Urokinase Plasminogen Activator/Urokinase-specific Surface Receptor Expression and Matrix Invasion by Breast Cancer Cells Requires Constitutive p38α Mitogen-activated Protein Kinase Activity*

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Overexpression of urokinase plasminogen activator (uPA) and its receptor (uPAR) has been well documented in a wide variety of tumor cells. In breast cancer, expression of uPA/uPAR is essential for tumor cell invasion and metastasis. However, the mechanism responsible for uPA/uPAR expression in cancer cells remains unclear. In the studies reported here, we show that endogenous p38 MAPK activity correlates well with breast carcinoma cell invasiveness. Treatment of highly invasive BT549 cells with a specific p38 MAPK inhibitor SB203580 diminished both uPA/uPAR mRNA and protein expression and abrogated the ability of these cells to invade matrigel, suggesting that p38 MAPK signaling pathway is involved in the regulation of uPA/uPAR expression and breast cancer cell invasion. We also demonstrated that SB203580-induced reduction in uPA/ uPAR mRNA expression resulted from the destabilization of uPA and uPAR mRNA. Finally, by selectively inhibiting p38α or p38β MAPK isoforms, we demonstrated that p38α, rather than p38β, MAPK activity is essential for uPA/uPAR expression. These studies suggest that p38α MAPK signaling pathway is important for the maintenance of breast cancer invasive phenotype by promoting the stabilities of uPA and uPAR mRNA.

In multicellular organisms, invasion plays a pivotal role in diverse physiological and pathological processes such as tissue remodeling associated with embryonic development (1), inflammation (2, 3), angiogenesis (4), wound healing (5), and tumor metastasis (6–9). In studies conducted in a number of experimental models, it appears that cell migration and the production of proteases including plasminogen activator and metalloproteinases (MMPs)1 are essential components of the invasion process (6, 10, 11). Among the large number of proteases involved in invasion, urokinase plasminogen activator (uPA) and urokinase-specific cell surface receptor (uPAR) are of particular importance (12). uPA is a serine protease and, when bound to its receptor, uPAR, initiates the activation of MMPs as well as the conversion of plasminogen to plasmin (13, 14). These proteases confer on the cells the ability to degrade the extracellular matrix, thus allowing them to overcome the constraints of cell-cell and cell-matrix interactions (14). uPA and uPAR are overexpressed in various malignancies including breast, ovarian, and prostate cancers and have been clearly demonstrated to be essential in the maintenance of invasive and metastatic phenotypes (15). Expression of uPA and uPAR can be up-regulated by mitogen, growth factors, oncogenes, and ligation of integrin with extracellular matrix protein (12). For example, PMA stimulates uPA and uPAR expression in colon cancer cells (16). Epidermal growth factor and insulin-like growth factor I and II induce uPA expression in keratinocytes (17, 18) and transforming growth factor β increases uPAR expression in lung carcinoma cells (19). Oncogenes such as ErbB-2, c-Ha-Ras, and Tpr-Met increase uPA expression in lung carcinoma cells and fibroblast (20–22). Ligation of integrin αvβ3 with vitronectin enhances uPA expression in melanoma cells (23), and leukocyte integrin αvβ2 engagement promotes uPAR expression in T lymphocytes (24). However, the mechanism involved in the maintenance of uPA and uPAR overexpression in cancer cells remains unknown.

The mitogen-activated protein kinases (MAPKs) have been shown to transduce extracellular signals into cellular responses and play important roles in cell proliferation, apoptosis, differentiation, cell migration, and cytoskeleton remodeling (25). Mammalian cells express at least three types of MAPKs, ERKs, p38 MAPKs, and JNKs (26). The p38 MAPKs include at least four isoforms (α, β, γ, and δ) (27). Despite the structural similarity among the member of p38 MAPK family, differences in the activation profile and substrate specificity have been observed (27). The p38 MAPKs can be activated by a wide spectrum of stimuli, including cellular stress, bacterial lipopolysaccharide, the proinflammatory cytokines IL-1 and tumor necrosis factor α and growth factors such as transforming growth factor β (25, 27). Recently, p38 MAPK signaling pathway has been shown to be important for the induction of several metalloproteinases by extracellular stimuli. For example, p38 MAPK pathway signals for the expression of collagenase-3 (MMP-13) in human skin fibroblast cultured on three-dimensional collagen gels (28). In squamous carcinoma cells of head and neck, a specific inhibitor of p38 MAPK, SB203580, was found to inhibit PMA-induced MMP9 expression (29). Moreover, p38 MAPK pathway was also reported to mediate both okadaic acid and tumor-derived extracellular matrix metalloproteinase inducer-stimulated collagenase-1 (MMP-1) expression in fibroblasts (30, 31). Together, these studies suggest that p38 MAPK signaling may be important for cell invasive properties.

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‡The abbreviations used are: MMP, metalloproteinase; uPA, urokinase plasminogen activator; uPAR, urokinase-specific surface receptor; PMA, phorbol 12-myristate 13-acetate; MAPK, mitogen-activated protein kinase; 3′-UTR, 3′-untranslated region; IL, interleukin; DMEM, Dulbecco’s modified Eagle’s medium; PBS, phosphate-buffered saline; MOPS, 4-morpholinopropanesulfonic acid; mAb, monoclonal antibody; UTR, untranslated region.
In this paper, we investigated the role of p38 MAPK in breast cancer cell invasion. We found that the endogenous p38 MAPK activity correlated well with the *in vitro* invasiveness of breast carcinoma cells. Moreover, the ability of highly invasive BT549 cells to invade matrigel was almost completely abrogated by specific p38 MAPK inhibitor SB203580. We also provide substantial evidence that p38α MAPK pathway signals for the stability of uPA and uPAR mRNA and thereby may enhance breast cancer cell invasiveness.

**EXPERIMENTAL PROCEDURES**

**Material and Cell Culture**—SB203580, SB202474, U0126, and actinomycin D were purchased from Calbiochem (San Diego, CA). Polyclonal antibodies to activated p38 MAPK (Thr<sup>180</sup>/Tyr<sup>182</sup>-phosphorylated p38 MAPK), p38 MAPK, and MAPKAPK-2 were purchased from Bio-Labs (Beverly, MA). Polyclonal antibody to uPA and monoclonal antibody to uPAR were purchased from American Diagnostics (Greenwich, CT). cDNAs for uPA, uPAR, MMP2, and MMP9 were purchased from American Type Culture Collection (Manassas, VA). Breast cancer cells SK-BR-2, T47D, MDA231, MDA435, MDA436, and BT549 (American Type Culture Collection) were maintained at 37 °C in a humidified atmosphere of 5% CO<sub>2</sub> supplemented with 10% fetal calf serum.

**Matrigel Invasion Assay**—Cell invasion assay was analyzed using BIOCOAT matrigel invasion chamber (Becton-Dickinson, Bedford, MA) according to the protocol provided by the manufacturer. Briefly, 2 × 10<sup>5</sup> cells in 300 μl were added to each chamber and allowed to invade matrigel for 20 h at 37 °C, 5% CO<sub>2</sub> atmosphere. The noninvading cells on the lower surface of membrane were removed from the chamber, and the invading cells on the lower surface of membrane were stained with Quick-Diff stain kit (Becton-Dickinson). After two washes with water, the chambers were allowed to air dry. The number of invading cells were counted using a phase-contrast microscope. To determine the effect of p38 kinase inhibitor SB203580 on BT549 invasion, cells were treated with 10 μM SB203580 for 24 h and then added into invasion chambers, and the number of invading cells was subsequently determined 20 h after.

**Cell Migration**—To measure cell migration, the undersurfaces of Transwells (Costar, Corning, NY) were coated with 10 μg/ml of laminin (Sigma) or collagen I (Upstate Biotechnology, Lake Placid, NY) overnight at 4 °C. Coated wells were then placed into a 24-well plate containing 500 μl of serum-free DMEM containing 0.1 mg/ml bovine serum albumin. Cells were detached with 10 mM EDTA and washed several times with serum-free DMEM medium. Cells were suspended 5 × 10<sup>5</sup> cells/ml in serum-free DMEM containing 0.1 mg/ml bovine serum albumin, added into transwells (200 μl of 1 × 10<sup>5</sup> cells/ml cell suspension in each well), and allowed to migrate for 8 h at 37 °C. Crystal swabs were then used to remove cells in the upper surface of the transwells, and migratory cells attached on the undersurface were stained with crystal violet solution. Wells were gently rinsed with water, dried, and the crystal violet-stained attached cells were solubilized with 100 μl of 10% acetic acid and quantitated on a microplate reader at 600 nm.

**Immunoblotting**—Cells were cultured in 6-well plates and then washed with ice-cold PBS and lysed in RIPA buffer (PBS containing 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 1 mM Na<sub>3</sub>VO<sub>4</sub> and 100 μg/ml phenylmethylsulfonyl fluoride). Cell lysates (50 μg of protein) were boiled in nonreducing SDS sample buffer, electrophoresed on a 10% polyacrylamide SDS gel, and transferred onto polyvinylidene difluoride membranes (Immobilon-P, Millipore). The activated p38 MAPK (the Thr<sup>180</sup>/Tyr<sup>182</sup>-phosphorylated p38 MAPK) and p38 MAPK were detected by phospho-p38 and p38 polyclonal antibodies, respectively. uPA was detected using a uPA polyclonal antibody.

**Analysis of Kinase Activity**—BT549 cells were treated with SB203580 for 24 h and then lysed using RIPA buffer. Cells lysates were immunoprecipitated using an anti-MAPKAPK-2 polyclonal antibody, and the immunoprecipitates were used for *in vitro* kinase assays using recombinant Hsp27 (heat shock protein 27) as a substrate as described previously (32).

**RNA Analyses**—The expression of uPA, uPAR, MMP2, MMP9 and GAPDH was analyzed by Northern analysis as described previously (33). To examine the role of p38 MAPK on protease expression, BT549 cells were treated with 5, 10, and 20 μM SB203580 to inhibit p38 MAPK activity for 24 h, and total RNA was then isolated using Trizol reagent (Life Technologies, Inc.). RNA (20 μg) was electrophoresed on a paraformaldehyde/MOPS agarose gel (1.2%) and then transferred onto a Nitran membrane (Schleicher & Schuell) using 20× SSPE. The membrane was UV cross-linked and hybridized with 32P-labeled cDNA probes for uPA, uPAR, MMP2, MMP9, and GAPDH, respectively. The membrane was then washed and exposed to x-ray film.

To study the role of p38 MAPK on uPA and uPAR mRNA stability, 1 μg/ml of actinomycin D was added to untreated or treated BT549 cells (10 μM) for 24 h. Total RNAs were isolated from these cells at varying times (1–12 h), and Northern analysis was then performed to detect uPA and uPAR expression.

**Flow Cytometry**—To detect cell surface uPAR expression, BT549 cells were suspended in PBS containing 1% fetal calf serum and 0.2% sodium azide and incubated with uPAR MAbs for 30 min on ice. The cells were washed twice and further incubated with fluorescein-conjugated goat anti-mouse secondary antibody for 30 min on ice. Following three additional washes with PBS, cells were resuspended in 500 μl and analyzed by flow cytometry (FACScan; Becton-Dickinson). Data analyses were performed using the CellQuest program (Becton-Dickinson).

**Dominant Negative p38α, p38β, JNK1, and Erk2 Mutant-encoding Adenovirus Constructs—**All dominant negative MAPK mutant-encoding adenovirus constructs have been described elsewhere previously (32, 34, 35). To determine the effect of dominant negative MAPK mutants on uPA and uPAR mRNA expression, BT549 cells were infected with each of these adenovirus constructs (10 plaque-forming units/cell) or control Ad-RSV (no insert) for 48 h. The Northern analyses were then performed on the total RNA isolated from these cells to detect the level of uPA and uPAR. The level of p38 MAPK expression was detected by immunoblotting using anti-uPA polyclonal antibody, and uPAR was detected by flow cytometry using anti-uPA mAb.

**uPA and uPAR Promoter Reporter Gene Constructs and Promoter Assay—**A 2170-base pair fragment containing the human uPA promoter (~2139 to +30 relative to the transcription start site) (36) was cloned into the XhoI site of pGL2-basic vector (Promega, Madison, WI), which contains a luciferase reporter gene. A 1604-base pair fragment containing the human uPAR promoter (~1552 to +51 relative to the transcription start site) (37) was cloned into the XhoI site of the pGL2-basic vector. To analyze uPA or uPAR promoter activity in BT549 cells, 2 μg of reporter gene plasmid, and 0.5 μg of pRSVβ (containing the β-galactosidase gene, Promega) were co-transfected into BT549 cells plated on 6-well plate (5 × 10<sup>5</sup> cells/well seeded overnight) using 5 μl of LipofectAMINE (Life Technologies, Inc.) for 24 h and further cultured in the presence or absence of SB203580 for another 24 h. Cells were then lysed, and the luciferase activity was determined using Luciferase Assay System (Promega). The β-galactosidase activity was determined using O-nitrophenyl β-D-galactopyranoside as a substrate, as described previously, and used to standardize uPA and uPAR promoter activity.

**Construction of uPA Expression Vector and Transfection—**The uPA coding sequence was amplified by reverse transcription-polymerase chain reaction from total RNA isolated from BT549 cells using the primers HUKa (5′-TACCGCTGACCGCCACCATGAGCCCTGCTGCGG-3′, the underlined sequence is an XhoI site) and HUKb (5′-TACCGCTGACTCAAGGGCCAGCGCATTCTTCTCTC-3′, the underlined sequence is a SflI site). The amplified uPA cDNA lacks 5′- and 3′-untranslated sequences. The primers were designed based on published sequence (GenBank™ accession number X02419) (38). The polymerase chain reaction fragment was digested with XhoI/SalI and subcloned into pCI-neo mammalian expression vector (Promega) and designated as pCI/uPA. The sequence of uPA in pCI/uPA was confirmed by automated sequence analysis.

To generate uPA stable transfectants, BT549 cells were transfected with pCI/uPA, using LipofectAMINE (Life Technologies, Inc.). The transfected cells were selected with 1 mg/ml G418 for 3 weeks. Individual stable transfectants were isolated using cloning cylinders, and uPA mRNA expression was then determined by Northern analysis using uPA cDNA as probe. One clone, which expressed high level of uPA (data not shown), was collected and designated as BT549/uPA. Control transfectants were established by transfecting BT549 cells with pCI/neo and designated as BT549/neo.

**RESULTS**

**Endogenous p38 MAPK Activity Is Associated with Breast Cancer Cell In Vitro Invasiveness**—The p38 MAPK signaling pathway has been shown to mediate the expression of several metalloproteinase induced by various extracellular stimuli (28–31, 39). We, thus, hypothesized that constitutive p38 MAPK signaling might be important for tumor cell invasiveness. To test this hypothesis, we first examined whether a...
correlation existed between the constitutive activity of p38 MAPK and invasion of human breast cancer cells. Because p38 MAPK is activated through dual phosphorylation of threonyl and tyrosyl residues at TGY motif by upstream MAPK kinases including MKK3 and MKK6 (27), we used the antibody, which strictly recognizes Thr/Tyr-phophorylated p38 MAPK kinases including MKK3 and MKK6 (27), we used the antibody, which strictly recognizes Thr/Tyr-phophorylated p38 MAPK, to detect the endogenous constitutive activity of p38 MAPK in six breast cancer cell lines. T47D and MDA-MB-435 lines, which contained little p38 MAPK activity (Fig. 1A), were unable to invade matrigel (Fig. 1B), a matrix containing laminin, collagen I, and other matrix proteins (information provided by manufacturer). In contrast, BT549, MDA231, and MDA-MB-436 lines, which exhibited high endogenous p38 MAPK activity, showed significant matrigel invasion (Fig. 1). SK-BR2 cells displayed low p38 MAPK activity and were also only slightly invasive (Fig. 1). The low p38 MAPK activity in T47D and MDA-MB-435 lines was not due to lack of p38 MAPK protein expression because immunoblotting using anti-p38 antibody, which strictly recognizes Thr/Tyr-phophorylated p38 MAPK, showed similar levels of p38 MAPK protein expression in these lines (Fig. 1A). These results thus showed a good correlation between constitutive p38 MAPK activity and the invasiveness of human breast cancer cells.

p38 MAPK Activity Is Required for BT549 Cell Invasion—Several related pyridinyl imidazole compounds have been found to be highly specific inhibitors of p38 MAPK (40). SB203580, a member of this group, is a highly selective inhibitor of p38 MAPK, which does not alter the activity of other MAPKs including ERKs and JNKs (41–43). We thus used this inhibitor to investigate the role of p38 MAPK activity in invasion of BT549 breast cancer cells. We first examined whether SB203580 could inhibit p38 MAPK activity in BT549 cells by analyzing its ability to activate MAPKAPK-2, a kinase that lies immediately downstream of p38 MAPK (43). Lysates from BT549 cells treated with a increasing amount of SB203580 showed decreased ability to phosphorylate heat shock protein 27 (Hsp27), a substrate of MAPKAPK-2. SB203580 at 10 μM was capable of inhibiting over 80% of Hsp27 phosphorylation (Fig. 2A). The reduced Hsp27 phosphorylation was not due to decreased levels of p38 MAPK or MAPKAPK-2 because SB203580 treatment had no effect on the level of either protein (Fig. 2A). In further experiments, we also examined the effect of U0126, a MEK1 inhibitor, and SB202474, a control compound that lacks the ability to inhibit p38 MAPK activity. No inhibition of Hsp27 phosphorylation was observed with these agents (data not shown). These results demonstrate that SB203580 was capable of specifically blocking p38 MAPK activity in BT549 cells. To investigate the effect of SB203580 on BT549 cell invasion, we next treated cells with SB203580 and then added them to matrigel invasion chambers for 20 h. SB203580 at 10 μM blocked BT549 cell invasion by over 90% and at 20 μM completely diminished BT549 cell invasion on matrigel (Fig. 2B). In a control experiment, we treated cells with the same concentration of control compound SB202474 and did not detect any significant inhibitory effect on BT549 cell invasion (Fig. 2B). To rule out the possibility that SB203580 was toxic to BT549 cells, we examined cell growth of both untreated and SB203580-treated cells. SB203580 up to 20 μM showed little effect on BT549 cell proliferation as determined by MTT assay (data shown) (33). These results suggest that constitutive p38 MAPK activity is necessary for breast cancer cell invasion.

p38 MAPK Activity Is Required for uPA and uPAR Expression—Cell migration and protease production are the two essential processes of cell invasion. We thus investigated the effect of SB203580 on BT549 cell migration on extracellular matrix laminin and collagen I. Untreated or SB203580-treated BT549 cells were added into transwells and allowed to migrate to laminin or collagen I for 8 h. BT549 cells migrated very well...
on both collagen and laminin-coated wells. Treatment of cells with SB203580 showed only slight inhibitory effect on migration (Fig. 3). These results suggest that p38 MAPK activity is not essential for BT549 cell migration. We next investigated whether p38 MAPK activity was required for the expression of proteases in BT549 cells. Cells were treated with various concentrations (5, 10, and 20 \( \mu \)M) of SB203580, and then total RNA was isolated and the steady-state levels of uPA, uPAR, MMP2, and MMP9 mRNAs were analyzed by Northern blotting. Expression of MMP2 mRNA was not detected (data not shown), whereas MMP9 mRNA was readily detected and not significantly affected by SB203580 treatment (Fig. 4A). Interestingly, SB203580 significantly inhibited the steady-state levels of uPA and uPAR mRNA in BT549 cells (Fig. 4A). We also observed a decrease in cell-associated uPA protein (assayed by immunoblotting; Fig. 4B) and cell surface uPAR expression (assayed by flow cytometry; Fig. 4C) on BT549 cells upon SB203580 treatment. These results suggested that the constitutive p38 MAPK activity is essential for both uPA and uPAR expression in BT549 cells.

**SB203580 Destabilizes uPA and uPAR mRNA**—The steady-state level of mRNA can be affected by the level of gene transcription and/or the stability of mRNA. To investigate whether SB203580 inhibited transcription of uPA and uPAR, we constructed the luciferase reporter gene plasmid constructs under the transcriptional control of the uPA and uPAR promoters, respectively. BT549 cells were transfected with either uPA or uPAR promoter reporter gene plasmid construct and then treated with SB203580. The cells were then lysed, and promoter-mediated luciferase activities were determined. There was no significant reduction in either uPA or uPAR promoter activity upon SB203580 treatment (Fig. 5). The lack of inhibition in promoter activity was not caused by a lower expression of uPA and uPAR mRNA due to suppression of transcription.

We next investigated whether SB203580 altered the stability of uPA and uPAR mRNA. Actinomycin D (1 \( \mu \)g/ml) was added into BT549 cells cultured in the presence or absence of SB203580, the total RNA was then isolated at varying times (1–12 h), and uPA and uPAR expression was determined by Northern blotting. In untreated BT549 cells, the time required for a 50% loss of uPA and uPAR mRNA was >12 h (Fig. 6). However, treatment of SB203580 reduced the half-life to approximately 3 and 2 h, respectively, for uPA and uPAR mRNA (Fig. 6). These results suggest that signaling processes initiated by p38 MAPK pathway alter the stability of uPA and...
p38 MAPK-mediated destabilization of uPA mRNA involves the 3′-UTR of uPA.

p38α Isoform of MAPK Is Required for uPA and uPAR Expression—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 (27). To examine whether p38α, p38β, or both molecules were required for uPA and uPAR expression, dominant negative mutants of p38α or p38β MAPK were expressed in BT549 cells using recombinant adenovirus. Total RNA and cellular protein were extracted from these cells 48 h post-infection and analyzed for the levels of uPA and uPAR mRNA and protein expression. Northern blot analyses showed that a dominant negative p38α, but not p38β, significantly inhibited the steady-state levels of uPA and uPAR mRNA (Fig. 8A). Similarly, the levels of cell-associated uPA and cell surface uPAR were also significantly inhibited by the expression of dominant negative p38α mutant (Fig. 8, B and C). These results suggest that p38α, rather than p38β, MAPK signaling pathway participates in the regulation of uPA and uPAR expression. In control experiments, we also examined the effect of dominant negative Erk2 or JNK1 mutant on uPA and uPAR expression. The expression of uPA was not affected by either dominant negative Erk2 or JNK1 mutant (Fig. 8). Interestingly, both dominant negative ERK2 and JNK1 mutants inhibited uPAR expression, although to a lesser degree than dominant negative p38α mutant (Fig. 8, A and C). These results suggest that the expression of uPA is specifically regulated by p38α MAPK signaling pathway, whereas all three known MAPK signaling pathways may participate in the regulation of uPAR expression.

DISCUSSION

Tumor cell invasion is associated with the destruction of the basement membrane and extracellular matrix. This process is associated with proteases, such as uPA and MMPs, secreted by malignant cells and stroma cells (6–9). Several recent studies have demonstrated that expression of several metalloproteinases including MMP-9 and MMP-1 induced by extracellular stimuli is mediated by p38 MAPK signaling pathway. These earlier findings prompted us to investigate whether p38 MAPK pathway is important for breast cancer cell invasion and metastasis. We determined that the presence of endogenous p38 MAPK activity correlated with in vitro invasiveness of six breast cancer cell lines as indicated by invasion of matrigel (Fig. 1). Treatment of highly invasive breast cancer BT549 cells with p38 MAPK inhibitor SB203580 nearly abolished the invasiveness of these cells (Fig. 2), suggesting the importance of p38 MAPK signaling pathway in breast cancer cell invasion.

Tumor cell invasion involves two essential components: cell migration and the production of proteases including uPA and MMPs (6, 10, 11). We found that SB203580 only marginally inhibited BT549 cell migration on both collagen I and laminin matrix (Fig. 3). Instead, SB203580 significantly reduced both
mRNA and protein expression of uPA and uPAR in BT549 cells (Fig. 4). Several early studies using in vitro and in vivo animal models showed that neutralizing uPA and uPAR function or blocking expression of these molecules significantly inhibited breast cancer invasion and metastasis (47, 48). Therefore, we considered the possibility that reduction in breast cancer invasion by SB203580 resulted from down-regulation of uPA and uPAR expression, an event regulated by p38 MAPK signaling pathway.

In this study, we also investigated whether p38 MAPK activity regulated MMP2 and MMP9 expression in breast cancer BT549 cells. MMP2 mRNA could not be detected in BT549 cells, whereas MMP9 mRNA was readily detected. However, MMP9 expression was not affected by SB203580 treatment in BT549 cells (Fig. 4). This is of interest given the recent study showing that SB203580 treatment abolishes PMA-induced MMP9 expression in head and neck squamous cells (29). The difference in these studies may be explained by (i) the different cell systems used and (ii) the fact that the p38 kinase MAPK pathway may be important for induction of MMP9 expression but not for the constitutive expression.

The expression of uPA and uPAR mRNA may be regulated at either the transcriptional or mRNA stability levels or both. In terms of transcriptional regulation, oncogene c-Ha-Ras and epidermal growth factor have been reported to transcriptionally up-regulate uPA expression via AP-1, PEA3, and ETS binding motifs in uPA promoter region (21, 49). PMA was found to transcriptionally up-regulate uPAR expression dependent on AP-1 site in uPAR promoter region (16). In terms of mRNA stability, previous studies reported that the half-life of uPA mRNA in pig epithelial LLC-PK1 cells was 70 min, whereas in contrast its half-life in breast cancer MDA-MB-231 cells is 17 h, suggesting that uPA expression can be regulated at the level of mRNA stability (45). Cross-linking αβ2 integrin in T lymphocytes was also found to promote uPAR mRNA stability and uPAR expression (24). Furthermore, both studies indicated that uPA and uPAR mRNA stability is controlled through the AU-rich sequences in 3′-UTR of uPA and uPAR mRNA (24, 45).

In our studies, we found that p38 MAPK activity had little effect on uPA and uPAR promoter activities (Fig. 5), suggesting that p38 MAPK signaling pathway is not required for uPA and uPAR transcription. When we measured the stability of uPA and uPAR mRNAs in BT549 cells, we found that they both had a half-life of over 12 h (Fig. 6). However, SB203580 treatment reduced the half-life of uPA and uPAR to 3 and 2 h, respectively (Fig. 6). These results clearly demonstrate that p38 MAPK activity is required for the stability of uPA and uPAR mRNA.

Two recent studies showed that p38 MAPK regulates IL-6, IL-8, and granulocyte-macrophage colony-stimulating factor mRNA stabilization via the AU-rich element-targeted mechanism (50, 51). Removal of AU-rich elements in the 3′-UTR of tumor necrosis factor α gene has been reported to abrogate tumor necrosis factor α mRNA responsiveness to p38 MAPK modulation (52). Although it is not clear whether AU-rich element-targeted mechanisms are responsible for stabilization of uPA and uPAR mRNA, the fact that the expression of uPA transgene (lacking 3′-UTR) in BT549 cells was not affected by SB203580 (Fig. 7) suggests that p38 MAPK pathway may also impact the stability of uPA and uPAR mRNA through the AU-rich elements in 3′-UTR.

The activity of p38α and p38β MAPK can be inhibited by SB203580. High concentration of SB203580 (20 μM or higher) has been shown to inhibit JNK activity (27, 40). We showed that a dominant negative p38α mutant, rather than dominant negative p38β or other MAPK mutants, was capable of significantly down-regulating both uPA mRNA and protein expression (Fig. 8). These results strongly suggest that constitutive uPA expression may be strictly regulated by p38α MAPK pathway. Interestingly, we also showed that dominant negative JNK1 and Erk2 mutants were all capable of inhibiting uPAR mRNA and protein expression (Fig. 8). These results indicate that all three MAPK signaling molecules may participate in constitutive uPAR expression. Two recent studies have demonstrated that JNK1 signaling pathway stabilizes IL-2 in human T cells (53) and IL-3 mRNA in mast cells (54), and thus it is possible that JNK1 pathway may also regulate uPAR expression by stabilizing mRNA.

Substantial evidence has been obtained that uPA and uPAR are overproduced in malignancies including breast, ovary, and prostate tumors (7, 14, 15). Moreover, high levels of uPA and uPAR are found to associate with disease progression and poor prognosis and have been proposed as prognostic factors for breast cancer (55). Because of the importance of uPA/uPAR in cancer invasion and metastasis, our study suggests that p38α MAPK signaling pathway may be a potential therapeutic target for anti-cancer treatment.

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