Membrane Type 1 Matrix Metalloproteinase Regulates Collagen-Dependent Mitogen-Activated Protein/Extracellular Signal-Related Kinase Activation and Cell Migration

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ABSTRACT

Mitogen-activated protein kinase-extracellular signal-related kinase (ERK) kinase 1 (MEK1)/ERK signaling has been implicated in the regulation of tumor cell invasion and metastasis. Migration of HT1080 cells on type I collagen was suppressed by the matrix metalloproteinase (MMP) inhibitors BB94 and tissue inhibitor of metalloproteinase (TIMP)-2 but not by TIMP-1. TIMP-2-specific inhibition suggests that membrane type 1 MMP (MT1-MMP) is likely involved in this process. Activation of ERK was induced in HT1080 cells adhered on dishes coated with type I collagen, and this was inhibited by BB94. MMP-2 processing in HT1080 cells, which also was stimulated by cultivation on type I collagen, was inhibited by MEK inhibitor PD98059. Expression of a constitutively active form of MEK1 promoted MMP-2 processing concomitant with the increase of MT1-MMP levels, suggesting that MT1-MMP is regulated by MEK/ERK signaling. In addition, expression of the hemopexin-like domain of MT1-MMP in HT1080 cells interfered with MMP-2 processing, ERK activation, and cell migration, implying that the enzymatic activity of MT1-MMP is involved in collagen-induced ERK activation, which results in enhanced cell migration. Thus, adhesion of HT1080 cells to type I collagen induces MT1-MMP-dependent ERK activation, which in turn causes an increase in MT1-MMP levels and subsequent cell migration.

INTRODUCTION

Cell migration is a complex process that can be regulated by multiple mechanisms, including mitogen-activated protein kinase (MAPK)/extracellular signal-related kinase (ERK), phosphatidylinositol 3-kinase, and extracellular matrix (ECM)-degrading proteinases such as matrix metalloproteinases (MMPs; Refs. 1–6). Adhesion-mediated ERK activation depends on integrin engagement (7, 8). ERK activity has been implicated in ECM-dependent cell spreading and migration, concomitant with a role for ERK in the regulation of integrin-dependent adhesion/cytoskeletal organization (3, 4). Constitutive activation of ERK by v-src, v-ckr, and constitutively active form of mitogen-activated protein/ERK kinase (CA-MEK) promotes not only cell migration but also MMP-2 activation (9–11). However, the precise mechanism by which ERK activation promotes MMP-2 processing is largely unknown.

MMPs are a family of Zn2+-dependent enzymes that have been involved in multiple physiologic and pathologic processes (12, 13). Among MMPs, MMP-2 has been implicated in tumor invasion and metastasis. Membrane type 1 (MT1)-MMP was identified originally as an MMP-2 activator and was shown later to degrade various ECM components, including type I, II, and III collagen (1, 13–15). Six MMPs identified to be anchored to the plasma membrane now are subgrouped into the MT-MMP subfamily (13–17). Of all of the MT-MMPs, MT1-MMP expression correlates most closely with the invasive phenotype of human tumors (13, 15, 18). MT1-MMP expression also correlates with epithelial-mesenchymal transdifferentiation, which is associated with an increase in migration and invasion (19, 20). MT1-MMP-deficient mice show impaired processing of MMP-2 and display severe defects in skeletal development and angiogenesis, suggesting an essential role for MT1-MMP in the processes of angiogenesis and bone growth (21).

MT1-MMP expression is induced during tubule formation stimulated with hepatocyte growth factor in Madin-Darby canine kidney epithelial cells and by embedding tumor cells or fibroblasts in a three-dimensional collagen gel (10, 22). Invasion of COS-1 cells into a collagen gel requires the activity of MT1-MMP (23). ERK activation is essential for cell migration and is induced by overexpression of MT1-MMP in COS-7 cells (24). Transformation by v-src or CA-MEK in Madin-Darby canine kidney epithelial cells up-regulates MT1-MMP expression (10, 22). MEK1/ERK signaling seems consequently to be important for MT1-MMP expression. In this study, we provide evidence that for HT1080 cells cultured on type I collagen, MT1-MMP plays an essential role in the activation of ERK, and activated ERK in turn up-regulates MT1-MMP. Thus, MT1-MMP functions in a positive feedback loop to induce sustained ERK activation and subsequent MT1-MMP accumulation, which collectively promotes cell migration on type I collagen.

MATERIALS AND METHODS

Cell Culture and Reagents. HT1080 cells were maintained in DMEM supplemented with 5% fetal bovine serum. The synthetic MMP inhibitor BB94 was prepared as described previously (25). PD98059 was purchased from Sigma-Aldrich (St. Louis, MO), and recombiant TIMP-1 and -2 were purchased from Daichi Fine Chemical (Takaoka, Japan). Antibodies used were anti-phospho-p44/42 mitogen-activated protein kinase and anti-p44/42 mitogen-activated protein kinase (Cell Signaling Technology, Beverly, MA); anti-ERK2 and antipaxillin (Transduction Laboratories, Lexington, KY); anti-actin, anti-α-tubulin, and anti-FLAG M2 (Sigma-Aldrich); anti-MT1-MMP (Chemicon, Temecula, CA); and antianthemagglutinin (HA) antibody (Santa Cruz Biotechnology, Santa Cruz, CA).

Expression Plasmids. pSG5-FLAG epitope tagged-MT1-MMP (MT1F); pSG5-MT1F-Pex (MT1F-Pex), which lacks the catalytic domain of MT1-MMP; pSG5-MT1-E/A (MT1-E/A), which is a catalytic inactive mutant of MT1-MMP [Glu (240) to Ala]; and pcDNA-C-A-MEK1 were constructed as described previously (26, 27). pcDNA-HA-ERK2, pRK-GFP, and pHA262pur plasmids were gifts from Dr. Kenneth M. Yamada (NIH, Bethesda, MD). Transient transfections were performed by the calcium phosphate method.

Cell Migration Assay. HT1080 cells were cotransfected with 1.0 μg of pSG-MT1F or 0.5 μg of pcDNA-C-A-MEK1 with 0.5 μg each of pRK-GFP and pH2A62pur plasmids. The cells were cultured in 1.5 μg/ml puromycin-containing medium for 36 h. HT1080 cells and puromycin-selected cells were replated on 35-mm glass-bottom dishes coated with 750 μg/ml of type I collagen in 1 mg/ml BSA/DMEM with indicated inhibitors. Two h after replating, cell migration was monitored using an Olympus inverted microscope (Tokyo, Japan) for 3 h. Video images were...
collected at 5-min intervals, digitized, and analyzed using Move/Tr/2D software (Library Co. Ltd., Tokyo, Japan).

**Cell Adhesion Assay.** Cell adhesion assays were performed as described previously (28). Briefly, 96-well plates were coated with BSA, poly-L-lysine (PLL), or type I collagen. Cells in suspension with or without BB94 were allowed to adhere to the plates for 1 h.

**Immunofluorescence Staining.** The cells replated onto glass coverslips coated with type I collagen were incubated for 4 h in DMEM containing 1 mg/ml BSA in the presence or absence of BB94 (1 μM), fixed with 4% paraformaldehyde for 15 min, and permeabilized with 4% paraformaldehyde/0.5% Triton X-100 for 5 min. The cells were then stained with an antipaxillin antibody, rhodamine-phalloidin, and Cy3-conjugated goat antitomouse IgG (Molecular Probes, Eugene, OR) and observed using confocal laser microscopy (Carl Zeiss, Oberkochen, Germany).

**Immunoprecipitation and Immunoblotting.** The cells were washed with ice-cold PBS, homogenized in a buffer containing 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM EGTA, 1 mM phenylmethylsulfonyl fluoride, 1 mM Na3VO4, 1 mM NaF, and 1% NP-40. The samples were used for immunoprecipitation with anti-HA antibody for 2 h at 4°C, followed by sedimentation with GammaBind Plus Sepharose (Amersham Pharmacia Biotech, Piscataway, NJ). The cells embedded in a three-dimensional collagen gel were homogenized directly in SDS-PAGE sample buffer. The samples then were analyzed by immunoblotting using indicated antibodies.

**Gelatin Zymography.** Gelatin zymography was performed with an SDS-polyacrylamide gel containing 0.1% gelatin as described previously (16).

**Three-Dimensional Cell Culture.** One ml of type I collagen containing 5 × 10⁶ cells was polymerized in each well of a 12-well plate and then covered with 500 μl of serum-free DMEM with DMSO, BB94, or PD98059 for 12 h.

### RESULTS

**MT1-MMP Is Involved in Cell Migration.** To study whether MT1-MMP and ERK signaling are involved in the migration of HT1080 cells on type I collagen, the effect of MMP inhibitors (BB94 and TIMP-1 and -2) and an MEK inhibitor (PD98059) was examined. As shown in Fig. 1A, HT1080 cell migration was reduced significantly by the MMP inhibitors BB94 and TIMP-2 to 69% and 62% of control cells, respectively, but not by TIMP-1. HT1080 cells are known to express MT1-MMP, which is inhibited selectively by TIMP-2 but not by TIMP-1 (15). Transfection of MT1-MMP into HT1080 cells resulted in an increase of cell migration to 127% of control cells. Treatment of MT1-MMP-transfected cells with BB94 suppressed migration to almost the same levels as BB94-treated control cells. HT1080 cell migration also was suppressed by treatment with PD98059 to 65% of control cells. HT1080 cells expressing CA-MEK showed elevated migration to 166% of control cells. These results suggest strongly that MT1-MMP and the MEK/ERK pathway are involved in migration of HT1080 cells on type I collagen.

Next, the effects of BB94 on cell adhesion and morphology on type I collagen were examined. Adhesion of HT1080 cells to dishes coated with PLL or collagen was equivalent and was not altered by BB94 treatment (Fig. 1B). However, a distinct morphology was evident when grown on either PLL or collagen-coated dishes (Fig. 1C). HT1080 cells spread well on type I collagen at 1 h after plating, whereas the cells spread poorly on PLL-coated dishes. To analyze the effects of BB94 on cell spreading onto type I collagen, HT1080 cells were stained with an antipaxillin antibody to detect focal adhesions and with phalloidin to visualize filamentous (F)-actin at 4 h after plating onto collagen. HT1080 cells showed well-organized actin stress fibers and focal adhesion formation in the absence of BB94; however, treatment of HT1080 cells with BB94 suppressed organization of stress fibers and focal adhesion formation (Fig. 1D).

**MT1-MMP Is Involved in Collagen-Induced ERK Activation.** The morphologic changes of HT1080 cells induced by BB94 suggest that BB94 may affect the signals generated by absorption onto type I collagen. This led us to examine the effects of BB94 on ERK activation. Phosphorylation of ERK was observed in HT1080 cells plated onto collagen-coated dishes but not in cells on PLL-coated dishes (Fig. 2A). The ERK phosphorylation induced at 30 min after plating onto type I collagen was not altered significantly by BB94; however, BB94 apparently suppressed ERK activation at 6 h after plating (Fig. 2B). These results suggest that MMP activity is required for sustained ERK activation induced by type I collagen in HT1080 cells. As described previously, MT1-MMP is active in HT1080 cells, which suggests that MT1-MMP is involved in sustained ERK activation induced by collagen. To confirm whether the catalytic activity of MT1-MMP is involved in collagen-induced ERK activation, expression plasmids for wild-type or the catalytic inactive mutant (E/A) of MT1-MMP were cotransfected with HA-ERK2 into HT1080 cells, and collagen-induced ERK activation in these cells was analyzed by immunoblotting. As shown in Fig. 2C, the cells expressing wild-type MT1-MMP showed two-fold higher ERK activation induced by collagen than those of control cells or the cells expressing MT1-E/A. These results suggest that MT1-MMP is involved in sustained ERK activation induced by cultivation on type I collagen. It should be noted that the amount of active MT1-MMP increased in cells cultured on collagen, whereas the processed form of MT1-E/A was detected in cells cultured in suspension or on collagen-coated dishes at comparable levels (Fig. 2C, bottom panels).
Collagen-Induced ERK Activation Enhances MT1-MMP Activity. MT1-MMP was shown to play an essential role not only in MMP-2 processing but also in sustained ERK activation in HT1080 cells cultured on type I collagen. We next examined MT1-MMP activity in HT1080 cells cultured on type I collagen by monitoring MMP-2 processing. HT1080 cells spontaneously secreted latent (M₉₆,000), activation intermediate (M₉₄,000), and fully active (M₆₈,000) forms of MMP-2 when cultured on uncoated or PLL-coated dishes (Fig. 3A). When HT1080 cells were cultured on dishes coated with high concentrations (750 ng/ml) of type I collagen, MMP-2 activation was increased, and this was blocked by BB94 treatment.

In parallel with collagen-induced MMP-2 activation, the MT1-MMP protein levels increased in HT1080 cells cultured on type I collagen compared with those on PLL-coated dishes (Fig. 3B). This is consistent with the aforementioned data that the active form of MT1-MMP accumulates in MT1-MMP-transfected cells cultured on collagen (Fig. 2C).

To clarify the relevance between collagen-dependent MMP-2 processing mediated by MT1-MMP and ERK activation, the effect of an MEK inhibitor PD98059 on collagen-stimulated MMP-2 processing was examined. As shown in Fig. 3C, treatment of HT1080 cells with PD98059 suppressed sustained ERK activation induced by collagen in a dose-dependent manner. This inhibition was accompanied by a reduction of collagen-stimulated MMP-2 processing. The effects of BB94 and PD98059 on MMP-2 processing and ERK activation induced by a three-dimensional collagen gel also were studied (Fig. 3D). As reported previously, culture of cells within a three-dimensional collagen gel stimulated MMP-2 activation, which was inhibited completely by BB94 and partially by PD98059 treatment (30). ERK activation also was induced in HT1080 cells cultured in a threedimensional collagen gel, which was suppressed not only by PD98059 but also by BB94. These results suggest that ERK activation induced by collagen in HT1080 cells enhances MT1-MMP activity.

Sustained ERK Activation Up-Regulates MT1-MMP. To confirm that sustained ERK activation stimulates MT1-MMP activity in HT1080 cells, CA-MEK was used to activate ERK independently of collagen adhesion. Sustained ERK activation induced by type I collagen in control cells was suppressed by BB94 treatment; however, ERK activation in CA-MEK expressing cells was higher than that of control cells and was not suppressed by BB94 treatment (Fig. 4A). Concomitant with enhanced ERK activation, MMP-2 processing was enhanced markedly in CA-MEK expressing HT1080 cells. BB94 treatment suppressed the MMP-2 processing in mock and CA-MEK transfectants.

**Fig. 2.** MT1-MMP is involved in collagen-induced ERK activation. A, HT1080 cells were replated onto culture dishes coated with poly-L-lysine (PLL) or collagen for 30 min. The lysates were analyzed by immunoblotting using anti-phospho-p44/42 MAPK (Blot: pERK) and anti-ERK2 (Blot: ERK2) antibodies. B, HT1080 cells were plated onto collagen-coated dishes in serum-free DMEM with or without BB94 (1 μM) for 0.5 or 6 h. MT1-MMP (MT1) or MT1-MMP/E/A (MT1-E/A) was cotransfected with MT1-MMP (MT1) or MT1-MMP/E/A (MT1-E/A) into HT1080 cells. At 24 h after transfection, the cells were serum starved for 12 h, and then the cells were kept in suspension in serum-free DMEM for 20 min (S) and then replated and cultured on collagen-coated dishes in serum-free DMEM for 6 h (C). The cells were homogenized, immunoprecipitated with an anti-HA (IP: HA) antibody, and analyzed by immunoblotting using anti-phospho-p44/42 MAPK (Blot: pERK) or anti-p44/42 MAPK (Blot: ERK2) antibodies. WCLs were immunoblotted with an anti-MT1-MMP (Blot: MT1) or anti-α-tubulin (Blot: Tubulin) antibody. Numeric values represent the relative density of the phosphorylated ERK bands calculated relative to the control samples and normalized to 1.0.

**Fig. 3.** Collagen-induced ERK activation promotes MT1-MMP activity. A, HT1080 cells were allowed to adhere to dishes coated with poly-L-lysine (PLL) or type I collagen (Collagen) and cultured in serum-free DMEM with or without BB94 (1 μM) for 20 h. Mock-treated cells also were cultured in serum-free DMEM (on dish) for 20 h. The conditioned media were analyzed by gelatin zymography. L, latent; 1, activation intermediate; and A, active form of MMP-2. B, HT1080 cells cultured on PLL (PLL) or type I collagen (Collagen) for 20 h and HT1080 cells transfected with MT1-MMP (MT1-MMP) were cotransfected with MT1-MMP (MT1-MMP) or anti-α-tubulin (Blot: Tubulin) antibodies. C, HT1080 cells were kept in suspension for 20 min, plated onto collagen-coated dishes (Collagen), and then incubated in serum-free DMEM containing DMSO, or PD98059 (2.5 or 25 μM) for 6 h. Whole-cell lysates (WCLs) were immunoblotted with anti-phospho-p44/42 MAPK (Blot: pERK) and anti-ERK2 (Blot: ERK2) antibodies. The conditioned media were analyzed by gelatin zymography. D, HT1080 cells were pretreated with DMSO, BB94 (1 μM), or PD98059 (25 μM) for 2 h and then embedded in a three-dimensional collagen gel for 12 h in serum-free DMEM containing DMSO, BB94, or PD98059. The WCLs were immunoblotted with anti-p44/42 MAPK (Blot: pERK) and anti-ERK2 (Blot: ERK2) antibodies. The conditioned media were analyzed by gelatin zymography.
expressing cells. The MT1-MMP protein levels in mock-transfected cells were low and were augmented by BB94 treatment because of the inhibition of MT1-MMP autodegradation. In contrast, MT1-MMP levels in CA-MEK-expressing cells were relatively high compared with mock-transfected cells and were no longer enhanced by BB94 treatment. Moreover, the ectopic expression of MT1-MMP promoted MMP-2 processing, which was blocked by BB94 treatment. Coexpression of CA-MEK with MT1-MMP augmented MT1-MMP-induced MMP-2 processing concomitant with an increase in the protein levels of MT1-MMP active form as demonstrated by immunoblotting (Fig. 4B). These results suggest that constitutive ERK activation by CA-MEK expression increases MT1-MMP protein levels in HT1080 cells.

Hemopexin-Like Domain of MT1-MMP Inhibits Collagen-Induced ERK Activation and Cell Migration. The hemopexin-like domain of MT1-MMP plays a crucial role in MT1-MMP homophilic complex formation, binding to native type I collagen, and MMP-2 processing. Thus, the expression of the hemopexin-like domain of MT1-MMP (MT1-Pex) has been shown to modulate MT1-MMP activity (29, 38). To confirm the involvement of MT1-MMP on collagen-induced ERK activation and cell migration, the effects of MT1-Pex expression on ERK phosphorylation was examined. Expression of MT1-Pex in HT1080 cells suppressed significantly collagen-induced MMP-2 activation (Fig. 5A). As expected, the cells expressing MT1F-Pex showed impaired collagen-dependent ERK activation (Fig. 5B). This suppression of MMP-2 processing and sustained ERK activation by MT1F-Pex expression was accompanied with the attenuation of collagen-induced cell migration to 39.5% of controls (Fig. 5A). Down-regulation of MMP-2 processing and sustained ERK activation by MT1F-Pex suggests that MT1-MMP activity is involved in collagen-induced ERK activation, which results in enhanced migration in HT1080 cells.

**DISCUSSION**

MEK1/ERK signaling has been involved in the regulation of the invasive and metastatic potential of tumor cells by affecting the actin-myosin system (3, 4, 31). In the present study, we demonstrated that HT1080 cells cultured on type I collagen up-regulates MT1-MMP, which plays an important role in cell migration on type I collagen. HT1080 cells formed well-organized actin stress fibers and focal adhesions when cultured on type I collagen, which might gen-

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**Fig. 4.** Sustained ERK activation induces MT1-MMP expression. A, pcDNA-CA-MEK or pcDNA3 (Ctr) were cotransfected into HT1080 cells with pRK-GFP and pHA262pur plasmids. The cells selected with puromycin were plated onto collagen-coated dishes and incubated in serum-free DMEM with or without BB94 (1 μM) for 6 h. The whole-cell lysates (WCLs) were immunoblotted with anti-phospho-p44/42 ERK MAPK (Blot: pERK), anti-p44/42 ERK MAPK (Blot: tERK), anti-MT1-MMP (Blot: MT1-MMP), or antia-actin (Blot: Actin) antibodies. The conditioned media were analyzed by gelatin zymography. B, MT1F-MMP (MTIF) and CA-MEK (CA-MEK) plasmids were cotransfected into HT1080 cells as indicated with prk-GFP and pHA262pur plasmids. The cells selected with puromycin were replated onto collagen-coated dishes and incubated in serum-free DMEM with or without BB94 (1 μM) for 20 h. The conditioned media were analyzed by gelatin zymography, and the cell lysates were analyzed by immunoblotting using anti-FLAG (Blot: FLAG) or anti-a-tubulin (Blot: Tubulin) antibodies.

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**Fig. 5.** Effect of MT1-MMP hemopexin-like domain. A, HT1080 cells were transfected with pSG5 (mock) or pSG5-MTIF-Pex (MTIF-Pex) plus pRK-GFP and pHA262pur plasmids. At 48 h after transfection, the cells were replated onto collagen-coated dishes and incubated in serum-free DMEM with or without BB94 (1 μM) for 20 h. The conditioned media were analyzed by gelatin zymography. B, HT1080 cells were transfected with MTIF-Pex or pSG5 plus pcDNA-HA-ERK2 plasmids. At 48 h after transfection, the cells were kept in suspension for 20 min (S) and then replated on collagen-coated dishes in serum-free DMEM for 6 h (C). The cells were homogenized, immunoprecipitated with an antihemagglutinin (IP: HA) antibody, and analyzed by immunoblotting using anti-phospho-p44/42 MAPK, Blot: pERK or anti-p44/42 MAPK (Blot: tERK) antibodies. The whole-cell lysates (WCLs) were immunoblotted with an anti-FLAG M2 (Blot: FLAG) antibody. C, cell migration assays were performed using HT1080 cells transfected with pSG5 (mock) or pSG-MTIF-Pex (MTIF-Pex) as described in “Materials and Methods.” Error bars indicate SD for at least 30 cells per condition. *P < 0.0001 versus mock.
generate a sustained signal through the MEK/ERK pathway (Fig. 1D). Treatment of HT1080 cells with concanavalin A, a tetravalent lectin, promotes MMP-2 processing, in which expression of focal adhesion kinase and its phosphorylation is essential (32, 33). BB94 did not block adhesion of HT1080 cells to type I collagen nor ERK activation induced by the initial binding (30 min) to type I collagen (Fig. 1B and Fig. 2B). MT1-MMP may be involved in the turnover of focal adhesions on type I collagen, thus explaining why BB94 interfered with sustained ERK activation resulting from constitutive signal transduction from focal adhesions. Induction of sustained ERK activation and enhanced MMP-2 processing in HT1080 cells required higher concentrations of type I collagen or a three-dimensional collagen gel. Constitutive degradation and reconstruction of the collagen environment may be essential for sustained ERK activation through cell-collagen interactions. The precise role of MT1-MMP in generating signals to activate ERK through cell-collagen interactions remains to be determined.

Interestingly, enhanced ERK signaling in turn increased MT1-MMP levels. MT1-MMP levels on the cell surface are subject partially to autodegradation because cell surface MT1-MMP accumulates by treatment with MMP inhibitors (e.g., BB94 and TIMP-2; Ref. 34). However, the levels of MT1-MMP enhanced by CA-MEK were no longer augmented by BB94 treatment. This suggests that ERK signaling blocks the autodegradation of MT1-MMP. Although the autodegradation of MT1-MMP is blocked by CA-MEK in HT1080 cells, MMP-2 processing by MT1-MMP was not inhibited but rather enhanced in parallel with the increase in MT1-MMP levels. One possible explanation for this is that MEK/ERK activation recruits TIMP-2 to form the trimolecular complex with MT1-MMP and MMP-2, which not only stabilizes MT1-MMP but also accelerates MMP-2 processing (25). Alternatively, MT1-MMP localization may have been shifted from the intracellular compartment to the cell surface by ERK signaling, resulting in the increase in MMP-2 processing, as has been shown previously for HT1080 cells treated with concanavalin A (35, 36).

We demonstrated here that collagen-induced ERK activation and cell migration were inhibited by the expression of the hemopexin-like domain of MT1-MMP. This domain is known to be essential not only for its homophilic complex formation but also for association with other proteins, such as the hyaluronic receptor CD44, the tenascin CD63, and native type I collagen (29, 37–39). Tam et al. (38) reported that recombinant MT1-MMP hemopexin-like domain reduced the degradation of native type I collagen by MT1-MMP in vitro and in vivo and implied that collagen might assemble MT1-MMP on the cell surface via binding that the hemopexin-like domain, resulting in the increase of the local concentration of MT1-MMP required for pericellular collagen degradation and efficient MMP-2 processing. This may be supported by our findings that the high concentration but not low concentration of type I collagen used in this study could promote MMP-2 processing in HT1080 cells, which was suppressed by the expression of MT1-MMP hemopexin-like domain (Fig. 3A and Fig. 5A). Expression of the hemopexin-like domain of MT1-MMP may compete with MT1-MMP for the interaction with type I collagen, thus resulting in the suppression of collagen cleavage by MT1-MMP and subsequent inhibition of ERK activation and cell migration.

Thus, MT1-MMP may be essential for invasive tumor cells not only to adapt to the growth conditions within a three-dimensional meshwork of ECM macromolecules but also to induce ERK activation in two- and three-dimensional collagen. Additional studies are needed to examine whether MT1-MMP-mediated ERK activation leads to tumor invasion and proliferation in the two- and three-dimensional collagen ECM.

In conclusion, we demonstrated that MT1-MMP positively regulates cell-collagen interactions, which generate a sustained signal through the MEK/ERK pathway, and this sustained ERK activation in turn up-regulates MT1-MMP. Thus, MT1-MMP functions in a positive feedback loop to induce sustained ERK activation, which consequently stimulates cell migration on type I collagen. Understanding the role of MT1-MMP in cell migration may provide a new strategy to inhibit tumor invasion.

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