The Hemopexin Domain of Membrane-type Matrix Metalloproteinase-1 (MT1-MMP) Is Not Required for Its Activation of proMMP2 on Cell Surface but Is Essential for MT1-MMP-mediated Invasion in Three-dimensional Type I Collagen*

Received for publication, August 9, 2004, and in revised form, September 16, 2004
Published, JBC Papers in Press, September 20, 2004, DOI 10.1074/jbc.M409074200

Ping Wang, Jing Nie, and Duanqing Pei‡
From the Department of Pharmacology, University of Minnesota School of Medicine, Minneapolis, Minnesota 55455

Membrane-type matrix metalloproteinase-1 (MT1-MMP) plays a key role in tumor invasion and metastasis by degrading the extracellular matrix and activating proMMP2. Here we show that the conserved hemopexin domain is required for MT1-MMP-mediated invasion and growth in three-dimensional type I collagen matrix but not proMMP2 activation. Deletion of the hemopexin domains in MT1-, MT2-, MT3-, MT5-, and MT6-MMP does not impair their abilities to activate proMMP2. In fact, hemopexin-less MT5- and MT6-MMP activate proMMP2 better than their wild type counterparts. On the other hand, hemopexin-less MT1-MMP fails to promote cell invasion into type I collagen but retains the capacity to enhance the growth of Madin-Darby canine kidney cells as cysts in three-dimensional collagen matrix. Moreover, the hemopexin domain is also required for MT1-MMP-mediated invasion/scattering of MCF-7 cells in three-dimensional collagen matrix. Because growth and invasion in a three-dimensional model may correlate with tumor invasiveness in vivo, our data suggest that the hemopexin domains of MT-MMPs should be targeted for the development of anti-cancer therapies by employing screening assays developed for three-dimensional models rather than their enzymatic activity toward proMMP2.

Malignant cancer cells must degrade the surrounding extracellular matrix (ECM) including type I collagen and the basement membrane to invade and metastasize to other tissues (1,2). Matrix metalloproteinases (MMPs), a zinc-dependent proteinase family with more than 25 members, play a key role in various forms of ECM destruction (3). MMPs can be classified into two major groups according to their subcellular localization: membrane-type MMPs (MT-MMPs) and secreted ones (3).

MT-MMPs, especially MT1-MMP, have attracted considerable attention because they possess an enhanced ability to degrade various pericellular ECMs under most physiological and pathological conditions (4). MT-MMPs are also important activators for several secreted MMPs such as proMMP2 (5) and proMMP13 (6). Six MT-MMPs have been identified so far including MT1-, MT2-, MT3-, and MT5-MMP, which are anchored on the cell membrane via a type I integral transmembrane domain followed by a short cytosolic tail, and MT4- and MT6-MMP, which are anchored on the cell membrane by glycosylphosphatidylinositol (GPI) anchors (4). Otherwise, MT-MMPs have similar extracellular structures, including a signal peptide, a prodomain, a catalytic domain, a hinge region, and a hemopexin domain (4). Despite a relatively high degree of similarity, their activity toward different substrates, including proMMP2 and type I collagen, is quite different (3). The structural basis for this difference remains unknown.

Activation of proMMP2, which is implicated in tumor invasion and metastasis, is the most significant function of MT-MMPs. MT1-, MT2-, MT3-, MT5-, and MT6-MMPs have been reported to be able to activate proMMP2 in cell-based assays (5,7–10). Intense efforts have been made to study the mechanism of MT1-MMP-mediated proMMP2 activation over the past decade. It is now clear that MT1-MMP can form a triple complex with proMMP2 through TIMP2. The complex then functions as a receptor for soluble proMMP2 and another TIMP2-free MT1-MMP then cleaves the proMMP2 (4, 11, 12). Different domains of MT1-MMP have been demonstrated to play distinct roles in proMMP2 activation (13). Our previous data showed that the cytoplasmic tail of MT1-MMP may serve as a negative regulator for proMMP2 activation by mediating its internalization from cell surface (14). Recently, it has been reported that homophilic complex formation is necessary for MT1-MMP-mediated surface activation of proMMP2 (15) and that either a hemopexin domain or cytoplasmic tail is required for complex formation (15–17). Although the domain-swapping approach has been regarded as a useful method to study the structure-function relationship of MT1-MMP, some of those results appear to be inconsistent and subject to different interpretations. For example, the substitution of the MT1-MMP hemopexin domain with that of MT4-MMP greatly impairs its ability to activate proMMP2 (15), whereas the same domain of MT3 is a successful substitute (13). Therefore, further studies, perhaps employing different strategies, should be performed to clarify this inconsistency.

Type I collagen, the most abundant ECM protein in human, is a critical component of many tissues including blood vessels, bone, and skin (18). Several MMPs including MMP1, MMP8, MMP13, and MT1-MMP possess robust collagenolytic activity...
in vitro (19, 20). Mice deficient in MT1-MMP exhibit severe aberrations in type I collagen abundant tissues such as bone and skin, demonstrating an essential role for MT1-MMP in collagen metabolism in vivo (21, 22). Reports from several groups including our own have recently shown that MT-MMPs, but not secreted MMPs, can overcome the suppressive effects exerted by type I collagen lattice to promote cell growth and proliferation in three-dimensional type I collagen lattice when expressed in various cells including tumor cells (8, 23, 24). Therefore, a better understanding of MT1-MMP-mediated collagenolysis may generate novel strategies targeting various forms of MT1-MMP activities in a specific manner.

In this report, we provide direct evidence to show that the hemopexin domain of MT1-MMP is not required for its activity toward proMMP2, yet is essential for MT1-MMP mediated invasion in three-dimensional type I collagen. These results suggest that the hemopexin domain of MT1-MMP should be targeted for the development of therapies against tumor invasion and metastasis.

MATERIALS AND METHODS

Reagents—General chemicals were purchased from Sigma or Fisher (Pittsburgh, PA). Rabbit anti-MT1-, MT5-, and MT6-MMP antibodies were raised against the purified catalytic domain and affinity-purified as described previously (14, 25, 26). Mouse anti-FLAG M2 monoclonal antibody and goat anti-rabbit or mouse secondary antibodies were purchased from Sigma. MT3-MMP anti-catalytic domain antibody and GM6001 (a broad hydroxamate inhibitor of MMP) were from Chemicon (Temecula, CA). Fluorescein isothiocyanate-conjugated secondary antibody was from Molecular Probes (Eugene, OR). Purified proMMP2 and TIMP2 were obtained from R&D Systems.

Plasmids—cDNAs encoding full-length MT1-, MT3-, MT5-, MT6-MMP, and MT1αC were cloned into the expression vector pCR3.1-1Uni as described previously (7, 8, 14, 20). Full-length MT2-MMP cDNA (342–532) and a fragment MT3Pex (318–535) were inserted into the expression vector pCR3.1-1Uni by PCR. A FLAG tag was inserted exactly after the furin site in MT2-MMP using PCR based methods. Hemagglutinin-tagged MT2-MMP was kindly provided by Dr. Stephen J. Weiss (23). The deletion mutants MT1Pex-(318–535), MT2Pex-(336–535), MT3Pex-(342–532), MT5Pex-(347–538), and MT6Pex-(347–538) and point mutation E240A of MT1-MMP were generated by PCR using Pfu polymerase. All of the mutants were verified by DNA sequencing.

Cell Culture and Transfection—HEK 293 cells, COS-7, MCF-7 cells, MDCK cells, and TIMP2−/− cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal bovine serum (FBS). PC3 cells were cultured in RPMI 1640 medium with 10% FBS. All of the cells were transfected using the calcium phosphate/DNA co-precipitation method as described previously (27) or FuGENE 6. For all of the transfection experiments, pcDNA3 or pCR3.1-1Uni vector was used to compensate for total DNA input.

Generation of Stable Cell Lines—Various plasmids were transfected into MDCK or MCF-7 cells using FuGENE 6, and stable clones were selected in the presence of 1 mg/ml G418 as described previously (13). The stable clones were screened for proMMP2 activation by a zymography assay and with Western blot analysis using anti-MT1 catalytic domain antibody. At least two of the representative clones were selected for further analysis.

Zymography and Western Blotting—Zymography was performed as described previously (13). Cells were cultured in a 12-well plate and transfected as indicated. After 24 h, cells were washed three times with PBS and the media were changed to a 5% fetal bovine serum medium. Cells were cultured with 25 ng/ml purified proMMP2. After 48 h of incubation, the media were harvested and cleared by centrifugation at 12,000 rpm for 10 min and subjected to analysis by SDS-PAGE impregnated with 1 mg/ml gelatin as described previously (20). The gels were incubated at 37 °C overnight, stained with Coomassie Blue, destained, and then scanned. For Western blotting, the cells cultured in media with or without 5 μM of GM6001 were lysed in lysis buffer (50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 10 mM EDTA, 1% Triton X-114, and a mixture of protease inhibitors), cleared by centrifugation, and analyzed with a specific antibody as detailed in the figure legends.

Immunostaining and Confocal Microscopy—MDCK cells were grown on glass coverslips and transfected with the indicated plasmids. After being cultured with 5 μM of GM6001 for 24 h, cells were fixed with 4% polyformaldehyde for 20 min and incubated with PBS containing 0.1% Triton X-100 for 5 min. After blocking with bovine serum albumin/PBS, 0.2 μg/ml anti-MT1-MMP catalytic domain antibody was added to the cells and then the cells were incubated at 4 °C overnight. Fluorescein isothiocyanate-labeled secondary antibody was then used to detect the primary antibody. After three washes with PBS, the coverslips were mounted with NO-FADE (10% glycerol in PBS, 0.1% p-phenylenediamine, pH 8.0). Confocal images were collected from a Bio-Rad MRC 1024 system attached to an Olympus microscope (Melville, NY) with a ×80 oil objective at the Biomedical Image Processing Laboratories at the University of Minnesota.

Growth of MDCK and MCF-7 Cells in Three-dimensional Collagen Lattice—MDCK or MCF-7 stable transfectants (1×10^5) were mixed with 500 μl of type I collagen (2.5 mg/ml, Collaborative Research, Bedford, MA) and allowed to gel at 37 °C in 24-well plates to give rise to three-dimensional collagen lattice. Fresh medium containing 10% fetal bovine serum and with or without 5 μM GM6001 was added to the cells and changed every 2 days. After 7 days, cells were photographed with a video camera at the University of Minnesota Biomedical Image Processing Laboratories and then quantified and analyzed as described previously (8).

RESULTS

The Hemopexin-less MT1-MMP Activates proMMP2 Efficiently on the Cell Surface—To understand the role of the hemopexin domain of MT1-MMP in proMMP2 activation, several deletion constructs of MT1-MMP were made as shown in Fig. 1. When detected using an antibody against the catalytic domain of MT1-MMP, the mutants expressed protein species as expected (Fig. 2, A and B). Interestingly, the deletion of its hemopexin domain led to a slightly higher level of expression (Fig. 2A, lane 3 versus 2). This is in agreement with a previous report that the hemopexin domain of MT1-MMP can associate with CD63, which leads to accelerated degradation (28). We next tested whether the deletion of the hemopexin domain affects the subcellular localization of MT1-MMP. As shown in Fig. 2B, deletion of the cytoplasmic tail (MT1ΔC) notably shifted more MT1-MMP from the cytosol to the cell surface compared with wild type MT1-MMP, which is consistent with our previous report (14). In contrast, deletion of the hemopexin domain in either wild type MT1 (MT1ΔPex) or in MT1ΔC

![Image](image_url)
(MT1ΔPex/C) had little effect on their subcellular localization, indicating that the hemopexin domain does not regulate MT1-MMP localization.

Since a previous report showed that the hemopexin domain of MT1-MMP is crucial to its activity toward proMMP2 (15), the ability of these mutants to activate proMMP2 on the cell surface was examined. Surprisingly, both MT1ΔPex and MT1ΔPex/C activate proMMP2 efficiently when transfected into HEK 293 cells (Fig. 2C, upper panel, lanes 3 and 5). Similar results were obtained when tested in other cells such as MDCK, PC3, and MCF-7 cells (Fig. 2C, lower panels, lanes 8, 10, 13, 15, 18, and 20). To further confirm this surprising finding, the activation of proMMP2 by these mutants was also tested in COS-7 cells using either 5% serum media or purified proMMP2 as described previously (15). As shown in Fig. 2D, the deletion of the hemopexin domain had little effect on MT1-MMP-mediated activation of proMMP2 from either source (lanes 3, 5, 9, and 11). Thus, we conclude that the hemopexin domain of MT1-MMP is not required for proMMP2 activation. However, our data contradict the previous report that the
hemopexin domain is required for proMMP2 activation, which was based on the observation that the substitution of the MT1-MMP hemopexin domain with that of MT4-MMP completely impaired its ability to activate proMMP2 (15). To clarify this discrepancy, we made several additional constructs in which MT4- and MT6-MMP hemopexin domains were separately introduced into MT1-MMP and their activities toward proMMP2 were analyzed. Consistent with the previous report, the MT4-MMP hemopexin domain with or without its hinge region impairs the ability of MT1-MMP to activate proMMP2 when swapped (Fig. 2E, lanes 2 and 4) (15). Interestingly, the MT6-MMP hemopexin domain did not interfere with MT1-MMP-mediated proMMP2 activation when swapped either alone or with its hinge region to MT1-MMP (Fig. 2E, lanes 3 and 5). This is also in agreement with our recent report that the MT3-MMP hemopexin domain can functionally replace the MT1-MMP hemopexin domain in proMMP2 activation (13). These results suggest that the hemopexin domain of MT4-MMP may have an unknown inhibitory effect on MT1-MMP activity toward proMMP2.

It has been reported that TIMP2 is required for MT1-MMP-mediated proMMP2 activation (15, 29). To this end, we tested proMMP2 activation mediated by MT1APex in TIMP2-deficient mouse embryonic fibroblast cells (30). As shown in Fig. 2F, little proMMP2 was activated by transfected wild type MT1-MMP or MT1APex in the absence of TIMP2. TIMP2 supplied with increasing concentrations enhanced the activation of purified proMMP2 by both MT1-wild type and MT1APex, suggesting that TIMP2-dependent proMMP2 activation mediated by MT1-MMP is independent of its hemopexin domain.

The Hemopexin Domains in Other MT-MMPs Are Not Required for Their Ability to Activate proMMP2—We and others (18, 23, 25, 26, 31, 32) have demonstrated that MT2-, MT3-, MT5-, and MT6-MMP can also activate proMMP2 efficiently in cell-based assays. It is not clear whether or not their hemopexin domains are required for this ability. Compared with MT1-MMP, the structural bases for these MT-MMPs in proMMP2 activation are largely unknown. Moreover, the mechanisms by which these MT-MMPs mediate proMMP2 activation may be quite different. For example, compared with MT1- and MT3-MMPs, MT2-MMP appears to mediate proMMP2 activation independent of TIMP2 (31, 32). Therefore, to investigate the role of their hemopexin domains in promoting proMMP2 activation, the hemopexin domains were deleted from MT2-, MT3-, MT5-, and MT6-MMP and the activities of deletion mutants toward proMMP2 were analyzed using gelatin zymography assays. As shown in Fig. 3, the deletion did not affect the ability of MT2-MMP (Fig. 3A) and MT3-MMP (Fig. 3B) to mediate proMMP2 activation. Surprisingly, the same deletion in MT5-MMP increased its activity toward proMMP2 on the cell surface significantly as did the deletion of its cytosolic tail (Fig. 2C). Our recent reports have shown that MT6-MMP, a GPI-anchored MT-MMP, can activate proMMP2 in a co-transfection assay, although much weaker than MT1-MMP and MT5-MMP (26). Interestingly, the deletion of the hemopexin domain from MT6-MMP greatly enhances its ability to mediate proMMP2 activation when co-expressed in HEK 293 cells (Fig. 2D). Thus, we conclude that the hemopexin domains of MT-MMPs are not required for proMMP2 activation. In fact, the hemopexin domain may behave as a negative regulator for MT5- and MT6-MMP-mediated proMMP2 activation.

Membrane Anchoring but Not the Anchoring Mechanism per se Is Required for MT1-MMP-mediated proMMP2 Activation—It has been reported that secreted MT1-MMP loses its ability to activate extracellular proMMP2, indicating that the C terminus is required for its activity toward proMMP2 (33). It is interesting to know whether the transmembrane domain is required for the ability of MT1-MMP to activate proMMP2. To answer this question, several mutants were made and analyzed in HEK 293 cells. As shown in Fig. 4, the secreted ectodomains and catalytic domains of MT1-MMP completely lost their ability to activate extracellular proMMP2, suggesting that a membrane anchor is required for this activity in cell-based assays. To test this idea, we engineered the MT6-MMP GPI domain to the carboxyl end of MT1-MMP catalytic domain (CatGPI). As shown in Fig. 4, MT1-CatGPI was able to activate proMMP2 effectively on the cell surface. These data suggested that a membrane anchor instead of the transmembrane domain is sufficient for the MT1-MMP catalytic domain to mediate proMMP2 activation on cell surface.

Minimal Domain Requirement for MT1-MMP-mediated proMMP2 Activation on the Cell Surface in Stably Expressed Cells—To further characterize the MT1-MMP mutants, we established stable clones expressing moderate levels of these mutant enzymes in MDCK cells. As shown in Fig. 5A, MDCK cells expressing MT1APex, MT1APex/C, and CatGPI activate proMMP2 efficiently (lanes 3, 5, and 6), whereas cells expressing the mutant MT1-E240A, which lost the catalytic activity, could not activate proMMP2 as expected (Fig. 5A, lane 7). Similar results were also obtained using the MCF-7 stable cell lines (Fig. 5B). These results demonstrate that the catalytic domain is the minimal domain sufficient to activate proMMP2 when attached to the cell surface.

The Hemopexin Domain Is Required for MT1-MMP-mediated Invasion into Type I Collagen Lattice—It has been reported that MT1-MMP can promote cells to invade into type I collagen lattice (23). To identify the domains involved in invasion, various MDCK stable cells were cultured on top of type I collagen matrix and their invasiveness was monitored and quantified by microscopy. As shown in Fig. 6, both wild type MT1 and MT1AC transfectants could efficiently invade into the collagen lattice and the MMP inhibitor GM6001 was able to completely block their invasion. As expected, the cells expressing MT1-E240A failed to invade. These data indicated that the catalytic activity is required for MT1-MMP-mediated invasion. In contrast to proMMP2 activation, both MT1APex and MT1APex/C completely lost their ability to promote cell invasion into type I collagen lattice, suggesting that the hemopexin and catalytic domains of MT1-MMP are both required.

The Hemopexin Domain of MT1-MMP Plays a Crucial Role in Cyst Formation and Growth in Three-dimensional Type I Collagen Lattice—MDCK cells can form cysts when grown in type I collagen lattice. The growth of cysts can be greatly enhanced by stably expressing MT1-MMP (8, 24) through an unknown mechanism. To delineate the role of the hemopexin domain in cyst growth, we assessed the growth of the stable cells described above in three-dimensional type I collagen lattice. As shown in Fig. 7A, whereas MDCK cells expressing wild type MT1-MMP grew into significantly larger cysts than MDCK cells (panel b versus a), the cells with MT1APex grew less robustly (panel c versus b), suggesting that the hemopexin domain is important for mediating cyst growth in type I collagen lattice. As expected, the MT1-E240A mutant completely lost the ability to enhance cyst formation (Fig. 7A, panel g), indicating that MT1-MMP-dependent cyst growth requires its catalytic activity. In agreement with our previous report that the cytosolic tail is a negative regulator for collagen growth, MT1AC-expressing cells grew into much larger cysts than MT1-wild type expressing cells (13). To our surprise, MT1APex/C and MT1-CatGPI transfectants also grew significantly larger cysts than the control cells (Fig. 7A, panels e and f). The MMP inhibitor GM6001 efficiently inhibited their
growth in type I collagen three-dimensional gels (Fig. 7B). These data demonstrated that the hemopexin domain is important to but not required for MT1-MMP-mediated cyst growth in type I collagen lattice.

The Hemopexin Domain Is Required for MT1-MMP-mediated MCF-7 Scattering in Three-dimensional Collagen Lattice—MT1-MMP has been reported to be expressed in various tumor types including lung, gastric, colon, breast, cervical carcinomas, gliomas, and melanomas (4). To further test the role of MT1-MMP domains in tumors cells, its wild type and mutants were stably expressed in MCF-7 cells. As shown in Fig. 7C, control-transfected MCF-7 cells grew into cyst-like shapes when cultured in three-dimensional type I collagen lattice, which is consistent with our previous report (27). Surprisingly, MCF-7 cells stably expressing MT1-MMP or MT1ΔC failed to form any cysts, instead invading and scattering in the threedimensional lattice (Fig. 7C, panel b). However, MCF-7 cells expressing the hemopexin-less mutant (MT1ΔPex) grew as cysts and did not invade or scatter in three-dimensional collagen lattice, indicating that the hemopexin domain is important to MT1-MMP-mediated cell scattering in three-dimensional collagen lattice.

DISCUSSION

MT1-MMP is a key enzyme involved in tumor invasion and metastasis (4). The best characterized function of MT1-MMP is to activate proMMP2 and degrade components of the ECM, especially type I collagen (4). Our previous data showed that the hemopexin domain of MT1-MMP could be successfully substituted by that of MT3-MMP to cleave proMMP2 rather than
degrade type I collagen (13). In this report, we further provided direct evidence to show that the hemopexin domain of MT-MMPs were not required for their activity toward proMMP2 activation. Instead, the membrane-anchored catalytic domain together with the hinge region of MT1-MMP proved to be an efficient activator for proMMP2. On the other hand, we showed that the hemopexin domain of MT1-MMP is critical for cell invasion and cyst growth in three-dimensional collagen lattice.

proMMP2 activation is one of the most important functions for MT-MMPs and thus has been investigated extensively (13). It has been reported that formation of a homophilic complex via the hemopexin-like domain or C-terminal is important for MT1-MMP mediated proMMP2 activation (15, 16, 34). The substitution of an MT1-MMP hemopexin domain with that of MT4-MMP greatly impaired its ability to activate proMMP2 (15). Our present data clearly showed that the hemopexin domains of MT-MMPs are not required for MT1-, MT2-, MT3-, MT5-, and MT6-MMP-mediated proMMP2 activation. Moreover, the introduction of the hemopexin domain of MT4-MMP in contrast to MT3-MMP and MT6-MMP, greatly inhibited MT1-MMP-mediated proMMP2 activation for an unknown reason (Fig. 2E). However, compared with other MT-MMPs, full-length MT4-MMP cannot activate proMMP2 in a cell-based assay (10), suggesting that the hemopexin domain of MT4-MMP may play a negative role in proMMP2 activation. Interestingly, we indeed found that the hemopexin domains of MT5- and MT6-MMP negatively regulate proMMP2 activation because deletion of their hemopexin domains greatly increased their activity toward proMMP2. This is also in agreement with our recent report that the substitution of the MT1-MMP catalytic domain with that of MT6-MMP greatly increased MT6-MMP catalytic activity compared with wild type MT6-MMP (26). It is notable that the hinge region is critical for MT1-MMP-mediated proMMP2 activation. Deletion of the MT1-

MMP hinge region totally impaired its activation ability.\(^2\)

Thus, we have provided direct evidence that the hemopexin domain is not involved in proMMP2 activation by MT-MMPs.

ECM degradation plays key roles in malignant tumor invasion and metastasis (1). Several MMPs such as MMP-1, MMP-8, MMP-13, and MT1-MMP exhibit significant activity toward native type I collagen (19). However, only MT-MMPs including MT1- and MT2-MMP can confer the invasion-incompetent cells with the ability to invade type I collagen gel (23). In this report, we showed that MT1-MMP mutants without hemopexin domains completely lost their ability to promote cell invasion into type I collagen matrix but were potent to activate proMMP2. This is in agreement with the previous report that secreted MMP-2 and MMP-13 cannot promote cells to invade the collagen matrix, although they possess the ability to degrade type I collagen (23). This is also consistent with our

\(^2\) P. Wang, J. Nie, and D. Pei, unpublished data.
MT-MMPs Activate proMMP2 Independent of Hemopexin-like Domains

FIG. 6. Hemopexin domain is required for MT1-MMP-mediated invasion into type I collagen gel. MDCK stable clones were cultured on top of 2.5 mg/ml type I collagen in 24-well plates with or without 5 μM GM6001 for 7 days. The invasion of cells was measured and photographed under a microscope. WT, wild type.

previous report that introducing the MT3-MMP hemopexin domain into MT1-MMP greatly impaired its activity (13). In this report, we further demonstrated that the catalytic activity is absolutely required for MT1-MMP-dependent cell invasion by employing both MMP inhibitors and E240A mutants. Tam et al. (35) have recently shown that the hemopexin domain of MT1-MMP is responsible for type I collagen binding and that purified hemopexin domain can inhibit MT1-MMP-mediated collagen degradation. Purified MT1 catalytic domain itself cannot cleave native type I collagen in vitro (36). Thus, it is plausible that the deletion of the hemopexin domain incapacitates MT1-MMP to bind and degrade type I collagen and consequently impairs cell invasiveness.

MT1-MMP can confer a cell growth phenotype in three-dimensional collagen lattice (24). In this report, we showed that the deletion of the hemopexin domain from MT1-MMP greatly impaired its ability to promote cyst growth in a three-dimensional model. Our previous data showed that MT3- and MT5-MMP can also promote cyst growth in three-dimensional collagen gel but much less than MT1-MMP (8, 13). Interestingly, neither MT3-MMP nor MT5-MMP has been shown to degrade native type I collagen in vitro (25, 37). Thus, it is possible that other mechanisms together with collagen degradation may be involved in cyst growth, although metalloproteinase activity is required for MT-MMP-mediated cell growth in three-dimensional collagen (8, 13). MMP2, which can be activated by all of the MT1-MMP mutants, possesses weak activity toward type I collagen (19). However, activated MMP2 has been shown to have little effect on three-dimensional cell growth (24). In fact, in addition to degrade matrix protein, MT1-MMP has been shown to be a sheddase for several membrane proteins, including CD44 (34), αv integrin (38, 39), syndecan-1 (40), E-cadherin (41), and tissue transglutaminase (42). Moreover, MT1-MMP can regulate signaling pathways such as the mitogen-activated protein kinase (43, 44), Cdk3 (24), and focal adhesion kinase (39) pathways and regulate gene expression such as vascular endothelial growth factor and MT1-MMP (43, 45). Thus, it is conceivable that an MT1-MMP mutant without a hemopexin domain still has the ability to regulate some cell growth pathways by cleaving some of these membrane substrates. Based on the varying activities of MT1-MMP mutants to mediate invasion and three-dimensional growth, we suggest that MT1-MMP mediates cell growth through mechanisms other than proMMP2 activation and type I collagen degradation.

Since MT1-MMP can promote cyst growth in several cell lines such as MDCK and SCC-1 cells in three-dimensional type I collagen lattice (24), it is interesting to note that MT1-MMP confers a rather different phenotype to MCF-7 cell behavior in three-dimensional type I collagen lattice. Wild type MCF-7 cells can form cysts when cultured in three-dimensional collagen gel (27). However, MT1-MMP significantly enhances MCF-7 scattering in a three-dimensional collagen matrix (Fig. 7). This is quite different from MT5-MMP, which can only promote cyst growth in three-dimensional collagen gel as in our previous report (27). The scattering phenotype is highly relevant in our effort to understand the role of MT-MMPs in tumor invasion and metastasis. Further studies are needed to dissect the cellular and molecular events controlling the scattering behavior for tumor cells. Taken together, we propose that cellular phenotypes such as growth and scattering in three-dimensional collagen lattice should be used as screening assays to search for compounds that may potentially block tumor invasion and metastasis. On the other hand, the hemopexin domain of MT1-MMP represents a good target for designing novel and specific therapeutic agents against malignant cancer.

Acknowledgments—We thank for Dr. Paul D. Soloway for providing us with TIMP2−/− mouse embryonic fibroblast cells, Dr. Stephen J. Weiss for providing the MT2-hemagglutinin plasmids. We also thank Xing Wang, Kristen England, Ping Cui, and Zhen Hua Geng for their kind technical help. We thank Jennifer Idziorek for help in editing the paper.
