Inhibition of Membrane-Type 1 Matrix Metalloproteinase at Cell-Matrix Adhesions

Takahisa Takino, Hiromi Saeki, Hisashi Miyamori, Tomoya Kudo, and Hiroshi Sato

Abstract
Membrane-type 1 matrix metalloproteinase (MT1-MMP) has been implicated in tumor invasion and metastasis. We previously reported that extracellular matrix degradation by MT1-MMP regulates cell migration via modulating sustained integrin-mediated signals. In this study, MT1-MMP–expressing cells were plated onto fibronectin-coated plates and monitored for cell-matrix adhesion formation and fibronectin degradation. The fibronectin was degraded and removed in line with the cell migration track. The migrating cells showed a polarized morphology and were in contact with the edge of fibronectin through the leading edge, in which cell-matrix adhesions are concentrated. Expression of MT1-MMP targeted to cell-matrix adhesions by fusing with the focal adhesion targeting (FAT) domain of FAK promotes the initial fibronectin lysis at the cell periphery immediately after adhesion. These results suggest that fibronectin is degraded by MT1-MMP located at cell-matrix adhesions, which are concentrated at the leading edge of the migrating cells. To inhibit MT1-MMP at cell-matrix adhesion, the dominant negative form of MT1-MMP (MT1-Pex) was targeted to the cell-matrix adhesion by fusing with the FAT domain (MT1-Pex-FAT). MT1-Pex-FAT accumulated at cell-matrix adhesions and inhibited fibronectin degradation as well as FAK phosphorylation more effectively than parental MT1-Pex. MT1-Pex-FAT was also shown to suppress the invasion of tumor cells into three-dimensional collagen gel more strongly than MT1-Pex. These results suggest that MT1-MMP–mediated extracellular matrix lysis at cell-matrix adhesions induces the establishment of cell polarity, which facilitates cell-matrix adhesion turnover and subsequent cell migration. This model highlights the role of MT1-MMP at the leading edge of migrating cells.

Introduction
Tumor cell invasion is a complex process that includes cell adhesion to the extracellular matrix (ECM), ECM degradation, and cell migration (1, 2). Cell adhesion to ECM governs cellular functions, including gene expression, cell differentiation, proliferation, survival, and migration, and induces the formation of cell-matrix adhesions such as focal complex, focal adhesion, fibrillar adhesion, or three-dimensional adhesion. The integrin family of transmembrane proteins forms heterodimers that function as receptors for ECM components and regulates cellular adhesiveness through cell-matrix adhesion formation. The engagement of integrins by cell attachment to ECM stimulates the activation of focal adhesion kinase (FAK), a ubiquitously expressed nonreceptor protein tyrosine kinase that functions as a scaffold to activate various signals (3). FAK plays a central role in integrin-mediated signaling by interacting with various signaling molecules such as Src, phosphoinositide-3 kinase, p130Cas, Crk, and JSAP1 (3–5). It also regulates cell migration, invasion, and cell-surface MT1-MMP. In fact, increases in the expression and phosphorylation of FAK have been shown in various tumors, and overexpression of the focal adhesion targeting (FAT) domain of FAK suppresses FAK auto-phosphorylation and the migration and invasion of tumor cells (3, 6–8).

Membrane-type 1 matrix metalloproteinase (MT1-MMP) was originally identified as a tumor-specific proMMP-2 activator (9) and is now known to activate proMMP-9 and proMMP-13, degrade a broad spectrum of ECM components including collagens, fibronectin, and laminins, and process cell-surface proteins such as integrins, CD44, tissue transglutaminase, and syndecan-1 (10, 11). Many clinical studies have revealed that MT1-MMP expression most closely correlates with the invasive phenotype of human tumors among MMP. The homodimerization of MT1-MMP is essential for MT1-MMP–mediated proMMP-2 activation and the cleavage of type I collagen fibers, which is mediated by intermolecular association through the MT1-MMP hemopexin domains (10, 11). Expression of MT1-MMP mutants lacking its catalytic domain (MT1-Pex) suppresses proMMP-2 activation, ECM degradation, and tumor cell invasion both in vitro and in vivo by disrupting the homodimerization of MT1-MMP (12, 13).

Cell-surface MT1-MMP is internalized by clathrin-dependent and clathrin-independent mechanisms, and regulation of these processes seems important in facilitating its functions (10, 11). MT1-MMP interacts and co-localizes with α3β1 and β3 integrins at the leading edge of migrating endothelial cells, where the dynamic turnover of cell-matrix adhesion and actin occurs (14), and cooperates with αvβ3 integrin in the full maturation of proMMP-2 (10, 15). Interestingly, FAK promotes the proliferation, migration, and invasion of tumor cells and is now known to up-regulate the level of cell-surface MT1-MMP by disrupting its internalization (16). Based on these studies, it seems reasonable to suppose that MT1-MMP conspires with integrins for efficient tumor invasion by modulating ECM degradation and integrin-mediated signals.

In this study, we localized MT1-MMP–mediated ECM degradation at cell-matrix adhesion sites during cell migration and invasion and showed that targeting MT1-Pex to cell-matrix adhesion sites augmented its inhibitory effect on MT1-MMP–mediated ECM degradation and the invasion of tumor cells.

Materials and Methods
Cell culture and reagents. All cell lines were obtained from the American Type Culture Collection. HT1080 and 293T cells were maintained...
in DMEM supplemented with 5% fetal bovine serum (FBS). HeLa and U87 cells were cultured in 10% FBS/DMEM.

To make the collagen solution, seven volumes of type I collagen (Nitta Gelatin) were mixed with two volumes of 5% concentrated DMEM and one volume of 0.05 N NaOH containing 2.2% NaHCO3 and 200 mmol/L HEPES. Cells were suspended in the collagen solution. Then, 350 μL of cells (2 × 105) in the collagen mixture were plated on the top of a cell-free gelled collagen layer. The collagen mixture was allowed to gel at 37°C, and then, the medium was added.

The synthetic MMP inhibitor BB94 was prepared as described previously (17). Fibronectin was purchased from Asahi Techno Glass. Matrigel was from BD Biosciences. The immunoregic reagents used were anti-FAK and anti-paxillin antibodies (BD Biosciences); an anti-β1 integrin antibody (Chemicon); anti-FLAG and anti-vascular stomatitis virus (anti-VSV) antibodies (Sigma-Aldrich); an anti–phospho-FAK (Tyroid) antibody (BioSource); an anti–c-Myc antibody (Cell Signaling Technology); rhodamine-phalloidin (Molecular Probes).

Expression plasmids. Expression plasmids for full-length MT1-MMP tagged with FLAG and FLAG-tagged FAK were constructed as described previously (4, 17). MT1-MMP tagged with the c-Myc epitope and FLAG-tagged MT1-Pex, which lacks the catalytic domain of MT1-MMP, were gifts from Dr. M. Seki (Institute of Medical Science, University of Tokyo, Tokyo, Japan). Plasmids encoding green fluorescent protein (pRK-GFP), HA-FAK, the VSV-tagged FAT domain of FAK, and the puroycin-resistance gene (pHA262pα) were gifts from Dr. Kenneth M. Yamada (NIH, Bethesda, MD).

A PCR product encoding the FAT domain of FAK cDNA (Ile304–Pro336 of FAK) was ligated into pEAK-MT1 or pEAK-MT1-Pex downstream of the transmembrane domain. Transient transfections were done by the calcium phosphate method or via Trans IT (PanVera) according to the manufacturer's protocols.

Immunoprecipitation and immunoblotting. The cells were washed twice with ice-cold PBS and homogenized in NP40 lysis buffer containing 50 mmol/L Tris-HCl (pH, 7.5), 150 mmol/L NaCl, 1 mmol/L EDTA, 1 mmol/L phenylmethylsulfonil fluoride, 2 mmol/L Na3VO4, 2 mmol/L NaF, and 1% NP40. Cell lysates were centrifuged to remove insoluble materials. Protein concentrations of cell lysates were determined, and samples were adjusted to an equal protein concentration and volume. For immunoprecipitation analysis, cell lysates were used for precipitation with FLAG affinity gel (Sigma-Aldrich) or anti-Myc antibody and protein G sepharose at 4°C.

Immunofluorescence staining. HeLa cells were transfected with MT1-MMP. At 24 h after transfection, cells were detached and kept in suspension for 30 min and then attached to fibronectin (10 μg/mL)-coated coverslips for 15 min, 40 min, or 3 h. Alternatively, cells were seeded onto coverslips coated with fibronectin and were transfected with plasmids as described. To detect cell-surface MT1-MMP, anti-FLAG antibody was added to the culture medium and reacted for 15 min. After washing, cells were fixed, permeabilized, and then exposed to primary antibodies and reacted with secondary antibodies. For three-dimensional collagen gel culture, HT1080 cells were transfected as described. At 24 h after transfection, cells were trypsinized and cultured in collagen gel for 2 days. An anti-FLAG antibody was then added to the culture medium and reacted for 1 h. Cells were rinsed, fixed, permeabilized, quenched with 50 mmol/L NH4Cl, and blocked with 0.7% gelatin/0.1% saponin. Samples were incubated with secondary antibody and rhodamine-phalloidin at 4°C overnight. Cells were then observed by confocal laser scanning microscopy (Carl Zeiss).

Fibronectin degradation by MT1-MMP. Glass coverslips were coated with fibronectin and FITC-labeled fibronectin as described previously (13). HeLa cells transfected with MT1-MMP were seeded onto FITC-coated coverslips and cultured for 40 min, 3 h, 12 h, or 24 h. Before washing, an anti-FLAG antibody was added to the culture medium and reacted for 15 min. After washing, cells were fixed, permeabilized, and exposed to primary antibodies. Fibronectin lysis was detected as the decrease of FITC-fibronectin using confocal laser scanning microscopy.

Cell invasion assay. Cell invasion was assayed using modified Boyden chambers consisting of Transwell membrane filters (Corning Costar) as described previously (7). HT1080 or puromycin-selected U87 cells (2 × 105 cells) were suspended in 100 μL of 0.5% FBS/DMEM and cultured for 24 or 30 h. After the fixation of membranes, the number of GFP-positive or crystal violet–stained cells on the lower surface was counted under a microscope. At least three independent experiments were carried out.

Results

MT1-MMP degrades fibronectin in close proximity to the leading edge. HeLa cells transfected with the MT1-MMP gene were plated onto fibronectin and examined regarding the distribution of MT1-MMP and focal adhesions (Fig. 1A). Cell attachment to fibronectin induces cell spreading, which is accompanied by the formation of cell-matrix adhesions toward the periphery of cells, as indicated by paxillin staining. At 3 h after attachment, polarized cell-matrix adhesion was organized in migrating cells. Cell-surface MT1-MMP was distributed evenly at the cell periphery during cell spreading and co-localized with paxillin at the leading edge in migrating cells (3 h). Co-localization of MT1-MMP with β1 integrin at the leading edge was also observed in migrating cells (Fig. 1B, 3 h). Similar results were obtained from an experiment using type I collagen as a substrate (data not shown). Thus, cell migration on ECM induces the redistribution of MT1-MMP to the leading edge, where the dynamic turnover of cell-matrix adhesion is known to occur.

The expression of MT1-MMP in HeLa cells promoted cell migration on fibronectin, and migrating cells digest fibronectin through the migration track (13). The cell-fibronectin adhesion and fibronectin lysis during the migration of HeLa cells expressing MT1-MMP were examined (Fig. 1C). Fibronectin lysis was not observed in spreading cells (40 min) regardless of MT1-MMP expression. At 3 h after plating, only MT1-MMP–expressing cells degraded fibronectin from the cell periphery, where cell-matrix adhesions accumulated. Fibronectin lysis was found as a migration track of MT1-MMP–expressing cells at 12 h after plating (Fig. 1C, 12 h). The migrating cells showed a polarized morphology and were in contact with the edge of coated fibronectin through the leading edge of the cells, where focal adhesions were concentrated. These results indicate that fibronectin lysis by migrating cells takes place at the leading edge where cell-matrix adhesions are concentrated.

Targeting MT1-MMP to cell-matrix adhesions. The targeting of FAK to cell-matrix adhesions is mediated by its FAT domain, which interacts with the cell-matrix adhesion-associated proteins paxillin and talin (6). To examine whether MT1-MMP localized at sites of cell-matrix adhesion is involved in fibronectin lysis, MT1-MMP and its dominant negative form were targeted to cell-matrix adhesions by fusing with the FAT domain (MT1-FAT and MT1-Pex-FAT, respectively; Fig. 2A). The expression of MT1-FAT induced proMMP-2 activation as effectively as MT1-MMP (Fig. 2B). Figure 2C shows that paxillin was coprecipitated with MT1-FAT and MT1-Pex-FAT, but not with MT1-Pex. Thus, both MT1-FAT and MT1-Pex-FAT were accumulated at cell-matrix adhesions, which were clearly co-localized with paxillin and served as the anchors for actin-stress fibers (Fig. 2D). Next, fibronectin lysis by the cells expressing MT1-MMP and MT1-FAT was compared. Fibronectin lysis at the cell periphery was faintly observed in MT1-MMP–expressing cells at 1 h after plating. Fibronectin lysis was clearly enhanced in MT1-FAT–expressing cells, and lysis spots were overlapped with cell-matrix adhesions, where MT1-FAT accumulated (Fig. 3A). At 12 h after plating, fibronectin lysis by MT1-MMP–expressing cells was found as a migration track, which was reduced in MT1-FAT–expressing cells (Fig. 3B). These results showed that the targeting of MT1-MMP to cell-matrix adhesions...
promotes initial fibronectin degradation after adhesion. However, fibronectin lysis by MT1-FAT–expressing cells did not develop, probably due to the reduced cell migration.

**MT1-Pex-FAT suppresses fibronectin lysis.** Immunoprecipitation analysis revealed that both MT1-Pex and MT1-Pex-FAT were coprecipitated with MT1-MMP (Fig. 4A). HeLa cells, which do not express endogenous MT1-MMP, were co-transfected with expression plasmids for MT1-MMP and MT1-Pex or MT1-Pex-FAT, and fibronectin degradation and cell migration were examined (Fig. 4B). Cells expressing MT1-MMP migrated on fibronectin, and fibronectin lysis was observed as a migration track (as shown in Fig. 1C). MT1-Pex-FAT but not MT1-Pex was concentrated at the periphery of cells, and MT1-Pex-FAT expression resulted in the loss of a migrating morphology and the decrease of fibronectin lysis.

Figure 1. MT1-MMP degrades fibronectin in close proximity to cell-matrix adhesions. A and B, HeLa cells transfected with MT1-MMP were detached and kept in suspension and then attached to fibronectin-coated coverslips for indicated periods. Cells were fixed and stained for cell-surface MT1-MMP with anti-FLAG antibody (MT1; green) and anti-paxillin (Paxillin; red; A) or anti-FLAG (MT1; green) and anti-β1 integrin (β1 integrin, red; B) antibodies as described in Materials and Methods. C, HeLa cells transfected with MT1-MMP were seeded on FITC-fibronectin–coated coverslips in 10% FBS/DMEM for 40 min, 3 h, or 12 h. Cells were fixed and stained for cell-surface MT1-MMP (MT1; blue) and cell-matrix adhesion with anti-paxillin antibody (Paxillin; red). Fibronectin lysis was detected as the decrease of FITC-fibronectin (green). Dotted line, margin of fibronectin lysis. Bar, 20 μm.
Figure 2. Targeting MT1-Pex to cell-matrix adhesion. A, a schematic representation of MT1-MMP and its deletion/chimeric mutants. MT1-MMP comprises the signal peptide (Signal), propeptide (Pro), catalytic domain, hemopexin domain, hinge region, transmembrane domain (TM), and cytoplasmic domain (CP). FAT, FAT domain of FAK; FLAG, FLAG epitope; HA, HA epitope; Myc, Myc epitope; VSV, VSV epitope. B, HeLa cells were transfected with MT1, MT1-FAT, and MT1-Pex-FAT. Conditioned media were analyzed with gelatin zymography. C, 293T cells were transfected with the indicated plasmids and subjected to immunoprecipitation with anti-FLAG affinity gel (IP: FLAG). The precipitates were analyzed by immunoblotting using anti-paxillin (Blot: Paxillin) and anti-FLAG (Blot: FLAG) antibodies. D, HeLa cells were transfected as indicated, cultured on fibronectin-coated coverslips, and stained for cell-surface MT1-MMP (MT1, green), paxillin (Paxillin; blue), and rhodamine-phalloidin (F-actin; red). Bar, 20 μm.
Coexpression of VSV-FAT with MT1-Pex also induced the loss of a migrating morphology, and MT1-MMP and MT1-Pex were not condensed at the leading edge. The expression of MT1-Pex attenuated fibronectin lysis by 41.1%, and coexpression of the VSV-FAT domain with MT1-Pex augmented the inhibition of fibronectin lysis (61.5%; Fig. 4C). However, MT1-Pex-FAT expression inhibited fibronectin lysis more strongly than the combination of MT1-Pex and FAT (80.7%). These results show that targeting MT1-Pex to cell-matrix adhesions by fusing with the FAT domain promotes the inhibition of MT1-MMP–mediated fibronectin lysis and cell motility.

MT1-Pex-FAT inhibits FAK autophosphorylation. Overexpression of the FAT domain is known to suppress the intermolecular autophosphorylation of Tyr397 of FAK by disturbing the accumulation of FAK at sites of cell-matrix adhesion (6). The effect of MT1-Pex-FAT on FAK phosphorylation was studied. Autophosphorylated FAK was localized at the periphery of MT1-Pex–expressing or control cells; however, it was notably absent from the periphery of MT-Pex-FAT–expressing cells where MT1-Pex-FAT accumulated (Fig. 5A). FAK autophosphorylation, as assessed by immunoblotting, was induced by cell attachment to fibronectin, and this was reduced by 18.7% in VSV-FAT domain–expressing, but not MT1-Pex–expressing, cells (Fig. 5B and C). FAK phosphorylation was more severely attenuated by MT1-Pex-FAT expression (50.5%) or by coexpression of the VSV-FAT domain and MT1-Pex (41.7%). These results show that MT1-Pex facilitates the suppression of fibronectin-induced FAK autophosphorylation by FAT.

MT1-Pex-FAT suppresses tumor invasion. The effect of MT1-Pex-FAT expression on the invasion of tumor cells into three-dimensional ECM was tested. MT1-MMP expression elevated HT1080 cell invasion into Matrigel to 131% of mock transfectants.
The rate of cell invasion was attenuated by 24.4% and 23.7% by the expression of MT1-Pex and VSV-FAT, respectively. MT1-Pex-FAT expression significantly reduced the invasion compared with MT1-Pex or VSV-FAT alone (P < 0.001) and coexpression of MT1-Pex and VSV-FAT (P < 0.05). The effect of MT1-Pex-FAT expression on the invasion of U87 glioma cells into three-dimensional type I collagen gel was also examined, which also express MT1-MMP endogenously (Fig. 6A). Expression of MT1-Pex, VSV-FAT, and VSV-FAT plus MT1-Pex in U87 cells also attenuated invasion into three-dimensional collagen matrix in a similar pattern. MT1-Pex-FAT expression most effectively attenuated the invasion compared with MT1-Pex or VSV-FAT alone (P < 0.001) and MT1-Pex plus VSV-FAT (P < 0.05). As shown in Fig. 6C, HT1080 cells grown in three-dimensional collagen gel formed protrusions. HT1080 cells transfected with the MT1-MMP gene exhibited a more invasive morphology, showing elongation and the formation of numerous cellular protrusions into the gel. Formation of protrusions was reduced in the cells expressing MT1-Pex, and the expression of MT1-Pex-FAT further reduced protrusion formation and induced cell rounding and cell death. These results show that the expression of MT1-Pex-FAT effectively suppresses MT1-MMP–promoted cell invasion into three-dimensional ECM.

**Discussion**

We previously reported that the lysis of ECM by MT1-MMP regulates focal adhesion stability and subsequent extracellular signal-regulated kinase activation, which, in turn, stimulates cell migration (13, 17). Our present study showed that the accumulation of MT1-MMP at sites of cell-matrix adhesion promotes early fibronectin lysis at the cell periphery, and that the inhibition of MT1-MMP at cell-matrix adhesions attenuates MT1-MMP–mediated fibronectin lysis and subsequent cell migration and invasion.

Our data suggest the model proposed in Fig. 6D. Cell attachment to ECM induces the formation of cell-matrix adhesions. During spreading, cell-surface MT1-MMP is recruited to newly formed cell-matrix adhesions where integrins are clustered. Fibronectin under the cell is digested from the cell periphery by MT1-MMP, and cell-fibronectin adhesions are concentrated in the fibronectin-rich area, which becomes the leading edge. MT1-MMP is transported toward the leading edge of a migrating cell, where cell-fibronectin adhesions are concentrated. Then, MT1-MMP may act with integrins for efficient ECM degradation at the leading edge, which results in the promotion of cell-matrix adhesion turnover and consequent cell movement. Thus, the inhibition of...
MT1-MMP at the leading edge of a migrating cell is effective in suppressing the motility and invasiveness of tumor cells (2).

We showed that the targeting of MT1-MMP to cell-matrix adhesions (MT1-FAT) promotes initial fibronectin lysis at the cell periphery following cell adhesion to fibronectin (Fig. 3). This suggests that MT1-MMP, localized at sites of cell-matrix adhesion, is involved in ECM degradation. Although MT1-FAT–expressing cells showed initial fibronectin lysis immediately after adhesion to fibronectin, cells did not migrate, and fibronectin lysis was not developed. The fused FAT domain not only targets MT1-MMP to cell-matrix adhesions to induce initial fibronectin lysis, but also down-regulates FAK to suppress MT1-MMP–induced cell migration. We also showed that the targeting of an MT1-MMP inhibitor, MT1-Pex, to cell-matrix adhesions (MT1-Pex-FAT) augmented the inhibition of MT1-MMP–mediated fibronectin lysis (Fig. 4B and C). Expression of the FAT domain or MT1-Pex significantly suppressed fibronectin lysis and invasion into three-dimensional ECM. MT1-Pex-FAT induced a more marked suppression than the combination of the FAT domain and MT1-Pex (Fig. 6A and B; \( P < 0.05 \)) and a loss of invasive morphology of HT1080 cells cultured in collagen gel (Fig. 6C). A recent study has shown that MT1-MMP is phosphorylated at Tyr573 by c-Src, which plays a key role in cell migration (18). It also showed that phosphorylated MT1-MMP is co-localized with phosphorylated caveolin-1 at Tyr14. Phosphorylated caveolin-1 is preferentially localized at sites of focal adhesion, forms a complex with MT1-MMP (19), involved in MT1-MMP transportation in endothelial cells (20), and induces the internalization of a cholesterol-enriched membrane microdomain upon cell detachment (21). Taken together with our results, MT1-MMP in cell-matrix adhesions may play an important role in ECM digestion and subsequent cell migration and invasion.

MT1-Pex-FAT also suppressed FAK autophosphorylation (Fig. 5B and C). MT1-Pex expression suppressed MT1-MMP–mediated fibronectin lysis, but did not significantly affect FAK autophosphorylation by itself. However, MT1-Pex augmented the inhibition of FAK phosphorylation when coexpressed with or fused to the FAT domain. The association of MT1-Pex with integrins may affect the FAK-FAT interaction and stimulate the suppression of FAK autophosphorylation by FAT.

MT1-MMP–mediated pericellular collagenolysis is required for angiogenesis, adipogenesis, and tumor invasion and proliferation in three-dimensional collagen matrix (22–25). Such collagenolysis optimally occurs at sites of cell-matrix adhesion to allow the cells to perceive altered collagen rigidity, as modulated by MT1-MMP. Our data support this concept. Focalized ECM degradation by MT1-MMP at the leading edge of migrating cells may be important to create not only a space but also to provide signals leading to cell migration and expansion.

In conclusion, MT1-MMP–mediated fibronectin lysis at the cell periphery, where cell-matrix adhesions are concentrated, initiates migration by generating a fibronectin concentration gradient. This facilitates the formation of the polarity on migrating cells and promotes cell-matrix adhesion turnover and consequent cell movement. Gene therapy delivering the MT1-Pex-FAT gene to the tumor, which inhibits both MT1-MMP and FAK, may provide a new strategy to suppress tumor invasion and metastasis.

Figure 5. Inhibition of FAK by MT1-Pex-FAT. A, HT1080 cells cultivated on fibronectin-coated coverslips were transfected with either MT1-Pex or MT1-Pex-FAT and were fixed and stained for cell-surface MT1-MMP mutants (\( \text{FLAG}; \text{red} \)) and phosphorylated FAK on Tyr977 (\( \text{pFAK}; \text{green} \)). *, non-transfected cells. Bar, 20 \( \mu \)m. B, HT1080 cells were co-transfected with MT1-Pex, MT1-Pex-FAT, VSV-FAT, MT1-Pex plus VSV-FAT or empty vector (Control), and pHA242pur. After selection with puromycin, cells were suspended in 0.5% FBS/DMEM for 30 min (S) and plated onto fibronectin-coated dishes for 6 h (F). The cell lysates were analyzed by immunoblotting using anti–phospho-FAK (Tyr977; Blot: pFAK), anti-FAK (Blot: tFAK), anti-FLAG (Blot: FLAG), or anti-VSV (Blot: VSV) antibodies. C, values represent the ratio of phosphorylated FAK/total FAK as calculated by image analysis. Columns, mean; bars, SE; \( n = 3 \).
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References


Figure 6. MT1-Pex-FAT suppresses tumor cell invasion. A, MT1, MT1-Pex, MT1-Pex-FAT, or VSV-FAT plus pRK-GFP were co-transfected into HT1080 cells as indicated. Cells were seeded onto transwell chambers coated with Matrigel, and cultured for 48 h. GFP-positive cells on the filter underside were counted. *, P < 0.001 versus control; **, P < 0.001 versus MT1-F-Pex-FAT. B, MT1-Pex, MT1-Pex-FAT, or VSV-FAT plus pRK-GFP and pH242pur were co-transfected into U87 cells as indicated. Puromycin-selected cells were seeded onto transwell chambers coated with type I collagen gel and cultured for 30 h. The number of invaded cells was counted. The arithmetic mean and SD of a representative of four independent experiments are shown. *, P < 0.001 versus control; **, P < 0.05 versus MT1-Pex plus VSV-FAT. C, HT1080 cells were transfected as indicated and cultured in collagen gel for 2 d. Cells were stained for cell-surface MT1-MMP (MT1; green) and rhodamine-phalloidin (F-actin; red). *, dead cells. Bar, 20 μm. D, model of the inhibition of MT1-MMP by MT1-Pex-FAT.
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