Mutation Analysis of Membrane Type-1 Matrix Metalloproteinase (MT1-MMP)

THE ROLE OF THE CYTOPLASMIC TAIL CYS574, THE ACTIVE SITE GLU240, AND FURIN CLEAVAGE MOTIFS IN OLGOMERIZATION, PROCESSING, AND SELF-PROTEOLYSIS OF MT1-MMP EXPRESSED IN BREAST CARCINOMA CELLS

Membrane type-1 matrix metalloproteinase (MT1-MMP) is a key enzyme in the activation pathway of matrix prometallproteinase-2 (pro-MMP-2). Both activation and autocatalytic maturation of pro-MMP-2 in trans suggest that MT1-MMP should exist as oligomers on the cell surface. To better understand the functions of MT1-MMP, we designed mutants with substitutions in the active site (E240A), the cytoplasmic tail (C574A), and the RRXX furin cleavage motifs (R89A, ARAA, and R89A/ARAA) of the enzyme. The mutants were expressed in MCF7 breast carcinoma cells that are deficient in both MMP-2 and MT1-MMP. Our results supported the existence of MT1-MMP oligomers and demonstrated that a disulfide bridge involving the Cys574 of the enzyme’s cytoplasmic tail covalently links MT1-MMP monomers on the MCF7 cell surface. The presence of MT1-MMP oligomers also was shown for the enzyme naturally expressed in HT1080 fibrosarcoma cells. The single (R89A and ARAA) and double (R89A/ARAA) furin cleavage site mutants of MT1-MMP were processed in MCF7 cells into the mature proteinase capable of activating pro-MMP-2 and stimulating cell locomotion. This suggested that furin cleavage is not a prerequisite for the conversion of pro-MTI-MMP into the functionally active enzyme. A hydroxamate class inhibitor (GM6001, or Ilomastat) blocked activation of MT1-MMP in MCF7 cells but not in HT1080 cells. This implied that a matrixin-like proteinase sensitive to hydroxamates could be involved in a furin-independent, alternative pathway of MT1-MMP activation in breast cancer cells. The expression of the wild type MT1-MMP enhanced cell invasion and migration, indicating a direct involvement of this enzyme in cell locomotion. In contrast, both the C574A and E240A mutations render MT1-MMP inefficient in stimulating cell migration and invasion. In addition, the C574A mutation negatively affected cell adhesion, thereby indicating critical interactions involving the cytosolic part of MT1-MMP and the intracellular milieu.

Received for publication, August 30, 2000, and in revised form, April 20, 2001
Published, JBC Papers in Press, May 2, 2001, DOI 10.1074/jbc.M007921200

Dmitry V. Rozanov‡, Elena I. Deryugina‡, Boris I. Ratnikov‡, Edward Z. Monosov‡, George N. Marchenko‡, James P. Quigley§, and Alex Y. Strongin¶

From ‡The Burnham Institute, La Jolla, California 92037 and the §Vascular Biology Department, The Scripps Research Institute, La Jolla, California 92037

MT1-MMP (MMP-14) is a member of a large family of zinc endoproteinases, matrixins or matrix metalloproteinases (MMPs) (1, 2). There are several structural features such as the modular domain structure and the existence of an N-terminal propeptide domain, a zinc-coordinating active site domain, and a C-terminal hemopexin-like domain that are characteristic for most MMPs (1–3). A subfamily of membrane type (MT)-MMPs including MT1-MMP is distinguished by a relatively short transmembrane domain and a cytoplasmic tail, which associate these enzymes with discrete regions of the plasma membrane and the intracellular compartment. MT1-MMP expression has been documented in many tumor cell types and strongly implicated in malignant progression (3, 4). In addition to its ability to directly cleave certain components of the extracellular matrix (5, 6), MT1-MMP initiates the activation pathway of the most widespread MMP, MMP-2, by converting pro-MMP-2 into an activation intermediate that further undergoes autocatalytic conversion to generate the mature enzyme of MMP-2 (7–9). Structure-function relationships of MT1-MMP (10–16) and the mechanisms of pro-MMP-2 activation to the mature enzyme (9, 17–20) are not understood in detail (21–23). An immediate proximity of at least two molecules of MT1-MMP (an “activator” and a “receptor”) on the cell surface is required for in trans activation of MMP-2 to the mature form (17, 19, 20, 24). However, there is no direct biochemical evidence to support the existence of MT1-MMP oligomers on cell surfaces. In addition, mechanisms involved in activation and trafficking of MT1-MMP are not well elucidated and remain controversial (10, 13–15, 25–28). Thus, furin, a serine proteinase of the trans-Golgi network, has been earlier assumed to function as a unique activator of MT1-MMP (25). However, evidence is emerging that there could be alternative pathways of MT1-MMP activation (27, 28). In this respect, it is not possible to rule out certain autocatalytic steps in MT1-MMP activation such as those involved in the activation pathway of pro-MMP-2 and pro-MMP-9 (8, 29–31).

To better understand functions of MT1-MMP, we con-
structured mutant MT1-MMPs and evaluated cell surface expression of the wild type and mutant enzymes in MCF7 breast carcinoma cells deficient in MT1-MMP and MMP-2. This allowed us to specifically identify the direct effects of MT1-MMP on cell locomotion. Here, we report novel mechanisms that may control dimerization, processing, and self-inactivating proteolysis of MT1-MMP in breast carcinoma cells.

MATERIALS AND METHODS

Proteins, Antibodies, and Inhibitors—Pro-MMP-2, essentially free from tissue inhibitors of metalloproteinases, was purified using the technique described by Alvarez et al. (30). After partial purification on metal-chelate chromatography, dimeric pro-MMP-2 was purified by a second metal-chelate step followed by gelatin zymography, SDS-PAGE, and proteolytic activity with sheep-type fibroblast collagenase. 

Activation of MMP-2—To evaluate MT1-MMP constructs in pro-MMP-2 activation, 2 x 10^5 cells of each stably transfected MT1-MMP mutant or wild type were added to 0.1 ml of 0.1 mg/ml collagen type I, washed with PBS, and blocked with 1% BSA-PBS (Sigma, St. Louis, MO). Cells were then incubated for 2 h at 37 °C followed by incubation with fluorescein isothiocyanate-conjugated Fab' 2 fragment of sheep anti-mouse IgG (Sigma). Population gates were set by using cells incubated with normal mouse IgG as a control. 

Cell Adhesion—Cells were harvested in the wells of a high-binding 96-well plate (Corning Glass) precoated with 1 μg/ml collagen type I (Vitrogen 100; Cohesion, Palo Alto, CA) overnight at 4 °C. Plates were washed with PBS and blocked for 1 h at 37 °C with 1% BSA in DMEM supplemented with 10 μg/ml aprotinin, 10 μg/ml leupeptin, 1 mM phenylmethylsulfonyl fluoride, 1 μM pepstatin A, 1 mg/ml leupeptin, 1 μM pepstatin A, 1 mM phenylmethylsulfonyl fluoride, 1 μM leupeptin, 1 μM pepstatin A, and 1% BSA (DBS/BSA), pH 7.2. Cells were stained with 2 μg/ml anti-hinge antibody. Further, cells were incubated with a fluorescein isothiocyanate-conjugated Fab'2 fragment of goat anti-rabbit IgG (Sigma). Population gates were set by using cells incubated with normal rabbit IgG.

Cell Migration in Transwells—The directional migration of cells in Transwells (Costar, Cambridge, MA) was analyzed under serum-free conditions as previously described (9). Staining procedures were done on ice in Dulbecco’s PBS supplemented with 1% BSA, 1 mM CaCl2, 1 mM MgCl2, and 1% BSA (DBS/BSA), pH 7.2. Cells were stained with 2 μg/ml anti-hinge antibody. Further, cells were incubated with a fluorescein isothiocyanate-conjugated Fab'2 fragment of sheep anti-mouse IgG (Sigma). Population gates were set by using cells incubated with normal mouse IgG as a control.

Cell Adhesion—Cells were harvested in the wells of a high-binding 96-well plate (Corning Glass) precoated with 1 μg/ml collagen type I (Vitrogen 100; Cohesion, Palo Alto, CA) overnight at 4 °C. Plates were washed with PBS and blocked for 1 h at 37 °C with 1% BSA in DMEM supplemented with 10 μg/ml aprotinin, 10 μg/ml leupeptin, 1 mM phenylmethylsulfonyl fluoride, 1 μM pepstatin A, 1 mg/ml leupeptin, 1 μM pepstatin A, 1 mM phenylmethylsulfonyl fluoride, 1 μM leupeptin, 1 μM pepstatin A, and 1% BSA (DBS/BSA), pH 7.2. Cells were stained with 2 μg/ml anti-hinge antibody. Further, cells were incubated with a fluorescein isothiocyanate-conjugated Fab'2 fragment of goat anti-rabbit IgG (Sigma). Population gates were set by using cells incubated with normal rabbit IgG.

Cell Migration in Transwells—The directional migration of cells in Transwells (Costar, Cambridge, MA) was analyzed under serum-free conditions as previously described (9). Staining procedures were done on ice in Dulbecco’s PBS supplemented with 1% BSA, 1 mM CaCl2, 1 mM MgCl2, and 1% BSA (DBS/BSA), pH 7.2. Cells were stained with 2 μg/ml anti-hinge antibody. Further, cells were incubated with a fluorescein isothiocyanate-conjugated Fab'2 fragment of sheep anti-mouse IgG (Sigma). Population gates were set by using cells incubated with normal mouse IgG as a control.
were plated in 0.15 ml of AIM-V medium (Life Technologies) per insert. The outer chamber was filled with 0.6 ml of AIM-V medium. Following incubation for 48 h, cells that migrated to the membrane’s undersurface were detached with trypsin/EDTA and counted.

In Vitro Cell Invasion—Cell invasion assays were performed in serum-free AIM-V in 6.5-mm Transwells with the 8-μm pore size membranes. The undersurface of the Transwell membrane was precoated with collagen type I at 20 μg/ml overnight at 4 °C. After washing with PBS, 50 μl of PBS containing 3 μg of Matrigel (Becton Dickinson, Bedford, MA) were dried overnight on the upper surface of the membrane at room temperature. Matrigel was reconstituted in PBS at 37 °C. Bedford, MA) were dried overnight on the upper surface of the membrane. The undersurface of the Transwell membrane was precoated with collagen type I at 20 μg/ml overnight at 4 °C. After washing with PBS, 50 μl of PBS containing 3 μg of Matrigel and 100 μl of AIM-V medium. Cells were allowed to invade Matrigel for 24–48 h at 37 °C in a CO2 incubator. Following incubation for 48 h, cells were detached with trypsin/EDTA and counted.

RESULTS

Dimerization of MT1-MMP on Cell Surfaces—To identify cell surface forms of MT1-MMP, MCF7 breast carcinoma cells were stably transfected with MT1-MMP-wt. We specifically selected these cells for our studies, since the parental cell line is deficient in both MT1-MMP and MMP-2 (Fig. 1A, zeo; Fig. 4A, lane 1). High levels of MT1-MMP expression in transfected cells relative to those of mock-transfected cells were verified by flow cytometry employing rabbit polyclonal antibodies directed against the hinge region of MT1-MMP (Figs. 2 and 3A). Since the hinge region is localized between the catalytic domain and the C-terminal hemopexin-like domain of MT1-MMP, the anti-hinge antibodies permit the detection of the full-length proenzyme and the active enzyme as well as its inactive forms lacking the catalytic domain.

The immunoprecipitation studies revealed that reduced MT1-MMP-wt was represented by the 63-kDa (the proenzyme), 60-kDa (the enzyme), 42-kDa, and 39-kDa protein bands (degradation products; these bands are the most prominent). Under nonreducing conditions, a relatively low amount of the protein material in the 39–42-kDa region was present, while the significant quantities of several high molecular weight MT1-MMP-wt forms (78–85 kDa and 120 kDa (the two major forms) and 170 kDa and 220 kDa (the two minor forms)) were observed (Fig. 1A, wt). MT1-MMP-wt was undetectable in the lysates of cells transfected with the original pcDNA3-zeo plasmid (Fig. 4A, lane 1A). No specific bands were detected in the samples immunoprecipitated with control rabbit IgG (data not shown).

To further address a question of whether the oligomers of MT1-MMP-wt preexisted on the cell surface or formed in cell lysates, cells expressing MT1-MMP-wt were surface-biotinylated and lysed in buffer containing 10 mM iodoacetamide. Incubation was carried out for 1 h to complete the modification of free cysteines. Residual iodoacetamide was blocked by excess cysteine (100 mM). When MT1-MMP-wt was immunoprecipitated and analyzed, the pattern of alkylated MT1-MMP was identical.
to that shown on Fig. 1A. Since alkylation during lysis had no effect, these findings confirmed that the observed MT1-MMP-wt oligomers preexisted on the cell surface.

To verify that the 78–85-kDa species of MT1-MMP-wt revealed under nonreducing conditions contained the 39- and 42-kDa MT1-MMP-wt monomers, we excised the 78–85-kDa band from the nonreducing gel and extracted the protein in PBS plus 0.1% SDS. Streptavidin-coated Dynabeads (Dynal, Lake Success, NY) were used to capture the biotin-labeled proteins from the extract. After washings, the captured proteins were eluted with 1% SDS, reduced with DTT, and rerun on the gel followed by Western blotting and developing by avidin-horseradish peroxidase (Fig. 1A, eluate). Two protein forms with apparent molecular masses of 39 and 42 kDa were identified after these procedures. These findings confirmed that the 39- and 42-kDa degradation products of MT1-MMP-wt form dimers and explained the broad width of the 78–85-kDa MT1-MMP-wt band that might include three combinations of disulfide-linked 39- and 42-kDa species. Accordingly, the 120-kDa MT1-MMP-wt form observed under nonreducing conditions probably corresponds to a dimer consisting of the 60- or 63-kDa MT1-MMP-wt monomers. Low nanogram (or high picogram) amounts of the 120-, 170-, and 220-kDa MT1-MMP-wt forms greatly complicate their direct isolation and analysis similar to that performed with the 78–85-kDa species.

Pretreatment of cells with Ilomastat, a specific hydroxamate inhibitor of MMPs (32), abrogated both activation and proteolysis of MT1-MMP-wt. Consequently, the 63-kDa band of the MT1-MMP proenzyme was the major species observed in reduced samples. Under nonreducing conditions, treatment with the inhibitor revealed increased levels of the 170- and 220-kDa MT1-MMP-wt bands that might include three combinations of disulfide-linked 39- and 42-kDa species. Accordingly, the 120-kDa MT1-MMP-wt form observed under nonreducing conditions greatly complicate their direct isolation and analysis similar to that performed with the 78–85-kDa species.

The data on transfected MCF7 cells showed that extensive maturation, dimerization, and degradation of cell surface MT1-MMP occurred. To specifically address the processing and dimerization of this proteinase, we designed and expressed mutant MT1-MMPs in MCF7 cells.

The expression plasmids encoding cDNA for mutant MT1-MMPs were constructed using a polymerase chain reaction-based QuickChange mutagenesis system (Stratagene) and cloned into the pcDNA3-zeo plasmid. The first mutant carries the sequence Ala<sub>100</sub>-Arg<sub>109</sub>-Ala<sub>110</sub>-Ala<sub>111</sub> (MT1-MMP-ARAA) that modifies the Arg<sub>108</sub>-Arg<sub>109</sub>-Lys<sub>110</sub>Arg<sub>111</sub> site susceptible to cleavage by furin, a reported activator of MT1-MMP (25). In the second mutant, the Arg<sub>202</sub> of the another putative furin cleavage motif (Arg<sub>202</sub>-Arg<sub>203</sub>-Pro<sub>204</sub>-Arg<sub>205</sub>-Cys<sub>206</sub>) (36) was replaced by alanine (MT1-MMP-R289A). The third mutant carried mutations in both furin cleavage motifs (MT1-MMP-R89A/ARAA). In the fourth mutant, the Glu<sub>89</sub> residue of the active site domain of MT1-MMP was replaced by alanine to create inactive MT1-MMP (MT1-MMP-E240A). Finally, to evaluate the possible role in oligomerization of the unique cysteine residue of the MT1-MMP’s cytoplasmic portion, the Cys<sub>119</sub> residue of MT1-MMP was replaced with alanine (MT1-MMP-C574A). The structure and relative positions of the mutations are schematically illustrated in Fig. 2.

MCF7 cells were transfected with mutant MT1-MMPs. According to flow cytometry (Fig. 3A) and efficiencies of the respective transfectant cells in MMP-2 activation (Fig. 4) and immunocapture (Figs. 1A and 3B), the levels of MT1-MMP-wt,
ARAA, -R89A, -R89A/ARAA, -E240A, and -C574A expression were highly similar. Further, these results were supported also by immunofluorescence and TIMP-2 binding studies (Fig. 5, A and B; Table I).

C574A Mutation Affects a Disulfide Bridge That Covalently Links MT1-MMP Monomers—The immunocapture analysis of MT1-MMP-C574A demonstrated the existence of the 60-kDa enzyme and its 39- and 42-kDa degradation forms in reduced samples. In contrast to MT1-MMP-wt, no 78–85-kDa or 120-kDa forms of MT1-MMP-C574A were observed in nonreduced samples. MT1-MMP Oligomerization, Proteolysis, and Processing

---

**Fig. 3. Expression and analysis of mutant MT1-MMPs.** A, flow cytometry analysis of MT1-MMP expression. Parental and mock-transfected (zeo) MCF7 cells as well as MCF7 cells expressing the wild type (wt) and mutant MT1-MMPs (E240A, C574A, and ARAA) were stained with control rabbit IgG (open histograms) and anti-hinge (shaded histograms). x axis, mean fluorescence intensity; y axis, cell number. Profiles are representative of several independent experiments. B, dimerization and proteolysis of mutant MT1-MMPs. MCF7 cells expressing MT1-MMP-wt and mutant E240A, C574A, R89A, R89A/ARAA, and ARAA constructs were lysed in 1% Triton X-114 and further analyzed by immunocapture as described in the legend to Fig. 1A. The apparent molecular weights of MT1-MMP forms are shown on the left. The positions of the molecular weight markers are on the right. Note the absence of C574A dimers and the presence of the 60-kDa protein and the major bands of the 39- and 42-kDa degradation products under nonreducing conditions.

-AARA, -R89A, -R89A/ARAA, -E240A, and -C574A expression were highly similar. Further, these results were supported also by immunofluorescence and TIMP-2 binding studies (Fig. 5, A and B; Table I).

**Fig. 4. Activation of pro-MMP-2 by the wild type and mutant MT1-MMPs.** A, MT1-MMP-E240A is incapable of MMP-2 activation. MCF7 cells transfected with the original pCNA3-zeo (zeo) plasmid (lanes 1 and 3) and MT1-MMP-wt, -E240A, -C574A, -ARAA, -R89A, and -R89A/ARAA (lanes 4–9, respectively) were incubated for 24 h in serum-free DMEM in the presence of 10 ng/ml purified pro-MMP-2 (lanes 3–9; lane 2, pro-MMP-2 alone, no cells). Aliquots of conditioned medium were analyzed by gelatin zymography. The molecular masses of the proenzyme, the intermediate, and the active enzyme of MMP-2 (68, 64, and 62 kDa, respectively) are shown on the right. B, the time course of pro-MMP-2 activation by cells expressing the wild type, ARAA, and C574A constructs. MCF7 cells stably expressing MT1-MMP-wt, -ARAA, and -C574A (upper, middle, and lower panels, respectively) were incubated for 0.5–8 h in serum-free DMEM in the presence of pro-MMP-2 (20 ng/ml). Aliquots of conditioned medium were analyzed by gelatin zymography. The molecular weights of the proenzyme, the intermediate, and the active enzyme of MMP-2 are shown on the right. C, gelatinolytic activity of MMP-2. Pro-MMP-2 (750 ng) was incubated for 1 h with 2.5 × 10⁵ MCF7 cells stably expressing MT1-MMP-wt, -ARAA, -C574A, and -E240A and mock cells (zeo) in 0.15 ml of serum-free DMEM. Further, cells were washed to remove unbound pro-MMP-2 and incubated with 75 pg of biotinylated gelatin in 0.5 ml of 0.1% BSA-DMEM for 4 h. Afterward, the gelatinolytic activity of MMP-2 was quantified as described earlier (33). Without pro-MMP-2, cells demonstrated low or no gelatinolytic activity. Data are mean ± S.E. of three independent experiments.
MT1-MMP Oligomerization, Proteolysis, and Processing

The subsequent analysis of the E240A protein showed the catalytically inactive MT1-MMP-E240A mutant. Immunocapture and proteolysis—Next, we analyzed molecular forms of the catalytically inactive MT1-MMP-E240A monomers to form this disulfide bridge.

The ability of MT1-MMP-C574A monomers to form this disulfide bridge.

60-kDa mature MT1-MMP as the major band in reduced samples (Fig. 3B, E240A). The existence of the dominant 60-kDa MT1-MMP-E240A protein excludes autocatalytic mechanisms of MT1-MMP activation. Since there is a complete absence of degraded forms (39–42-kDa reduced, 78–85-kDa non-reduced), we concluded that the E240A construct is incapable of self-proteolysis (Fig. 4A; lane 1).

Under nonreducing conditions, the E240A mutant showed relatively significant amounts of the 170- and 220-kDa MT1-MMP-specific bands (Fig. 3B, E240A). Similar high molecular weight forms were also observed in the nonreduced wild type plus Ilomastat samples from MCF7 cells (Fig. 1, A and B) and in the MT1-MMP samples from HT1080 fibrosarcoma cells (Fig. 1B).

Furin Cleavage Is Not Essential for Activation of MT1-MMP in Breast Carcinoma Cells—Recent controversial studies implicated furin, a serine protease of the trans-Golgi network, in the processing of the latent 63-kDa MT1-MMP proenzyme to the active enzyme by cleaving either the RRRKR11, the RRPR22, or both sequences in the propeptide domain (25–28, 36). To evaluate the effects of furin motif cleavage in the processing and dimerization of MT1-MMP, we constructed and analyzed MT1-MMP-R89A and MT1-MMP-ARAA mutants, each exhibiting a single respective modified furin motif, and the double MT1-MMP-R89A/ARAA mutant with no sites susceptible to furin cleavage. Immunocapture demonstrated that the pattern of MT1-MMP-ARAA, -R89A, and -R89A/ARAA on cell surfaces was highly similar to that of MT1-MMP-wt (Fig. 3B). Specifically, the mutants showed the 60-, 42-, and 39-kDa bands when reduced and the 78–85-kDa bands when unreduced.

Recent controversial studies implicated furin, a serine protease of the trans-Golgi network, in the processing of the latent 63-kDa MT1-MMP proenzyme to the active enzyme by cleaving either the RRRKR11, the RRPR22, or both sequences in the propeptide domain (25–28, 36). To evaluate the effects of furin motif cleavage in the processing and dimerization of MT1-MMP, we constructed and analyzed MT1-MMP-R89A and MT1-MMP-ARAA mutants, each exhibiting a single respective modified furin motif, and the double MT1-MMP-R89A/ARAA mutant with no sites susceptible to furin cleavage. Immunocapture demonstrated that the pattern of MT1-MMP-ARAA, -R89A, and -R89A/ARAA on cell surfaces was highly similar to that of MT1-MMP-wt (Fig. 3B). Specifically, the mutants showed the 60-, 42-, and 39-kDa bands when reduced and the 78–85-kDa bands when unreduced.

To additionally support our findings, we evaluated the effects of Ilomastat on MT1-MMP expressed in HT1080 cells. The samples of ILT1080 and MCF7 cells were run side-by-side to facilitate the comparison of MT1-MMP forms (Fig. 1B). Ilomastat induced the accumulation of the 63-kDa MT1-MMP proenzyme in MT1-MMP-wt cells (Fig. 1B, compare +DTT, MCF7-wt with MCF7-wt + Ilomastat). This correlated with the presence of higher levels of the 170- and 220-kDa species of MT1-MMP revealed under the nonreducing conditions (Fig. 1B, –DTT, MCF7-wt + Ilomastat). In contrast, Ilomastat failed to affect naturally expressed MT1-MMP in HT1080 (Fig. 1B). These

| Cell line | Cell-associated TIMP-2 levels (MFI) |
|-----------|-----------------------------------|
|           | No TIMP-2 added | TIMP-2 added |
| Mock      | Mouse IgG | Anti/TIMP-2 mAb |
| Wild type | 4.75      | 3.97        | 4.49       | 3.81          |
| ARAA      | 5.24      | 5.23        | 5.28       | 5.19          |
| C574A     | 4.10      | 5.56        | 5.26       | 60.01         |
| E240A     | 5.13      | 4.40        | 5.16       | 5.95          |

TABLE I
TIMP-2 binding by mutant MT1-MMP

Mock, MTI-MMP-wt, MTI-MMP-E240A, and MT1-MMP-ARAA caused the 60-kDa mature MT1-MMP as the major band in reduced samples (Fig. 3B, E240A). The existence of the dominant 60-kDa MT1-MMP-E240A protein excludes autocatalytic mechanisms of MT1-MMP activation.

Under nonreducing conditions, the E240A mutant showed relatively significant amounts of the 170- and 220-kDa MT1-MMP-specific bands (Fig. 3B, E240A). Similar high molecular weight forms were also observed in the nonreduced wild type plus Ilomastat samples from MCF7 cells (Fig. 1, A and B) and in the MT1-MMP samples from HT1080 fibrosarcoma cells (Fig. 1B).

Catalytically Inactive MT1-MMP-E240A Is Incapable of Self-proteolysis—Next, we analyzed molecular forms of the catalytically inactive MT1-MMP-E240A mutant. Immunocapture and the subsequent analysis of the E240A protein showed the 60-kDa mature MT1-MMP as the major band in reduced samples (Fig. 3B, E240A). The existence of the dominant 60-kDa MT1-MMP-E240A protein excludes autocatalytic mechanisms of MT1-MMP activation. Since there is a complete absence of degraded forms (39–42-kDa reduced, 78–85-kDa non-reduced), we concluded that the E240A construct is incapable of self-proteolysis (Fig. 4A; lane 1).

To additionally support our findings, we evaluated the effects of Ilomastat on MT1-MMP expressed in HT1080 cells. The samples of ILT1080 and MCF7 cells were run side-by-side to facilitate the comparison of MT1-MMP forms (Fig. 1B). Ilomastat induced the accumulation of the 63-kDa MT1-MMP proenzyme in MT1-MMP-wt cells (Fig. 1B, compare +DTT, MCF7-wt with MCF7-wt + Ilomastat). This correlated with the presence of higher levels of the 170- and 220-kDa species of MT1-MMP revealed under the nonreducing conditions (Fig. 1B, –DTT, MCF7-wt + Ilomastat). In contrast, Ilomastat failed to affect naturally expressed MT1-MMP in HT1080 (Fig. 1B). These
results are not surprising, since activation and processing of intrinsic MT1-MMP in HT1080 cells were specifically shown to involve furin (37). However, this does not rule out an existence of the furin-independent pathway(s) of MT1-MMP processing in other cell types (27, 28) including breast carcinomas. The furin-independent mechanisms involved in MT1-MMP activation in cancer cells are yet to be elucidated.

**MT1-MMP-E240A Is Incapable of MMP-2 Activation and TIMP-2 Binding—**Further, we assessed whether MT1-MMP mutants were capable of pro-MMP-2 activation. Since MCF7 cells are deficient in MMP-2 (Fig. 4A, lane 1), purified pro-MMP-2 (Fig. 4A, lane 2) was added to the cultures. After incubation for 24 h, aliquots of medium were analyzed by gelatin zymography to follow the conversion of the 68-kDa proenzyme of MMP-2 into the 64-kDa intermediate and the 62-kDa mature enzyme. As expected, mock-transfected cells and MT1-MMP-E240A cells both failed to process pro-MMP-2 (Fig. 4A, lanes 3 and 5, respectively). The other mutants, including MT1-MMP-C574A, -ARAA, -R89A, and -R89A/ARAA (Fig. 4A, lanes 6–9, respectively), as well as MT1-MMP-wt (Fig. 4A, lane 4) were able to convert the 68-kDa proenzyme into the mature 62-kDa enzyme via the 64-kDa activation intermediate. Transiently mock- and MT1-MMP-wt-transfected cells showed similar results in zymography studies as compared with the respective stably transfected cells (data not shown).

To evaluate the mutants in more detail, we assessed the time course of pro-MMP-2 activation by cells expressing the wild type, ARAA, and C574A constructs. Aliquots of medium were withdrawn in 0.5–8 h and analyzed by gelatin zymography. Fig. 4B shows that the wild type, ARAA, and C574A constructs (upper, middle, and bottom panels, respectively) were similarly efficient in activating pro-MMP-2.

To quantitatively confirm that activation of pro-MMP-2 by cells expressing mutant MT1-MMP results in gelatinolytic activity, we employed activity assay using biotinylated gelatin as a substrate (34). Since MCF7 cells do not produce any detectable gelatinolytic activity in serum-free conditions (Fig. 4A), purified pro-MMP-2 (Fig. 4A, lane 2) was added to the cultures. After incubation for 24 h, aliquots of medium were analyzed by gelatin zymography to follow the conversion of the 68-kDa proenzyme of MMP-2 into the 64-kDa intermediate and the 62-kDa mature enzyme. As expected, mock-transfected cells and MT1-MMP-E240A cells both failed to process pro-MMP-2 (Fig. 4A, lanes 3 and 5, respectively). The other mutants, including MT1-MMP-C574A, -ARAA, -R89A, and -R89A/ARAA (Fig. 4A, lanes 6–9, respectively), as well as MT1-MMP-wt (Fig. 4A, lane 4) were able to convert the 68-kDa proenzyme into the mature 62-kDa enzyme via the 64-kDa activation intermediate. Transiently mock- and MT1-MMP-wt-transfected cells showed similar results in zymography studies as compared with the respective stably transfected cells (data not shown).

To evaluate the mutants in more detail, we assessed the time course of pro-MMP-2 activation by cells expressing the wild type, ARAA, and C574A constructs. Aliquots of medium were withdrawn in 0.5–8 h and analyzed by gelatin zymography. Fig. 4B shows that the wild type, ARAA, and C574A constructs (upper, middle, and bottom panels, respectively) were similarly efficient in activating pro-MMP-2.

To quantitatively confirm that activation of pro-MMP-2 by cells expressing mutant MT1-MMP results in gelatinolytic activity, we employed activity assay using biotinylated gelatin as a substrate (34). Since MCF7 cells do not produce any detectable gelatinolytic activity in serum-free conditions (Fig. 4A), cells were supplemented with exogenous pro-MMP-2. For these purposes, cells were incubated with excess pro-MMP-2 to fully saturate the available MT1-MMP-TIMP-2 surface receptors. Next, cells were washed to remove unbound soluble pro-MMP-2 and any traces of the MMP-2 enzyme and free TIMP-2 that might have preexisted in the proenzyme samples. This significantly reduced the background activity and allowed us to follow the activation of pro-MMP-2 associated with the MT1-MMP-TIMP-2 surface receptors. Biotin-labeled gelatin was added to cells to examine the gelatinolytic activity of MMP-2 converted into the active enzyme by the MT1-MMP-TIMP-2 complexes. The gelatinolytic activity of MMP-2 generated by the cells expressing MT1-MMP-wt, -ARAA, and -C574A (Fig. 4C) correlated well with the results of zymography (Fig. 4B). Thus, the C574A mutant was almost as efficient in generating MMP-2's gelatinolytic activity as the ARAA mutant. As expected, MT1-MMP-E240A failed to demonstrate any gelatinolytic activity (Fig. 4C).

To evaluate whether MT1-MMP mutants were capable of TIMP-2 binding, transiently transfected MCF7 cells were pretreated with excess TIMP-2 followed by staining with anti-TIMP-2 mAb T2–101 and flow cytometry. Without TIMP-2 pretreatment, none of the cells were capable of binding anti-TIMP-2 mAb (Table I). In turn, if wild type, ARAA, and C574A cells were pretreated with TIMP-2, the levels of cell-associated TIMP-2 significantly increased relative to those of mock-transfected cells (Table I). These findings agreed with the results of gelatin zymography (Fig. 4, A and B), activity measurements (Fig. 4C), and immunocapture studies (Fig. 3B), confirming that there were no significant differences in the levels of active MT1-MMP expressed on the surface of wild type, ARAA, and C574A cells. Evidently, ARAA and C574A mutations did not affect TIMP-2 binding. In contrast, the E240A mutation in the enzyme's active site abolished the ability of MT1-MMP to bind TIMP-2. It is clear from the crystal structure of the MT1-MMP-TIMP-2 complex (38) and TIMP-1 binding studies with the Glu mutant of ministromelysin-1 (39) that the interaction of TIMPs with active MMPs does not rely on the Glu in the active site. Accordingly, our data suggest that the E240A mutation abolished TIMP-2 binding by significantly perturbing the overall structure of the enzyme's active site. The immunocapture of a 60-kDa form of the E240A mutant (Fig. 3B) suggests that the mutation did not affect the N-terminal processing of MT1-MMP. The recent data of Valtanen et al. (40), who have experimentally documented the proper processing of MT1-MMP-E240A mutant, support our suggestion.

**Cell Surface Localization of Mutant MT1-MMP—**To analyze the localization of MT1-MMP, cells expressing wild type, ARAA, C574A, and E240A constructs were plated on fibronectin-coated glass slides, fixed, and subjected to immunofluorescence staining with rabbit anti-hinge antibodies followed by fluorescence and confocal microscopy. We specifically employed permeabilized cells in these experiments to identify if there was any difference in both the intracellular and plasma membrane pools of mutant MT1-MMPs as compared with MT1-MMP-wt. A comparison of the phase contrast and fluorescence images indicated that endogenous expression of MT1-MMP in mock-transfected cells was not sufficient to generate any detectable specific fluorescence (Fig. 5A, see: upper right panel). Staining of any tested cells with control rabbit IgG was also negative (data not shown). In cells transfected with MT1-MMP-wt and MT1-MMP-C574A, the protein products were mainly localized to the cell surface. Cell localization and distribution across the plasma membrane of MT1-MMP-C574A was similar to that of the wild type enzyme (Fig. 5A). Cells expressing MT1-MMP-ARAA and -E240A exhibited a pattern of MT1-MMP staining similar to that of the wild type or C574A constructs (data not shown). ZX sections of stained cells (Fig. 5B) confirmed the cell surface localization of MT1-MMP in cells expressing the wild type enzyme and the C574A mutant.

However, there was a significant difference in the morphology of cells expressing MT1-MMP-C574A relative to cells expressing the wild type MT1-MMP. Under routine cell culture conditions, MT1-MMP-wt cells plated on plastic were well spread and demonstrated cell protrusions and ruffling, i.e. displaying a motile phenotype. C574A cells remained more round and appeared as cell clusters with smooth edges and almost no ruffling or spreading (Fig. 5C), thereby suggesting a lower migratory potential and indicating alterations in the cytoskeleton.

**MT1-MMP-C574A Does Not Support Cell Adhesion, Migration, and Invasion—**To analyze the effects of mutant MT1-MMPs on cell locomotion, we evaluated cells expressing the wild type construct and the mutants in a series of adhesion, migration, and invasion assays (Fig. 6). Expression of the wild type enzyme or MT1-MMP-E240A did not affect adhesive characteristics of cells. In contrast, expression of the C574A mutant significantly reduced the adhesive efficiency of cells onto type I collagen (Fig. 6A). Similar results were obtained when fibronectin and vitronectin were used as the substrates for cell attachment (data not shown).

Further, we evaluated the migratory efficiency of cells expressing wild type, E240A, and C574A constructs on collagen-coated surfaces. The expression of the wild type enzyme increased collagen-mediated migration of cells at least 2.5-fold

**MT1-MMP-Oligomerization, Proteolysis, and Processing**
compared with that of mock-transfected cells (Fig. 6B). The C574A and the catalytically inactive E240A mutants failed to facilitate cell migration.

To analyze the effects of mutant MT1-MMPs on the ability of cells to invade through basement membranes, we employed the Transwell cell invasion assay. Relative to mock-transfected cells, cell invasion through Matrigel was strongly enhanced by the expression of either MT1-MMP-wt or MT1-MMP-ARAA (Fig. 6C). Significant inhibition of cell invasion by Ilomastat additionally supported a direct role of MT1-MMP in cell locomotion. In contrast, both the C574A and catalytically inactive E240A mutants did not stimulate cell invasion. Since the C574A mutation affected the adhesive efficiencies of the transfected cells, low migration and invasion of the C574A mutant were not surprising. These findings indicate a significant functional role of the cytosolic portion of MT1-MMP in stimulating cell motility.

DISCUSSION

Given the central role of MT1-MMP in diverse aspects of malignancy (41–44), the localization of this enzyme to specific cell surface sites such as the invasive front and invadopodia (13, 23, 45–48) can efficiently regulate matrix proteolysis in the vicinity of cell surfaces. MT1-MMP has transmembrane and cytoplasmic domains, which target the enzyme to invasive front (3, 13, 45, 47, 49). In addition to its ability to directly degrade the extracellular matrix (3), MT1-MMP initiates activation of MMP-2 and MMP-13 (8, 50). These activation mechanisms are not understood in detail (9, 21, 51, 52). The in trans mechanisms of pro-MMP-2 activation implicate at least two molecules of MT1-MMP, a “receptor” molecule in a complex with TIMP-2 and an “activator” TIMP-2-free molecule. Accordingly, these two molecules of MT1-MMP should be co-localized in immediate proximity on the plasma membrane in order to bring together the binding and the activation of pro-MMP-2 (12, 17, 19, 20). Dimerization of MT1-MMP could accomplish this co-localization. However, direct evidence for dimerization of MT1-MMP has been missing.

To better understand functions of MT1-MMP, we designed MT1-MMP proteins with mutations in the active site, the two furin cleavage motifs and the cytoplasmic tail (MT1-MMP-E240A, -R89A, -ARAA, -R89A/ARAA, and -C574A, respectively). The wild type and mutant MT1-MMPs were expressed in MCF7 cells that are deficient in both MMP-2 and MT1-MMP. The absence of any MMP-2 and MT1-MMP activities in the parental cells facilitated the analysis of MT1-MMP in the transfected cells.

Our observations suggest that MT1-MMP is capable of oligomerization on cell surfaces. Homodimerization was most evident for the enzyme’s autolytic ectodomain forms. These inactive forms of MT1-MMP, 39 kDa (presumably, starting from Gly285) and 42 kDa (presumably, starting from Ile256) both lacking the zinc-binding catalytic site domain were identified and characterized in previous reports of other groups (14, 49). While our manuscript was in preparation, dimerization was demonstrated for MT1-MMP naturally expressed by platelets (7.5 × 10^5 cells/insert) with the undersurface membranes coated with 20 μg/ml collagen type I. After 48 h, cells that migrated onto the membrane undersurface were detached and counted. C, mock-transfected cells (zeo) and cells expressing MT1-MMP-wt, -E240A, and -C574A were plated into the Transwells with the undersurface membranes coated with 20 μg/ml collagen type I. After 48 h, cells that migrated onto the membrane undersurface were detached and counted. C, mock-transfected cells (zeo) and cells expressing MT1-MMP-wt, -E240A, and -C574A were plated into the Transwells with the undersurface membranes coated with 20 μg/ml collagen type I. After 48 h, cells that migrated onto the membrane undersurface were detached and counted. C, mock-transfected cells (zeo) and cells expressing MT1-MMP-wt, -E240A, and -C574A were plated into the Transwells with the undersurface membranes coated with 20 μg/ml collagen type I. After 48 h, cells that migrated onto the membrane undersurface were detached and counted. C, mock-transfected cells (zeo) and cells expressing MT1-MMP-wt, -E240A, and -C574A were plated into the Transwells with the undersurface membranes coated with 20 μg/ml collagen type I. After 48 h, cells that migrated onto the membrane undersurface were detached and counted.

FIG. 6. Effects of the wild type and mutant MT1-MMPs on cell adhesion (A), migration (B), and invasion (C). Data are presented as a percentage relative to invasion of mock-transfected cells (100%) and are mean ± S.E. from three experiments performed in triplicate. A, mock-transfected cells (zeo) and cells expressing MT1-MMP-wt, -E240A, and -C574A (5 × 10^4 cells/well) were allowed to adhere for 1 h to the wells of a 96-well plate coated with 1 μg/ml collagen type I. Adherent cells were fixed and stained with Crystal Violet in 10% ethanol. The incorporated dye was extracted, and the absorbance was measured at 540 nm. B, mock-transfected cells (zeo) and cells expressing MT1-MMP-wt, -E240A, and -C574A were plated into the Transwells with the undersurface membranes coated with 20 μg/ml collagen type I. After 48 h, cells that migrated onto the membrane undersurface were detached and counted.
Since the C574A mutant was quite proficient in MMP-2 activation, we suggest that this mutation and, accordingly, the absence of a covalent link between monomers, does not completely abolish dimerization of the enzyme. The existence of self-proteolyzed forms as well as efficacy of the mutant in pro-MMP-2 activation indirectly supports the presence of non-S-S dimers on the surface of cells expressing the MT1-MMP-C574A construct. Association of the homodimer is likely to be initiated by the motif involving the PRXXLYC\textsuperscript{574}RXXXXXV sequence of the cytoplasmic tail. This motif is fully conserved in MT1-, MT2-, and MT3-MMPs while MT5-MMP lacks several essential residues of this motif (54). MT4- and MT6-MMPs are entirely missing the motif (55–57). It cannot be excluded that protein-disulfide isomerase activity (58) is involved in the mechanisms that facilitate a disulfide bridge formation and stabilization of MT1-MMP dimers.

Further, there is evidence of the extensive self-proteolysis of MT1-MMP-wt, -R89A, -ARA, -R89A/ARA, and -C574A in our cell system that is devoid of MMP-2 (22). The catalytically inactive MT1-MMP-E240A protein was incapable of self-proteolysis. In agreement, a hydroxamate inhibitor, Iломastat, blocked autolytic cleavage of MT1-MMP. Autolysis of MT1-MMP that occurs under deficiency of TIMP-2 (14) is likely to be a mechanism for negative regulation of MT1-MMP. Our observations suggest that the soluble activity of MMP-2 is not a prerequisite for the degradation of MT1-MMP on cell surfaces (11, 13, 49, 59).

Recent studies suggested that furin might be a physiologically relevant activator of MT1-MMP (25–28). However, evidence is emerging that there could be alternative pathways of MT1-MMP activation (27, 28). Our data confirmed the hypothesis that furin cleavage of both putative RRXXR motifs of MT1-MMP is not necessary for the processing of MT1-MMP and the subsequent activation of pro-MMP-2 in breast carcinoma cells. In our experiments, MT1-MMP-ARA, -R89A, -R89A/ARA, and -wt displayed similar, if not identical pattern in immunocapture and MMP-2 activation studies. Resistance of the double MT1-MMP-R89A/ARA mutant to furin cleavage did not cause any accumulation of the respective proenzyme in MCF7 cells. However, MCF7 cells accumulated the MT1-MMP proenzyme in the presence of Iломastat. A putative matrixin-like proteinase involved in activation of MT1-MMP remains to be identified. These findings extend the physiological implications of the recent report that furin-independent pathway of MT1-MMP activation exists in rabbit dermal fibroblasts (27). In addition, we expressed MT1-MMP in furin-deficient LoVo lung carcinoma cells. Our studies correlate well with the observations of Yana and Weiss (36) and indicated that LoVo cells were capable of MT1-MMP activation (data not shown). These data support the existence of furin-independent cellular pathways involved in the processing of the full-length membrane-anchored MT1-MMP proenzyme.

MT1-MMP-wt, -ARA, and -C574A were efficient in TIMP-2 binding and, with the exception of MT1-MMP-C574A, facilitated migration and invasion of the respective cells through basement membrane-like matrices. The catalytically inactive E240A construct failed to promote cell locomotion. In agreement, Iломastat inhibited invasion of cells expressing MT1-MMP-wt. Thus, our results indicate that MT1-MMP is directly involved in cell invasion and migration and support our earlier report that, in functional cooperation with integrin \( \alpha_\beta_3 \), MT1-MMP facilitated migration of MCF7 cells devoid of MMP-2 (9, 22). In addition, our studies extend the recent observations that MT1-, MT2-, and MT3-MMP confer invasion-incompetent Madin-Darby canine kidney cells with the ability to penetrate collagen type I matrices (43). Hence, the previously underestimated function of MT1-MMP to support cell locomotion appears to be a general phenomenon (44, 60).

Intriguingly, the proteolytically active mutant MT1-MMP-C574A failed to stimulate migration and invasion of transfected cells. In contrast to all other MT1-MMP constructs, the expression of C574A also negatively affected the adhesive ability of the respective cells. Poor adhesion of C574A cells may result in their inefficient migration and invasion. Immunofluorescence, flow cytometry, TIMP-2 binding, and MMP-2 activation studies demonstrated that the expression levels of this mutant were similar to those of MT1-MMP-wt. However, MT1-MMP-C574A cells were unable to efficiently accomplish adhesion and locomotion. Similarly, a chimeric MT1-MMP protein containing the interleukin-2 receptor \( \alpha \) chain transmembrane and cytoplasmic domains failed to localize to invadopodia and to facilitate invasion of melanoma cells (45). Recent reports of Lehti et al. (13), who reported that a truncation of 10 amino acids that included the Cys\textsuperscript{574} decreased the invasion activity of melanoma cells by 30%, have pointed out that the middle portion of the cytoplasmic tail had an important role in cell invasion. In contrast, Hotary et al. (43) observed that truncation of the MT1-MMP cytoplasmic domain did not affect the invasive phenotype of Madin-Darby canine kidney cells stimulated with hepatocyte growth factor. However, the assays of Hotary et al. (43) were not strictly quantitative. Alternatively, Urena et al. (15) and Nakahara et al. (45) demonstrated that the cytoplasmic tail is critically involved in trafficking of MT1-MMP to discrete regions of the cell surface. In addition, our most recent finding clearly indicates that expression of either the C574A construct or the MT1-MMP mutant missing the entire cytoplasmic tail does not affect the locomotion of extremely migratory U-251 glioma cells (data not shown). Thus, although our results are not identical to what has been observed previously, we used a significantly different cell system that could account for the apparent disparity in findings.

Apparently, there are two distinct mechanisms that affect cell locomotion and involve MT1-MMP: the first where the proteolytic activity of MT1-MMP facilitates cell motility and the second where the cytoplasmic tail of the enzyme communicates with the putative intracellular components. Thus, the expression of the C574A mutant is likely to modify specifically the interactions of the MT1-MMP's cytoplasmic tail with the intracellular milieu, thereby affecting cell morphology, adhesion, and migration.

Hypothetically, translocations across the cell surfaces in migrating versus stationary cells indicate the direct critical interactions of MT1-MMP with the intracellular milieu (13, 23, 48, 61, 62). Since the putative cytoplasmic components that associate with cell surface MT1-MMP would not be biotinylated, they are not seen in our immunocapture experiments. Our most recent results indicate that the peptide derived from the cytoplasmic tail of MT1-MMP is capable of binding specifically with the p32\textsuperscript{gC\textsubscript{1q}R} multifunctional protein (63). This protein may be a compartment-specific partner of MT1-MMP. The p32 is likely to be involved in directional trafficking of MT1-MMP from the Golgi network to the plasma membrane. Further studies are needed to confirm direct interactions of MT1-MMP with the intracellular milieu via the enzyme's cytoplasmic domain.

In summary, we would like to emphasize that the existence...
of dimers and possibly, higher oligomers of MT1-MMP on cell surfaces correlates well with the mechanisms of pro-MMP-2 activation. Further, our data point to an important function of the cystolic portion of the MT1-MMP molecule in modulating cell adhesion and locomotion. There is growing evidence that MT1-MMP is a key enzyme involved in cancer cell invasion. Mutant MT1-MMPs characterized in this report may find further applications in structure-function analyses of MT-MMPs and other cancer-related studies.

REFERENCES

1. Massova, I., Kotra, L. P., Fridman, R., and Mobashery, S. (1998) FASEB J. 12, 1377–1383.
2. Nagase, H., and Woessner, J. F., Jr. (1999) J. Biol. Chem. 274, 21491–21494.
3. Seki, M. (1999) APMIS 107, 137–143.
4. Poel, J. M. and Birembaut, P. (1998) Int. J. Biochem. Cell. Biol. 30, 1195–1202.
5. Ohuchi, E., Imai, K., Fujiy, Sato, H., Seki, M., and Okada, Y. (1997) J. Biol. Chem. 272, 2446–2451.
6. d’Ortho, M. P., Will, H., Atkinson, S., Butler, G., Messent, A., Gavrilovic, J., Chen, W. T., Pei, D., and Weiss, S. J. (1996) J. Biol. Chem. 271, 614–621.
7. d’Ortho, M. P., Will, H., Atkinson, S., Butler, G., Messent, A., Gavrilovic, J., Chen, W. T., Pei, D., and Weiss, S. J. (1996) J. Biol. Chem. 271, 614–621.
8. Strongin, A. Y., Collier, I., Bannikov, G., Marmer, B. L., Grant, G. A., and Goldberg, G. I. (1995) J. Biol. Chem. 270, 5331–5338.
9. Deryugina, E. I., Ratnikov, B., Monosov, E., Postnova, T. I., DiScipio, R., Deryugina, E. I., Bourdon, M. A., Jungwirth, K., Smith, J. W., and Strongin, A. (1998) Cancer Res. 58, 124, 871–880.
10. Urena, J. M., Merlos-Suarez, A., Baselga, J., and Arribas, J. (1999) J. Biol. Chem. 274, 11635–11641.
11. Lehti, K., Vatanen, H., Wickett, S., Lehi, J., and Keski-Oja, J. (2000) J. Biol. Chem. 275, 15006–15013.
12. Hernandez-Barrancos, S., Toth, M., Brown, M. D., Yurkova, M., Gervasi, D. C., Raz, Y., Sang, Q. A., and Fridman, R. (2000) J. Biol. Chem. 275, 12080–12089.
13. Urena, J. M., Merlos-Suarez, A., Basegma, J., and Arribas, J. (1999) J. Cell Sci. 112, 555–563.
14. Maquoi, E., Frankenne, F., Baramova, E., Munaut, C., Sounni, N. E., Remacle, A., Noel, A., Murphy, G., and Foidart, J. M. (1999) J. Biol. Chem. 274, 339, 3270–3276.
15. Jo, Y., Yeon, J., Kim, H. J., and Lee, S. T. (2000) J. Biol. Chem. 275, 451–459.
16. Kirschke, P., Ziegler, M., Nisch, R., Breitkopf, K., Steurer, P., Klein, C. E., Nagase, H., and Woessner, J. F., Jr. (1999) J. Biol. Chem. 274, 35851–35858.
Mutation Analysis of Membrane Type-1 Matrix Metalloproteinase (MT1-MMP): THE ROLE OF THE CYTOPLASMIC TAIL CYS574, THE ACTIVE SITE GLU240, AND FURIN CLEAVAGE MOTIFS IN OLIGOMERIZATION, PROCESSING, AND SELF-PROTEOLYSIS OF MT1-MMP EXPRESSED IN BREAST CARCINOMA CELLS

Dmitry V. Rozanov, Elena I. Deryugina, Boris I. Ratnikov, Edward Z. Monosov, George N. Marchenko, James P. Quigley and Alex Y. Strongin

J. Biol. Chem. 2001, 276:25705-25714.
doi: 10.1074/jbc.M007921200 originally published online May 2, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M007921200

Alerts:

- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 63 references, 41 of which can be accessed free at http://www.jbc.org/content/276/28/25705.full.html#ref-list-1