in MCF10A cells [8]. To examine the role of p38 MAPK pathway in the transcriptional activation of MMP-2 induced by TGF-β, a promoter assay was performed on MCF10A cells treated with 10 ng/ml TGF-β in the presence of SB203580, a specific inhibitor of p38 MAPK. It was reported that p38 MAPK was inhibited by 50 μM SB203580 [27].

TGF-β-induced activation of MMP-2 transcription was significantly inhibited by SB203580 in a dose-dependent manner (Fig. 2a). MMP-2 promoter activity of TGF-β-treated cells was completely abolished by 50 μM of SB203580. To further confirm the role of p38 MAPK in TGF-β-induced transcriptional activation of MMP-2, we transfected the MCF 10A cells with a DNp38 construct in which both of the phosphorylation sites, Ser^{218} and Ser^{222}, were mutated to Ala [24]. TGF-β-induced

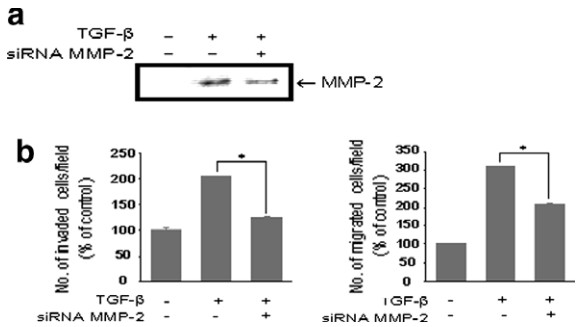

Fig. 1. MMP-2 is critical to TGF-β-induced invasion and migration. (a) MCF10A cells were transfected with control siRNA or MMP-2 siRNA in the absence or presence of 10 ng/ml TGF-β. MMP-2 expression was detected by immunoblot analysis. (b) The transfected cells were subjected to the *in vitro* invasion assay and transwell migration assay in the absence or presence of the 10 ng/ml TGF-β. The number of invaded or migrated cells per field was counted (400×) in thirteen arbitrary visual fields. The results represent means ± SE of triplicates. *Statistically different from control at $p < 0.05$ by the two-tailed Student's *t*-test.

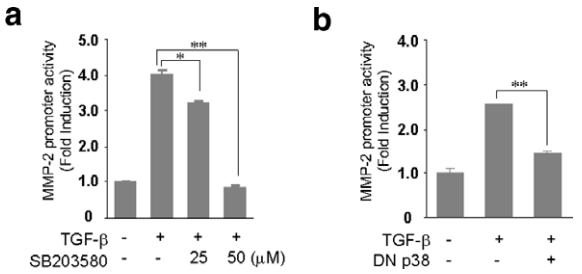

Fig. 2. TGF-β-induced MMP-2 transcriptional activation depends on p38 MAPK signaling. (a) Luciferase assay was performed to detect promoter activity of MMP-2 in MCF10A cells treated with TGF-β (10 ng/ml) for 24 h in the absence or presence of 25 or 50 μM SB203580. Luciferase activity, determined in triplicates, was normalized to β-galactosidase activity. (b) Luciferase activity was measured on cells co-transfected with MMP-2 promoter-luciferase construct and DN p38 construct and treated with TGF-β (10 ng/ml) for 24 h. The results represent means ± S.E. of triplicates. *,**Statistically different at $p < 0.05$ and $p < 0.01$, respectively, by the two-tailed Student's *t*-test.

MMP-2 transcriptional activation was markedly inhibited by DN p38 transfection (Fig. 2b), indicating that TGF-β-induced transcriptional activation of MMP-2 was dependent on p38 MAPK signaling.

3.3. Potential cis-acting elements for TGF-β-induced MMP-2 transcriptional activation were revealed by promoter deletion analysis

To assess the molecular basis for TGF-β-induced MMP-2 transcriptional activation, a promoter assay was performed on MCF10A cells treated with TGF-β using a series 5′-deletion mutants (D2–D7 and D9–D12) based on the −1659 bp MMP-2 promoter suggested by Qin et al [30]. Mutagenesis analysis of MMP-2 promoter (Fig. 3a) revealed that the deletion of the following four regions resulted in a significant reduction of MMP-2 promoter activity: $-1659 \sim -1611$ (Full length/D2), $-1591 \sim -1259$ (D3/D4), $-546 \sim -139$ (D7/D9), and $-139 \sim -85$ (D9/D10). Deletion of $-1659 \sim -1611$ (D2) region which contains potential binding sites for p53 and S1 elements resulted in 37% reduction of MMP-2 promoter activity compared with the full length MMP-2 promoter construct. TGF-β-induced MMP-2 promoter activity was significantly inhibited by deletion of the region that contains a potential AP-1 site (D4): 37% inhibition compared with the D3 construct. Less inhibition (19.5%) than the others was observed by deletion of the region spanning $-546 \sim -139$ which contains the binding sites for CREB, GCN-His, PEA3, suggesting that these elements were also important, to a lesser degree, for MMP-2 promoter activity. Deletion of $-139 \sim -85$ region containing the Sp1 (D10) site resulted in a prominent (76%) reduction compared with the D9 construct. Taken together, the data revealed the binding sites for p53, S1, AP-1 and Sp1, and to a lesser extent for CREB, GCN-His and PEA3, as essential *cis* elements for TGF-β-induced transcriptional activation of MMP-2 in MCF10A cells.

Although deletion of Sp1 binding site caused a more drastic inhibition of MMP-2 promoter activity than that of AP-1 site, this study has focused on the AP-1 site since our previous study demonstrated a critical role of the AP-1 binding site on the up-regulation of MMP-2 through MKK3/6-p38 MAPK pathway [22]. Further investigation would be needed to elucidate the role of Sp1 on TGF-β-induced up-regulation of MMP-2.

3.4. TGF-β increases DNA binding activity and transcriptional activity of AP-1

We then investigated the involvement of ATF2 in TGF-β-activated MMP-2 gene transcription. Although ATF2 can bind either to the AP-1 site or to the CREB site, we examined the role of ATF2 in AP-1 DNA binding activity since the deletion of AP-1 site caused a more drastic reduction of MMP-2 promoter activity than that of CREB site in MCF10A cells as shown in Fig. 3a. TGF-β increased the AP-1 DNA binding activity of MCF10A cells as evidenced by EMSA using a labeled oligonucleotide containing the AP-1 element (Fig. 3b), indicating the induction of DNA binding activity of AP-1 by TGF-β. To confirm the specific binding, a competition study with unlabeled AP-1 oligonucleotide was conducted. A gradual reduction in band intensity was observed by increasing the concentration of cold competitor AP-1 oligonucleotide while the non-specific cold competitor Sp1 oligonucleotide did not compete for the protein binding, confirming the specificity of AP-1 DNA binding.

Transcriptional activity of AP-1 in TGF-β-treated cells was measured by using a luciferase reporter gene (pAP-1-Luc) under the transcriptional control of a promoter containing a heptamer repeat of the AP-1 binding sequence. A significant increase of AP-1 luciferase activity was observed in TGF-β-treated cells (Fig. 3c), demonstrating that TGF-β increased AP-1 transcriptional activity in MCF10A cells.

3.5. TGF-β induces ATF2 activation

Involvement of ATF2 in TGF-β-increased DNA binding activity of AP-1 was examined by an immunoinhibition study with a specific antibody directed against ATF2. DNA binding activity of AP-1 enhanced by TGF-β was markedly decreased by addition of anti-ATF2 antibody (Fig. 4a). The result demonstrates that ATF2 is responsible for the TGF-β-increased AP-1 DNA binding activity. We then evaluated whether

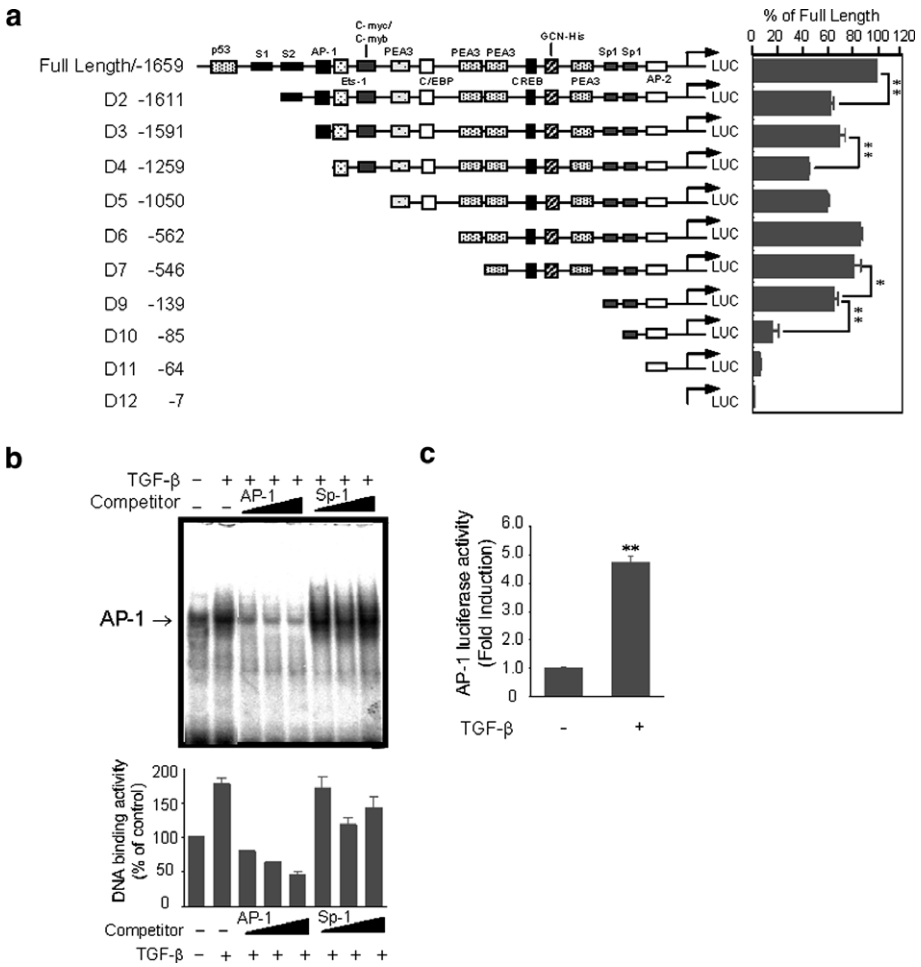

Fig. 3. AP-1 binding site is required for TGF-β-induced MMP-2 transcriptional activation. (a) Effects of 5′ deletion constructs on human MMP-2 promoter activity were examined by promoter assay. Cells were co-transfected with full length or truncated MMP-2 promoter construct and pMDV-lacZ galactosidase expression construct as indicated under Section 2. The transfected cells were allowed to recover for 12 h, and then were treated with TGF-β (10 ng/ml) for 24 h. MMP-2 promoter activity was determined in triplicate, and the luciferase activity of each construct was normalized to β-galactosidase activity. The activity of the full length MMP-2 construct is represented as 100%, and the activities of the deletion mutants compared with full length promoter were shown. Values indicate base pairs (bp). Data are the means ± SE from three independent experiments. *,**Statistically different from full length MMP-2 construct treated with TGF-β at $p < 0.05$ and $p < 0.01$, respectively. (b) Nuclear extracts from TGF-β-treated MCF10A cells were subjected to EMSA for the activated AP-1 using a radiolabeled oligonucleotide containing the AP-1 binding site. For competition analysis, nuclear extracts from the TGF-β-treated cells were preincubated with unlabeled oligonucleotide containing the AP-1 binding site as cold competitor or non-specific Sp1 binding site with an increasing concentration (50-fold, 100-fold, and 200-fold molar excesses of the unlabeled oligonucleotide) and subjected to EMSA. (c) Cells were transfected with a luciferase reporter plasmid with a promoter containing a heptamer repeat of the AP-1 binding sequence (pAP-1-Luc). AP-1 luciferase activity was measured in TGF-β-treated MCF10A cells. The results represent means ± SE of triplicates. **Statistically different from control at $p < 0.01$.

ATF2 was activated by TGF-β in MCF10A cells by detecting phosphorylated ATF2 in the nuclei of TGF-β-treated cells. A kinetic study showed that phosphorylated ATF2 was increased by TGF-β treatment in a time-dependent manner (Fig. 4b), consistently with the previous report that ATF2 phosphorylation occurred following exposure to TGF-β [21].

3.6. TGF-β-induced ATF2 activation requires p38 MAPK signaling

To investigate the role of p38 MAPK on TGF-β-induced activation of ATF2, cells were treated with TGF-β in the presence of 50 μM SB203580. Inhibition of p38 MAPK pathway by SB203580 markedly inhibited

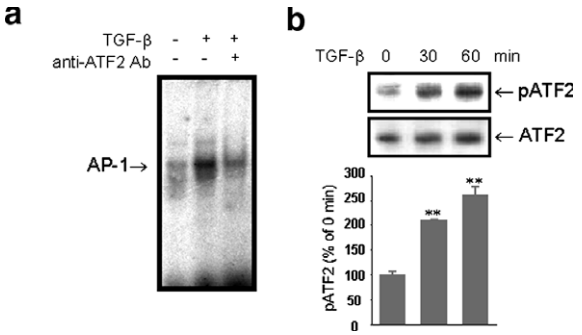

Fig. 4. ATF2 is activated upon TGF-β treatment. (a) Cells were treated with TGF-β (10 ng/ml) for 1 h, and nuclear extracts were prepared. Immunoinhibition study was performed on nuclear extracts from TGF-β-treated MCF10A cells by EMSA for the activated AP-1 with a specific antibody against ATF2. (b) Phosphoylated ATF2 and total ATF2 were detected on cells treated with TGF-β (10 ng/ml) for various periods of time by immunoblot analysis.

TGF-β-induced activation of ATF2 as shown in Fig. 5a. Similar results were obtained when the p38 MAPK pathway was inhibited by transfection with a DN p38 construct (Fig. 5b). These results indicate that TGF-β induces ATF2 phosphorylation through p38 MAPK signaling pathway in MCF10A cells.

3.7. ATF2 is required for TGF-β-induced transcriptional activation of MMP-2

We next asked whether ATF2 mediated TGF-β-induced MMP-2 transcriptional activation. To determine the functional importance of ATF2, a loss of function experiment was conducted using a DN ATF2, which acts by inhibiting the DNA binding of basic leucine zipper (bZIP) protein in a dimerization-dependent fashion [25]. The inhibitory effect of DN ATF2 transfection on ATF2 phosphorylation was determined by an immunoblot analysis of TGF-β-treated cells. A significant inhibition of ATF2 phosphorylation was observed by DN ATF2 transfection (Fig. 6a).

DN ATF2 inhibited the TGF-β-induced gelatinolytic activity of pro-MMP-2 (72 kDa) as evidenced by gelatin zymogram assay (Fig. 6b). Immunoblot analysis also showed a prominent inhibition of TGF-β-induced MMP-2 expression by DN ATF2 (Fig. 6c). Expression of MMP-9 was not affected by DN ATF2, indicating that ATF2 activation was required for TGF-β-induced up-regulation of MMP-2, but not that of MMP-9. Co-transfection with DN ATF2 and MMP-2 promoter construct resulted in a marked reduction of TGF-β-induced transcriptional activation of MMP-2 while it did not inhibit MMP-9 promoter activity (Fig. 6d). The results suggest that ATF2 mediates transcriptional activation of MMP-2 in response to TGF-β in MCF10A cells.

3.8. TGF-β-induced invasion and migration depends on ATF2

To assess the functional significance of TGF-β-activated ATF2 in MCF10A cell invasion and migration, we investigated whether interfering with ATF2 activation diminished TGF-β-induced invasiveness and migration *in vitro*. As shown in Fig. 7a, transfection of cells with DN ATF2 significantly inhibited the TGF-β-induced invasive phenotype of

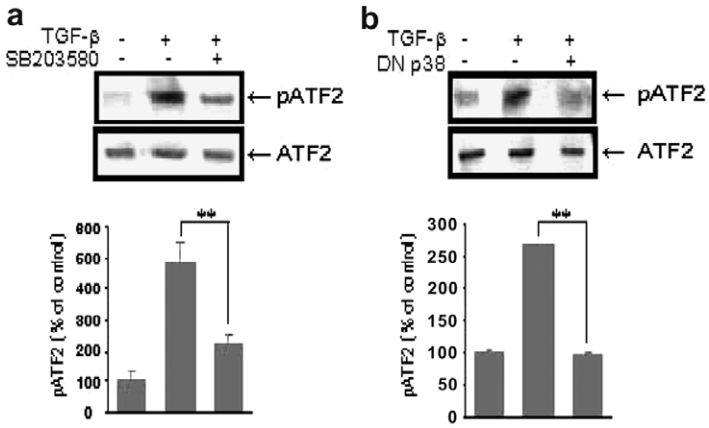

Fig. 5. TGF-β-induced activation of ATF2 depends on p38 MAPK. (a) Nuclear proteins of cells treated with TGF-β (10 ng/ml) for 1 h in the absence or presence of 50 μM SB203580 were analyzed for expression of phosphorylated and total forms ATF2 by immunoblot analysis. (b) Cells were transiently transfected with either the control vector or DN p38 construct. Immunoblot analysis was performed on the nuclear extract of the transfected cells treated with TGF-β (10 ng/ml) for 1 h. Relative band intensities were determined by using an Image analyzer and plotted. **Statistically different from control at $p < 0.01$.

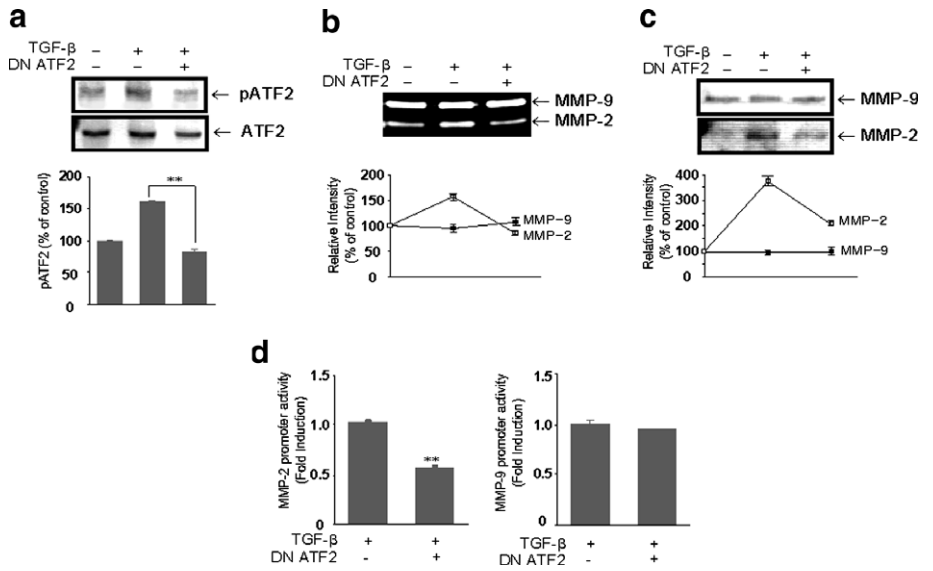

Fig. 6. ATF2 is required for TGF-β-induced transcriptional activation of MMP-2. (a) Cells were transiently transfected with either the control vector or DN ATF2 construct and treated with TGF-β (10 ng/ml) for 48 h. Nuclear protein was analyzed for phosphorylated and total forms ATF2 by immunoblot analysis. Gelatinolytic activities and expressions of MMP-2 and MMP-9 were determined in cells transiently transfected with DN ATF2 construct and treated with TGF-β (10 ng/ml) for 48 h by gelatin zymogram assay (b) and immunoblot analysis (c), respectively, (d) MMP-2 and MMP-9 promoter activities were measured in the cells transiently transfected with DN ATF2 and treated with TGF-β (10 ng/ml) for 24 h by luciferase assay. The results represent means ± SE of triplicates. **Statistically different from control at $p < 0.01$.

MCF10A cells. Migration of MCF10A cells treated with TGF-β was also inhibited by DN ATF2 transfection (Fig. 7b). The results show that the TGF-β-induced invasion and migration was mediated by MMP-2 up-regulation. Taken together, these data suggest that activation of ATF2 by TGF-β is important for TGF-β-induced breast epithelial cell invasion and migration.

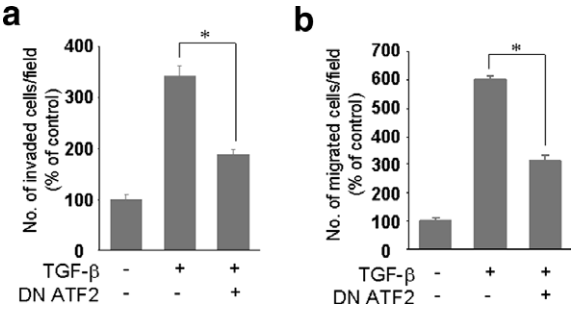

Fig. 7. ATF2 activation is crucial for TGF-β-induced invasion and migration. Cells transiently transfected with DN ATF2 were subjected to *in vitro* invasion assay (a) and migration assay (b) for 17 h in the presence of the TGF-β (10 ng/ml). The number of invaded or migrated cells per field was counted (100×) in thirteen fields. The results represent means ± SE of triplicates. *Statistically different from control at $p < 0.05$.

4. Discussion

The role of MMP-2 and MMP-9 in TGF-β-induced cell invasion and migration of epithelial tumor cells has been reported [6,7]. In MCF10A breast epithelial cells, we previously showed [8] that up-regulation of MMP-2 by TGF-β was due to transcriptional activation while that of MMP-9 might include other mechanism(s) such as increased mRNA stability as shown in human prostate cancer cells [33]. The present study aimed to elucidate the transcriptional regulation of MMP-2 by TGF-β.

Regulatory elements in the MMP-2 promoter have been suggested. The putative elements including p53, SI, S2, AP-1, Ets-1, C/EBP, CREB, GCN-His, PEA3, Sp1 and AP-2 binding sites were found in the promoter region of the MMP-2 gene [29,30,34]. AP-1 has been shown to be an effector molecule for TGF-β-dependent transcription [35,36]. It represents a heterogenous set of dimeric proteins consisting of members of the Jun, Fos, Maf and ATF subfamily members [37]. Our data indicate that the binding sites for p53, S1, AP-1 and Sp1 were crucial to MMP-2 promoter activation by TGF-β in MCF10A cells. CREB, GCN-His and PEA3, to a lesser extent, were also involved. Further investigation using MMP-2 promoter

constructs with point mutations in these sites would be required to determine whether these elements are indeed responsible for the TGF-β-induced transcriptional activation of MMP-2.

The transcription factor ATF2 has been shown to be a nuclear target of Smad and TAKl/p38 MAPK in TGF-β signaling [20]. It is phosphorylated and activated upon TGF-β treatment, mediating the cellular responses exerted by TGF-β [21]. Our data showed that ATF2 played an important role in TGF-β-induced MMP-2 up-regulation in human breast epithelial cells. ATF2 forms a homodimer or heterodimer and binds either to the CREB site or the AP-1 site. Heterodimerization alters ATF2 binding specificity and affinity for the binding sites. It has been shown that heterodimerization of ATF2 with c-Jun results in a higher affinity for AP-1 sites than for its usual CREB binding site [38]. The present study showed that AP-1 binding site was more critical than CREB binding site for TGF-β-induced MMP-2 promoter activity, suggesting that ATF2 might form a heteroduplex with c-Jun upon TGF-β treatment and bind to AP-1 site more efficiently than to CREB binding site. Further investigation on the nature of ATF2 homodimer or heterodimer complex regulating TGF-β-induced MMP-2 transcription remains to be performed.

The Sp1 transcription factor has been implicated in activation of target genes of TGF-β [39,40]. A strong contribution of Sp1 to the aberrant transcriptional response of transformed epithelial cells to TGF-β stimulation has been recently reported [41]. The proposed MMP-2 promoter structure contains two Sp1 binding sites [30]. Mutagenesis analysis of MMP-2 promoter showed that the deletion of the region containing the second Sp1 site resulted in the greatest reduction in MMP-2 promoter activity, suggesting the crucial role of Sp1 in TGF-β-induced transcriptional activation of MMP-2. It would be worthwhile to further examine the role of Sp1 in the transcriptional response by TGF-β leading to the malignant progression of human breast epithelial cells.

TGF-β has 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 [42,43]. Recent studies from several laboratories including ours suggest that TGF-β treatment may induce an oncogenic signaling pathway at early stage of tumor progression in normal mammary epithelial cells [8,44,45]. This study shows that TGF-β-induced MMP-2 expression, invasive and migratory phenotypes of MCF10A 'normal' epithelial cells require ATF2 activation. Based on these results and our previous study which demonstrated that ATF2 mediated MMP-2 transcriptional activation induced by p38 MAPK in MCF10Acells [22], we suggest that ATF2 may be a transcription factor responsible for TGF-β-induced MMP-2 up-regulation in MCF10A cells. Taken together, our study suggested ATF2 as a potential mediator for the TGF-β-induced invasive phenotypes, elucidating a molecular mechanism for the malignant progression of human breast epithelial cells exerted by TGF-β.

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