Matrix-dependent Proteolysis of Surface Transglutaminase by Membrane-type Metalloproteinase Regulates Cancer Cell Adhesion and Locomotion*

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Cell invasion requires cooperation between adhesion receptors and matrix metalloproteinases (MMPs). Membrane type (MT)-MMPs have been thought to be primarily involved in the breakdown of the extracellular matrix. Our report presents evidence that MT-MMPs in addition to the breakdown of the extracellular matrix may be engaged in proteolysis of adhesion receptors on tumor cell surfaces. Overexpression of MT1-MMP by glioma and fibrosarcoma cells led to proteolytic degradation of cell surface tissue transglutaminase (tTG) at the leading edge of motile cancer cells. In agreement, structurally related MT1-MMP, MT2-MMP, and MT3-MMP but not evolutionary distant MT4-MMP efficiently purified tTG in vitro. Because cell surface tTG represents a ubiquitously expressed, potent integrin-binding adhesion coreceptor involved in the binding of cells to fibronectin (Fn), the proteolytic degradation of tTG by MT1-MMP specifically suppressed cell adhesion and migration on Fn. Reciprocally, Fn in vitro and in cultured cells protected its surface receptor, tTG, from proteolysis by MT1-MMP, thereby supporting cell adhesion and locomotion. In contrast, the proteolytic degradation of tTG stimulated migration of cells on collagen matrices. Together, our observations suggest both an important coreceptor role for cell surface tTG and a novel regulatory function of membrane-anchored MMPs in cancer cell adhesion and locomotion. Proteolysis of adhesion proteins colocalized with MT-MMPs at discrete regions on the surface of migrating tumor cells might be controlled by composition of the surrounding ECM.

Remodeling of the extracellular matrix (ECM) is critical for cancer cell invasion and tumorigenesis (1–5). Membrane type matrix metalloproteinases (MT-MMPs) localized to the invasive front of highly motile cancer cells (6, 7) were shown to be directly involved in matrix breakdown (8–13). A cooperation involving MT-MMPs and cell adhesion receptors is likely to be essential to migrating cells (3, 14–16). So far, six members of the MT-MMP subfamily have been identified and partially characterized (2, 17–22). MT1-, MT2-, and MT3-MMP strongly contribute to tumor cell invasion (12). Recent studies demonstrated a functional significance and a direct role of MT1-, MT2- and MT3-MMP in cell locomotion on laminin-5 (11) and three-dimensional collagen type I lattice (8, 9, 12). In addition, MT-MMPs contribute indirectly to cell invasion by activating soluble secretory MMP-2 (23) and MMP-13 (24), which further cleave multiple matrix substrates (2, 5, 25–29).

Integrin adhesion receptors dynamically regulate cell-matrix interactions by the binding to matrix proteins and inside-out signaling (30, 31). This allows cells to discriminate any subtle alteration of the environment and to adjust cell locomotion accordingly. Direct interactions with multiple transmembrane and cell surface proteins (32) including integrin-associated protein-50 (33), TM4SF proteins (tetraspanins) (34) and tTG (35) further attenuate adhesive and signaling efficiency of integrins.

Cell surface tTG (protein-glutamine γ-glutamyltransferase, EC 2.3.2.13) promotes integrin-dependent adhesion and spreading of cells. By both direct associations with multiple β1 and β3 integrins and the binding with Fn, tTG independently mediates the interactions of integrins with Fn (35). The high affinity binding of tTG with Fn specifically involves the 42 kDa gelatin-binding domain of the Fn molecule, which consists of modules IgαII1,2I7–9 (36). The enhancement of integrin-mediated adhesion and spreading of cells on Fn is independent from the enzymatic activity of surface tTG (35). Intriguingly, reduced expression of tTG has been linked to aggressiveness and high metastatic potential of tumors, whereas overexpression of tTG in fibrosarcomas inhibited primary tumor growth (37, 38). Proteolysis of tTG at the normal tissue/tumor boundary was observed in invasive tumors (38).

Here, we report that depending on the structure of the ECM, MT-MMPs are capable of both positively and negatively regulating locomotion of cancer cells. Matrix-dependent proteolysis of surface tTG by MT1-MMP occurs on tumor cells of a diverse tissue origin, thereby representing a general phenomenon and a novel MT-MMP function. Our data suggest an existence of an unexpected link between tumor cell locomotion, the ECM and membrane-anchored MMPs. Regulatory proteolysis of cell surface adhesion proteins by the adjacent MT-MMP molecules is likely to play a significant functional role in cancer cell invasion.
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MATERIALS AND METHODS

Proteins, Antibodies, and Cell Lines—The tTG protein was purified from human red blood cells (39). GM6001 (Iomastat), tissue inhibitors of metalloproteinases TIMP-1 and TIMP-2, the individual catalytic domains of MT1-MMP, MT2-MMP, MT3-MMP, and MT4-MMP, and anti-MT1-MMP rabbit polyclonal antibody AB315 were purchased from Chemicon (Temecula, CA). Anti-β1 integrin mAb 9EG7 and sulfo-NHS-biotin (Pierce Chemical Co., Rockford, IL), respectively. Fn and its proteolytic fragments were obtained as described previously (27–29, 32). Vector- and MT1-MMP-transfected human HT1080 fibrosarcoma and U251 glioma cell lines were selected and reported earlier (35). Vector- and MT1-MMP-transfected human IL, respectively. Fn and its proteolytic fragments were obtained as described previously (27–29, 32). Vector- and MT1-MMP-transfected human HT1080 fibrosarcoma and U251 glioma cell lines were selected and reported earlier (35). Vector- and MT1-MMP-transfected human HT1080 fibrosarcoma and U251 glioma cell lines were selected and reported earlier (35).

Protein-incorporated radioactivity was determined by scintillation counting. Specific binding determined in presence of excess (10^3) unlabeled goat anti-rabbit IgG. For measurements of cell surface tTG, live nonpermeabilized transfected cells were incubated for 2 h at 37 °C without or with 20 μg/ml GM6001, 1 μg/ml TIMP-1, and 1 μg/ml TIMP-2 and then stained with 10 μg/ml rabbit polyclonal anti-tTG antibody and secondary conjugated IgG (25). Cells were analyzed using a FACScan flow cytometer (Becton Dickinson). At least three independent experiments were performed for each cell line.

Zymography—To evaluate the status of MMP-2, nonreduced aliquots of medium conditioned with HT-vector and HT-MT cells were analyzed by gelatin zymography. Zymography was performed in 0.1% gelatin and 10% polyacrylamide gels as described previously (29). After electrophoresis, SDS was replaced by Triton X-100, followed by incubation in a Tris-based buffer overnight. Gels were stained with Coomassie Brilliant Blue, and gelatinolytic activity was detected as clear bands in the background of uniform staining.

Measurement of Transglutaminase Activity—Transglutaminase activity was expressed on the surface of HT-vector and HT-MT cells as measured by the individual catalytic domain of MT-MMP (0.2 μg each) for 0.5–12 h at 37 °C in 0.05 M Tris-HCl buffer, pH 7.5, containing 50 mM NaCl, 1 mM CaCl2, and 10 μM ZnCl2. The reaction was stopped by SDS-sample buffer. After electrophoresis in 12% gels and transfer to an Immobilon membrane, cells were stained, excised, and subjected to the NH2-terminal microsequencing at the Protein Chemistry Facility of Washington University (St. Louis, MO).

Proteins, Antibodies, and Cell Lines—The tTG protein was purified from human red blood cells (39). GM6001 (Iomastat), tissue inhibitors of metalloproteinases TIMP-1 and TIMP-2, the individual catalytic domains of MT1-MMP, MT2-MMP, MT3-MMP, and MT4-MMP, and anti-MT1-MMP rabbit polyclonal antibody AB315 were purchased from Chemicon (Temecula, CA). Anti-β1 integrin mAb 9EG7 and sulfo-NHS-biotin (Pierce Chemical Co., Rockford, IL), respectively. Fn and its proteolytic fragments were obtained as described previously (27–29, 32). Vector- and MT1-MMP-transfected human HT1080 fibrosarcoma and U251 glioma cell lines were selected and reported earlier (35). Vector- and MT1-MMP-transfected human HT1080 fibrosarcoma and U251 glioma cell lines were selected and reported earlier (35). Vector- and MT1-MMP-transfected human HT1080 fibrosarcoma and U251 glioma cell lines were selected and reported earlier (35). Vector- and MT1-MMP-transfected human HT1080 fibrosarcoma and U251 glioma cell lines were selected and reported earlier (35). Vector- and MT1-MMP-transfected human HT1080 fibrosarcoma and U251 glioma cell lines were selected and reported earlier (35). Vector- and MT1-MMP-transfected human HT1080 fibrosarcoma and U251 glioma cell lines were selected and reported earlier (35).

RESULTS

tTG Is Degraded and Inactivated on the Surface of Cancer Cells Expressing MT1-MMP—HT1080 fibrosarcoma and U251 glioma cells that naturally express relatively low levels of MT1-MMP were transfected with the MT1-MMP cDNA (GenBank
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were plated in serum-containing medium at 2 days posttransfection. HT-MT and HT-vector cells were transfected with MT1-MMP cDNA. U41078) to up-regulate MT1-MMP and create HT-MT and U-MT pools of cells, respectively (27–29, 32). To avoid any undesirable clonal effects, our studies were performed with the corresponding pools of stable transfectants. Highly elevated levels of MT1-MMP in U-MT cells relative to U-vector control were confirmed previously by immunoprecipitation, flow cytometry, and functional assays (27–29, 32, 40). To corroborate these findings further, we evaluated the levels of MT1-MMP in HT-MT cells by immunoprecipitation, flow cytometry, and by analysis of the ability of cells to initiate the activation of the secretory MMP-2 proenzyme. Immunoprecipitation and flow cytometry studies employed rabbit polyclonal AB815 antibody directed against the hinge region of MT1-MMP. Immunoprecipitation confirmed an increase in the amounts of 60-kDa enzyme and the 42-kDa ectodomain form of MT1-MMP expressed on the surface of HT-MT cells, relative to those of HT-vector cells (Fig. 1a). Earlier, it has been shown that TIMP-2 regulates the amount of active 60-kDa MT1-MMP enzyme on the cell surface, whereas in the absence of TIMP-2 MT1-MMP undergoes autocatalysis to the 42-kDa form (41, 42). Apparently, our immunoprecipitation experiments showing high levels of the 42-kDa form point to an insufficiency of TIMP-2 relative to MT1-MMP in HT-MT transfectants (43).

Flow cytometry confirmed the immunoprecipitation data and demonstrated high cell surface levels of MT1-MMP expressed in HT-MT cells (Fig. 1b). As determined by gelatin zymography of conditioned medium samples, up-regulation of MT1-MMP correlated directly with the ability of HT-MT cells to activate the MMP-2 proenzyme (44). The intermediate and active forms of MMP-2 enzyme were present in the conditioned medium from HT-MT but not from HT-vector cells (Fig. 1c, middle and bottom arrows). Apparently, MT1-MMP transfection shifts a balance among the preexisting levels of TIMP-2, MT1-MMP, and MMP-2, thus inducing the activation of pro-MMP-2 by the cells (23). GM6001, a hydroxamate inhibitor of MMPs (45), failed to affect the total amounts of MT1-MMP expressed by HT-MT and HT-vector cells (Fig. 1b). In contrast, GM6001, by binding to the active site and, thereby inactivating MT1-MMP, completely abolished the MT1-MMP-induced activation of pro-MMP-2 (Fig. 1c). These findings correlate well with the results of our previous studies with U-MT and U-vector control cells (40) and demonstrate high levels of functionally active MT1-MMP enzyme expressed on the surface of cancer cells stably transfected with MT1-MMP cDNA.

Flow cytometry analyses demonstrated a 4-fold decrease in surface tTG expression in HT-MT cells relative to that of HT-vector control cells (Fig. 2a). GM6001 at 20 μM restored the expression of tTG on the surface of HT-MT cells to the control levels. Because GM6001 is a general inhibitor of MMP activity, we employed TIMP-2, an efficient inhibitor of MT1-MMP, and TIMP-1, which is a poor inhibitor of MT1-MMP, to distinguish the MT1-MMP effect on tTG. Importantly, TIMP-2 fully restored surface tTG on HT-MT cells. In turn, TIMP-1 was significantly less potent relative to TIMP-2 and GM6001 in its ability to restore the tTG expression on HT-MT cells (Fig. 2a). Any inhibitor had no effect on surface tTG of HT-vector cells. These findings indicate that MT1-MMP resistant to TIMP-1 inhibition is primarily involved in proteolysis affecting cell surface tTG in HT-MT cells.

To confirm the proteolytic degradation of surface tTG by MT1-MMP in cultured cells, the HT-vector and HT-MT transfectants were labeled with membrane-impermeable sulfo-NHS-biotin. The surface-biotinylated tTG was immunoprecipitated from cell lysates. In contrast to intact tTG (molecular mass ~80 kDa) found in HT-vector cells, its proteolytic fragments of ~70, ~53, ~41, and ~32 kDa were identified in untreated HT-MT cells (Fig. 2b). Inhibitors such as GM6001, TIMP-1, and TIMP-2 failed to affect the status of tTG in HT-vector cells. On the contrary, pretreatment with GM6001 completely abolished the degradation of surface tTG in HT-MT cells. In these assays, TIMP-2 was slightly less efficient compared with GM6001, whereas TIMP-1 failed to protect tTG from proteolysis in HT-MT cells (Fig. 2b). Together, our observations point to the main role of TIMP-1-resistant MT1-MMP in the proteolysis of cell surface tTG in HT-MT cells. However, MMP-2 and possibly other MMPs may also contribute to proteolytic degradation of surface tTG.

To demonstrate that degradation of cell surface tTG in HT-MT cells abolished its functional activities, we measured both the transglutaminase enzymatic (cross-linking) activity and the ability of surface tTG to bind specifically the 42-kDa gelatin-binding fragment of Fn (Fig. 2, c and d). We observed only a low residual level of transglutaminase activity on the surface of untreated HT-MT cells compared with that of control HT-vector cells. GM6001 restored the enzymatic activity of surface tTG in HT-MT cells to the level observed in HT-vector cells (Fig. 2c). The fact that the associations of surface tTG with Fn specifically involve the 42-kDa Fn fragment was established in our earlier work (35). Accordingly, the efficiency of HT-MT cells to bind the 42-kDa Fn fragment directly was drastically...
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FIG. 2. Proteolytic degradation by MT1-MMP destroys enzymatic and coreceptor activities of surface tTG. Panel a, flow cytometry analysis of surface tTG in HT-vector and HT-MT cells. Untreated cells and cells pretreated with 20 μM GM6001, 1 μg/ml TIMP-1, or TIMP-2 inhibitors were stained with anti-tTG antibody followed by secondary fluorescein-labeled IgG. The level of surface tTG in untreated HT-vector cells was taken as 100%. Shown are the means of triplicate determinations. Panel b, immunoprecipitation of surface tTG from HT-vector and HT-MT cells. Cells were incubated without or with the MMP inhibitors then surface biotinylated and lysed. tTG was immunoprecipitated from cell lysates with anti-tTG antibody and protein A-Sepharose. After SDS-PAGE under reducing conditions and transfer to a membrane, tTG was detected with neurtandinigerasiladrophere oxidase. Panel c, measurement of transglutaminase activity on the surface of HT-vector and HT-MT cells. Cells were incubated without or with 20 μM GM6001. The enzymatic activity of tTG expressed on the cell surface was quantified by measuring cell-mediated incorporation of [H]papetresine into N,N-dimethylcysteine. Bars show the means of triplicate determinations. Panel d, binding of the 42-kDa gelatin-binding fragment of Fn to HT-vector and HT-MT cells. Cells in suspension were preincubated without or with 20 μM GM6001 and then incubated with the 125I-labeled 42-kDa Fn fragment (1 μM) for 1 h at 37 °C. Cells were separated from excess labeled fragment by centrifugation through a layer of 20% sucrose. Cell-bound radioactivity was counted in a gamma counter. Shown are means of triplicate measurements. Panel e, immunostaining of surface tTG in HT-vector and HT-MT cells. Cells were cultured for 24 h in serum-free medium and then plated for 3 h on coverslips coated with Fn. Nonpermeabilized cells were stained with polyclonal anti-tTG antibody. Note the accumulation of tTG at the edge of lamellipodia in HT-vector cells (arrows) and the lack of tTG staining in HT-MT cells. Bar, ~50 μm.

reduced relative to that of control HT-vector cells. Again, inhibition of MMP activity by GM6001 fully restored the binding efficiency of HT-MT cells and failed to affect that of HT-vector cells (Fig. 2d). In agreement with our previous results (35), preincubation of HT-vector and HT-MT cells with polyclonal antibody against tTG abolished the binding of the 42-kDa fragment of Fn to cell surfaces (data not shown).

Immunofluorescent staining confirmed low levels of surface tTG in untreated nonpermeabilized HT-MT cells. In contrast, immunofluorescence of surface tTG was far more intense in HT-vector cells, particularly at the leading edge (Fig. 2e, arrows). Together, our results show that expression of MT1-MMP by cells strongly contributes to the degradation of surface tTG. The degradation of tTG correlates with the inactivation of both the enzymatic and coreceptor functions of this cell surface protein.

Proteolysis of Purified tTG in Vitro—To support our findings, we used enzymatically active individual catalytic domains of MT1-MMP (Fig. 3a) and related MT2-MMP, MT3-MMP, and MT4-MMP (Fig. 3b) to cleave the purified tTG protein in vitro. MT1-MMP, MT2-MMP, and MT3-MMP efficiently and specifically degraded tTG in vitro. The proteolytic fragments of tTG generated in vitro were similar to those detected on the cell surface (Figs. 2b, 3c, and 4a). This provides a key evidence that the MT1-MMP catalytic activity is essential for the proteolysis of surface tTG observed in cultured cells.

Extensive proteolysis of tTG by the catalytic domain of MT1-MMP resulted in four major (molecular masses of ~53, ~41, and ~32 kDa) and several minor cleavage products (Fig. 3a, marked by arrows and numbered). In contrast, the catalytic domain of MT4-MMP, a glycosylphosphatidylinositol-linked membrane enzyme that is structurally and functionally distant...
that cell surface tTG coprecipitated with β1 and β3 integrins (35). For these purpose, we immunoprecipitated β1 and β3 integrins from RIPA lysates and analyzed the precipitated samples for tTG. Large amounts of undegraded (~80 kDa) tTG, which comprised ~15–25% of total cellular tTG, coprecipitated with β1 and β3 integrins from the lysates of HT-110-MMP cells cultured on collagen in the presence of GM6001 or on Fn without this hydroxamate inhibitor (Fig. 4b, middle and bottom panels, respectively). In contrast, only levels of undegraded tTG, which accounted for ~1.8–2% of total cellular tTG, coprecipitated with β1 or β3 integrins if HT-MT cells were grown on collagen I without GM6001 (Fig. 4a, top panel, arrow). These results show that Fn specifically inhibited proteolysis of its own adhesion receptor, cell surface tTG, by MT1-MMP. A failure of collagen type I, a substrate more susceptible to MT1-MMP degradation than Fn (8, 45), to inhibit proteolysis of tTG, emphasizes a functional significance of the specific inhibitory effect of Fn.

Localization of tTG, β1 Integrins, and MT1-MMP on Cell Surfaces—Our earlier results indicated that tTG was colocalized with β1/β3 integrins at discrete regions of the cell surface (35). Apparently, surface tTG could be degraded by MT1-MMP only if this membrane-anchored proteinase is proximal to the tTG-integrin clusters. To demonstrate that tTG degradation by MT1-MMP might occur on cancer cells of a diverse tissue origin, we examined tTG, MT1-MMP, and β1 integrins on the surface of U-MT and U-vector cells derived from parental U251 human glioma cells. The status of MT1-MMP and MMP-2 in U-MT and U-vector cells has been characterized extensively in our previous work (39). When U-MT cells were plated for 24 h in serum-containing medium on Fn, that was shown to prevent proteolysis of tTG (Fig. 4, a and b), an extensive colocalization of tTG, MT1-MMP, and β1 integrins became evident at the periphery of U-MT cells (Fig. 5a, merge in yellow).

To corroborate these findings, we preincubated U-MT and U-vector cells in suspension for 2 h without or with GM6001 and then plated the cells for 3 h on Fn in serum-free medium containing cycloheximide. No MT1-MMP was detected on U-vector cells, whereas β1, integrins and tTG were localized at the cell periphery (Fig. 5b, left panels, arrows). In U-MT cells, MT1-MMP accumulated at the edges of lamellipodia (Fig. 5b, top panels, arrow, arrowhead). In the absence of GM6001 the peripheral staining of tTG in U-MT cells was consistently very weak or absent, pointing to degradation of the cell surface protein (Fig. 5b, middle panels, arrowhead). Localization of β1 integrins at the periphery of untreated U-MT cells was partially disrupted (Fig. 5b, arrowhead). In contrast, GM6001 restored both the peripheral staining and the colocalization of tTG with MT1-MMP and β1 integrin at the leading edge of U-MT cells (Fig. 5b, right panels, arrows).

Functional Significance of the Regulatory Proteolysis of Cell Surface tTG—To evaluate the functional significance of the proteolytic degradation of surface tTG by MT1-MMP, we employed cell function in vitro tests such as adhesion and migration assays. Surface tTG interacts with Fn via binding to its 42-kDa gelatin binding domain (35). Accordingly, we used intact Fn and its proteolytic gelatin-binding fragment of ~42 kDa in our adhesion and migration experiments. As a control, we employed the ~110-kDa Fn fragment that is incapable of binding tTG but associates with integrins (35).

Adhesion of untreated U-MT cells to Fn was ~30% lower relative to that of control U-vector cells (Fig. 6a). In the presence of MMP inhibitors such as GM6001 or TIMP-2, adhesion of U-MT cells was restored to the control levels. In contrast, TIMP-1, a poor inhibitor of MT1-MMP activity, showed only a weak effect in restoring adhesion of U-MT cells to Fn. Because...
the interaction of U-251 glioma cells with Fn involves multiple integrin receptors (27–29), elimination or blocking of a single receptor such as tTG cannot affect the overall adhesive properties of cells strongly. Indeed, we observed a moderate effect of tTG proteolysis on the adhesion of U-MT cell to Fn. To emphasize specifically the effect of tTG degradation on cell adhesion, we evaluated further the adhesion of U-vector and U-MT cells to Fn or its 42-kDa fragment (Fig. 6, c and d).

A more striking difference between U-MT and U-vector cells was identified when cells were allowed to adhere on this fragment of Fn. U-MT cells were about 7-fold less efficient in adhesion to this fragment compared with U-vector control.

MT1-MMP degradation caused the loss of tTG on U-MT cells cultured without GM6001 (lower panels, arrows). Note the colocalization of tTG with β1 integrin at the periphery of untreated U-vector and GM6001-treated U-MT cells (left and right panels, arrowheads).

**DISCUSSION**

MT-MMP activity that is mostly expressed in aggressive tumors (18, 19) greatly contributes to invasion of many tumor cell types (3, 12, 13). MT1-MMP has been thought to be exclusively involved in the breakdown of the ECM components including collagens (8) and laminin-5 (11), and in the activation pathway of soluble MMPs, i.e. MMP-2 (9, 23) and MMP-13 (24).

However, our report presents evidence that membrane-anchored MT-MMPs in addition to the breakdown of the ECM
may be directly engaged in the regulatory proteolysis of adhesion receptors on tumor cell surfaces.

Here we report that MT1-MMP and its structural homolog (MT2-MMP and MT3-MMP) specifically degrade tTG, a ubiquitous integrin-binding cell surface coreceptor for Fn (35). Our studies with inhibitors of MMP activity including a hydroxamic acid inhibitor GM6001 (45), TIMP-2, and TIMP-1, showed that MT-MMPs are likely to have a primary role in the degradation of cell surface tTG under the conditions of cell culture. Although our results were obtained with cancer cells overexpressing MT1-MMP, they demonstrate that tTG is a novel substrate for MT1-MMP and a potential target for cancer cell MT1-MMP in vivo. Further work is needed to assess a relative contribution of individual MMPs to the proteolytic degradation of surface tTG in vivo. Proteolysis of tTG by MT1-MMP specifically decreases adhesion and migration of cells on Fn. This underscores the significance of the coreceptor function of surface tTG on cancer cells. Our findings identified an unexpected function of MT-MMPs and a novel means of regulating cell locomotion. Trafficking and subsequent clustering of MT1-MMP with the tTG-integrin receptor complexes at the invasive front of tumor cells might be involved in the temporal and spatial control of cell locomotion. Importantly, Fn inhibited the proteolytic degradation of its own receptor, cell surface tTG by MT1-MMP, thereby supporting and enhancing cancer cell locomotion. This suggests that individual components of the ECM may reciprocally regulate proteolysis of their specific adhesion receptors on cell surfaces.

Recently, we reported that up to 40% of total cellular β1 integrins including α,β1, and other collagen-binding integrins, are directly associated with tTG on cell surfaces (35). Apparently, proteolysis by MT1-MMP of the tTG component of the cell surface tTG-integrin complexes is likely to alter the pattern of cell matrix recognition and to switch cells from the binding to Fn to a more efficient interaction with collagens or other ECM proteins. These versatile adjustment mechanisms may regulate migration of cells within composite matrices including connective tissue and tissue barriers such as the basement membrane.

Why do cancer cells, instead of aggressively invading the tissue, down-regulate their locomotion? Degradation of adhesion proteins including surface tTG by MT1-MMP may allow cells to avoid invasion of deficient, degraded matrices and, on the contrary, to trigger detachment and subsequent metastas-
sis. In this regard, our data explain previous findings that the reduced expression and proteolysis of tTG observed in vivo are linked to high metastatic potential of tumor cells (37, 38, 48).

A coordinated interplay of adhesion receptors, proteolytic enzymes, the ECM, and the cytoskeleton is likely to control spatially and temporarily invasion and the focalized proteolysis by tumor cells (3). Our work identifies a novel functional link between cell motility and the ECM composition. This link establishes a dual regulation of cell locomotion imposed by MT-MMPs. Evidently, depending on the structure and composition of the ECM, these proteinases may serve as both positive and negative regulators of cell motility. In accordance with the continually changing environment, MT-MMPs might be capable of differentially regulating cell locomotion by shifting molecular gears on the surface of migrating cancer cells. The regulatory proteolysis involving membrane-anchored MT-MMPs is likely to be critical to the status of adhesion proteins such as tTG and, possibly integrins (32). Reciprocally, proteolysis of ECM proteins and a deposition of tumor-specific components can modify the ECM structure. Jointly, these complex mechanisms are likely to control cancer cell invasion efficiently.

The existence of the ancestral membrane-anchored MMPs in plants (49) that have no ECM similar to that of mammals also indicates that matrix breakdown may not be a primary function of MT-MMPs. The cleavage specificity of MT-MMPs is not unique enough (2, 8, 9) to define an unique role of these membrane-anchored proteases in cell locomotion (11–13).

In contrast, targeting of MT-MMPs and the tTG-integrin complexes to the leading edge of migrating cancer cells where new transient cell matrix adhesive contacts are formed and most of the protrusive activity occurs, can modulate the efficiency of the regulatory proteolysis of adhesion proteins by MT-MMP. This localized proteolytic control of cell adhesion and migration is not possible for soluble MMPs. This hypothesis may partially explain a unique functional role of MT1-MMP, MT2-MMP, and MT3-MMP in tumor cell invasion (12).

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