Membrane-type matrix metalloproteinases (MT-MMPs) in tumor metastasis

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Membrane-Type Matrix Metalloproteinases (MT-MMPs) in Tumor Metastasis[1]

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Activated gelatinase A is reportedly associated with tumor spread. We identified novel matrix metalloproteinases that localize on the cell surface and mediate the activation of progelatinase A. Thus, these progelatinase A activators were named membrane-type matrix metalloproteinase-1 and -2 (MT-MMP-1 and -2, respectively). MT-MMP-1 is overexpressed in malignant tumor tissues, including lung and stomach carcinomas that contain activated gelatinase A. This suggests that MT-MMP-1 is associated with the activation of progelatinase A in these tumor tissues. The expression of MT-MMP-1 also induced binding of gelatinase A to the cell surface by functioning as a receptor. The cell surface localization of proteinases has advantages over pericellular proteolysis. MT-MMP-1 and its family may play a central role in the cell surface localization and activation of progelatinase A and *via* this mechanism, tumor cell use exogenous progelatinase A to mediate the proteolysis associated with invasion and metastasis.

Key words: gelatinase A, gelatinase A activator, matrix metalloproteinase, MT-MMP, tumor metastasis.

A major characteristic of malignant tumor cells is their ability to invade and form metastatic foci at distant sites in the body. The high frequency at which matrix metalloproteinase (MMP) transcripts or proteins is detected in invasive tumor cells and tissues suggests that these enzymes are closely associated with tumor invasion and metastasis (*1, 2*). The degradation of the extracellular matrix should be an essential step to allow the spread of the tumor cells.

Extracellular matrix has dynamic roles not only in supporting tissue structures but also in regulating cellular functions. For example, cell proliferation, differentiation, adhesion, and motility are tightly controlled by surrounding environment extracellular matrix. A number of studies have provided evidence for the involvement of MMPs and their inhibitors in developmentally regulated processes. These include ovulation (*3*), embryogenic growth and differentiation (*4*), and the development of organs (*5*). Thus, the expression of MMPs involved in the degradation of components of extracellular matrix must be tightly regulated and the overexpression of MMPs should be associated with various pathological events (*6, 7*).

The expression of MMP function is regulated mainly by 3 processes: transcription, activation of latent MMP, and inhibition by tissue inhibitor of MMPs (TIMP) (*8*). In addition to the overexpression of various MMPs in tumors, the close correlation between gelatinase A activation and metastatic progression in various tumors suggests that gelatinase A activator is a key enzyme triggering tumor spread (*9-12*). In this review we focus on the membrane-type MMP associated with activation of progelatinase A.

Modular structure of MMPs

The MMP gene family encodes 11 metal-dependent endopeptidases with activity against most, if not all extracellular matrix macromolecules. The enzymes have a similar structure composed of the five modular domains (Fig. 1A). The hydrophobic signal sequence is followed by a propeptide that is important to maintain the latency of the enzyme and which is cleaved when the enzyme is activated. The catalytic domain contains the two Zn^{2+}-binding sites that are essential for the catalytic function. The proline-rich hinge region marks the transition to the ~200 residue hemopexin- or vitronectin-like COOH-terminal domain that appears to play a role in encoding substrate specificity. Matrilysin/PUMP-1 does not possess this last domain and therefore is considerably smaller than the other members of the family. The two gelatinases have an insertion which has sequence similarity to the collagen-binding domains of fibronectin in the catalytic domain (*13, 14*). The fibronectin domain is involved in the substrate-binding properties of the molecules.

Activation mechanism of MMP

MMP is secreted in a proenzyme form which is enzymatically inactive. Van Wart's group has proposed a cysteine switch model for the mechanism of maintaining

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Abbreviations: aa, amino acid; APMA, aminophenyl-mercuric acetate; MMP, matrix metalloproteinase; MT-MMP, membrane-type MMP; PCR, polymerase chain reaction; TIMP, tissue inhibitor of MMP; TPA, 12-*O*-tetradecanoyl-phorbol acetate.

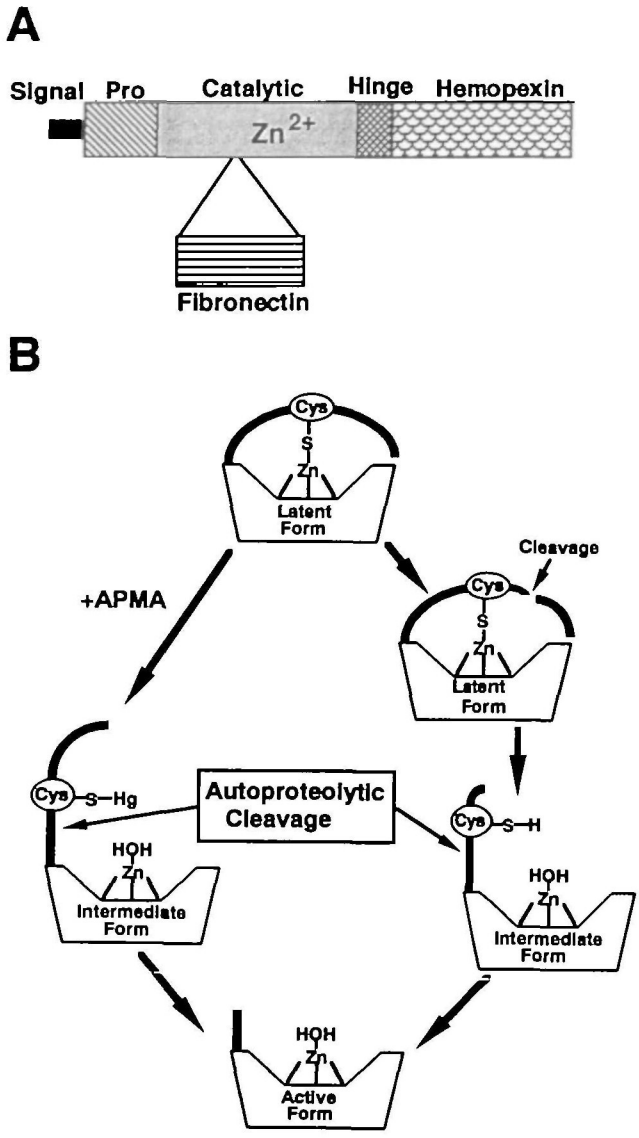

Fig. 1. **Scheme of the domain structures and cysteine switch mechanism for MMP activation.** A: The domain structure. Signal, signal peptides; Pro, the pro-peptide domain that contains PRCGVPD, the most conserved sequence in the pro-peptide domain and is necessary to mask the active site of enzymes; Catalytic, core domain essential for enzymatic activity; Zn^{2+}, Zn^{2+}-binding site; Fibronectin, a fibronectin-like domain of two gelatinases (gelatinases A and B); Hinge, proline-rich sequence potentially functioning as a hinge region; Hemopexin, hemopexin-like repeat. B: Cysteine switch mechanism. Proteolytic enzymes cleave the propeptide, ahead of the cysteine to generate intermediate forms. Alternatively, reagents that react with sulfydryl groups, such as organomercurials (*e.g.* aminophenyl-mercuric acetate, APMA), will modify the cysteine. In a second step, these intermediate forms can be autoproteolytically cleaved to remove the propeptide and confer permanent activity.

the latency that is shown in Fig. 1B (*15*). The "Pro" domain of the latent molecule is folded around so that the cysteine residue in the conserved PRCGVPD region can form a complex with the zinc molecule (*16*). Proteinases (trypsin, plasmin, chymotrypsin, neutrophil elastase, and plasma kalikrein) that attack a short basic sequence exposed on the surface of the molecule are thought to trigger the activation process of proMMPs *in vivo* (*17*). This initial cleavage causes a conformation change in the molecule that disrupts the cysteine-Zn^{2+} interaction and frees the Zn^{2+} to participate in the proteolytic cleavage. The mechanism is thus called "cysteine switch mechanism." The enzyme can then attack the peptide sequence downstream of the PRCGVPD in an autolytic manner and cleave it (by both intra- and intermolecular mechanisms). An alternative activation of the enzyme with mercurial compounds such as aminophenyl-mercuric acetate (APMA) causes chemical modification of the cysteine and generates free zinc at the catalytic center by the cysteine switch mechanism.

Gelatinase A is unique among MMPs in that it does not possess sequences susceptible to proteolytic activation by the above serine proteinases. The mechanism of progelatinase A activation has been attracted attention of many tumor biologists, because a close association between the expression of activated gelatinase A and tumor spread has been found in various types of tumors (*10, 11*).

Identification of membrane-type MMP

Activated gelatinase A is released *in vitro* from fibrosarcoma HT1080, breast tumor MDA-MB-321, and normal fibroblast cells stimulated with TPA or concanavalin A (*18-21*). The activation activity resides in the plasma membrane fraction and it is sensitive to EDTA and TIMP-2 which are inhibitors of MMP, thus suggesting that the activator might be a membrane-bound MMP.

All the known MMPs are in a soluble form and none of them is the membrane-type. The complexity of extracellular matrix components suggests that more MMPs are involved in the efficient turnover of extracellular matrix during tissue remodeling. However, conventional biochemical approaches are no longer useful for identifying unknown enzymes. Polymerase chain reaction (PCR) using a set of degenerate primers for conserved amino acid sequences is a powerful means with which to identify new members of a gene family. Amino acid sequences for the cysteine switch (PRCGVPD) and binding sites for zinc molecule are highly conserved among all known MMPs. Reverse-transcription PCR amplification of MMP gene fragments with degenerate primers for these sequences using mRNA from various sources as a template has amplified not only known MMP genes but also unique fragments homologous to MMP (*22*). One of these was derived from human placenta and the other from melanoma tissue. Two cDNAs have been isolated from a human placenta cDNA library using these fragments as probes (*22-24*). Long open reading frames encoding 582 and 604 amino acids (aa) have been identified; both of these were aligned with known MMP family members but they were most closely related to each other (Fig. 2). While these proteins have a common MMP domain structure, they have three unique insertions. First there is an insertion of 11 aa between the pro-peptide and the catalytic enzyme domains. Stromelysin-3/MMP-11 has a similar insertion at the same position (*25*). The conserved RXKR sequences precede the potential processing sites of these two MMPs. The peptide bond following RXKR is the processing site of many secretory proteins by the eukaryotic KEX2 family of endopeptidase (*26*). Stromelysin-3 is activated by furin, one of intracellular KEX2 family endopeptidases (*27*). The MT-MMPs have a second insertion of 8 aa in the catalytic enzyme domain which is not present in other MMPs. The

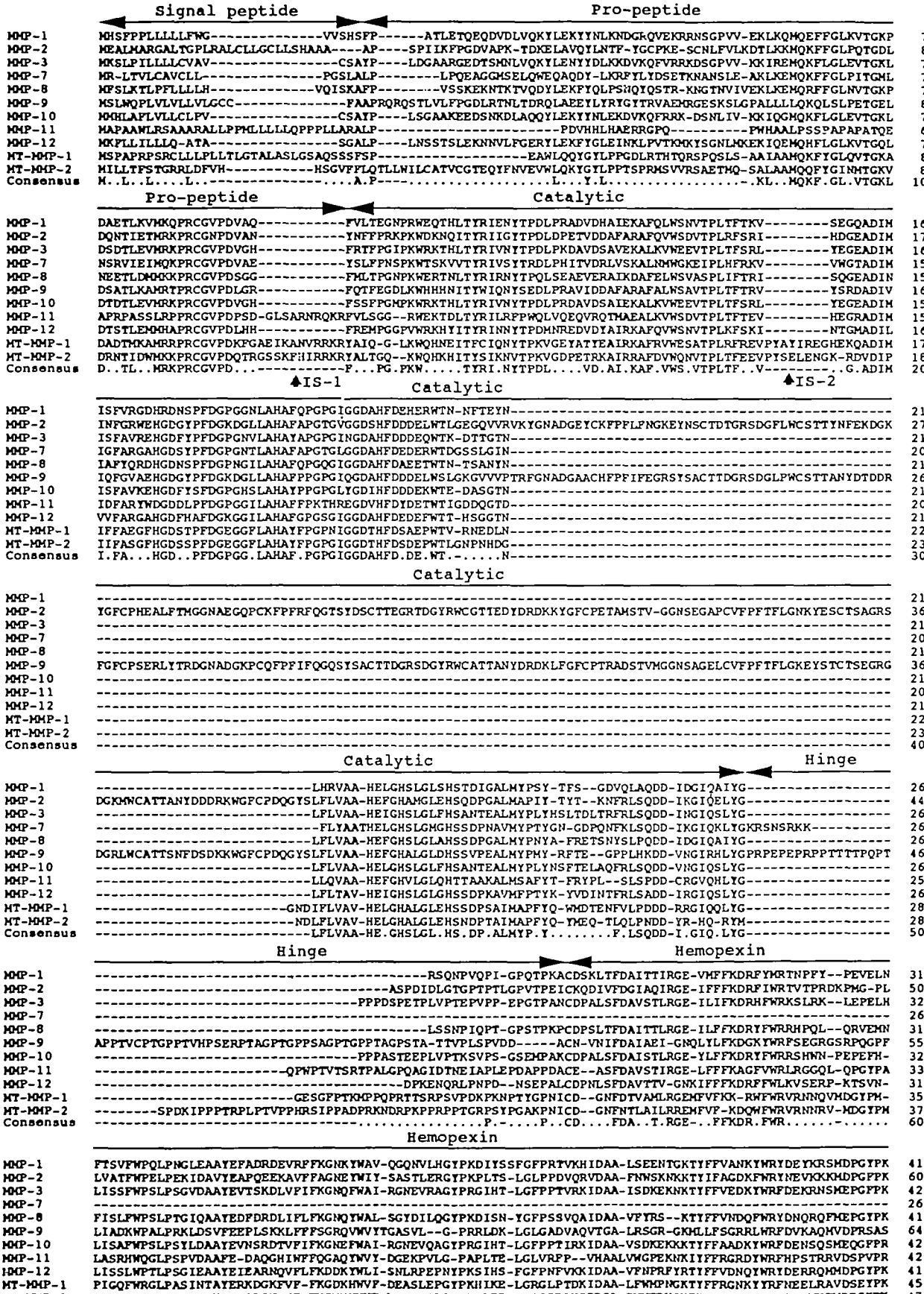

Fig. 2. (continued on next page)

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                                            Hemopexin
           -------------------------------------------------->
MMP-1      MIAHDFPGIGHKVDAVFMKDGFF--YFFHGTRQYKFDPKT-KRILTL-QKANS-WFNCRKN---------------------------------------  469
MMP-2      LIADAWNAIPDNLDAVVDLQGGGHSYFFKGAYYLKLENQS-LKSVKF-GSIKSDWLGC------------------------------------------  660
MMP-3      QIAEDFPGIDSKIDAVFEEFGFF--YFFTGSSQLEFDPNA-KKVTHT-LKSNS-WLNC------------------------------------------  477
MMP-7      ----------------------------------------------------------------------------------------------------  267
MMP-8      SISGAFPGIESKVDAVFQQEHFF--HVFSGPRYYAFDLIA-QRVTRV-ARGNK-WLNCRYG---------------------------------------  467
MMP-9      EVDRMFPGVPLDTHDVFQYREKA--YFCQDRFYWRVSSRSELNQVDQVGYVTYDILQCPED---------------------------------------  707
MMP-10     LIADDFPGVEPKVDAVLQAFGFF--YFFSGSSQFEFDPNA-RMVTHI-LKSNS-WLHC------------------------------------------  476
MMP-11     R-ATDWRGVPSEIDAAFQDADGYA-YFLRGRLYWKFDPVK-VKALEGFPRLVGPDFFGCAEPANTFL---------------------------------  488
MMP-12     LITKNFQGIGPKIDAVFYSKNKY-YYFFQGSNQFEYDFLL-QRITKT-LKSNS-WFGC------------------------------------------  470
MT-MMP-1   NIKVWE-GIPESPRGSFMGSDEVFTYFYKGNKYWKFNNQKLKVEPGYPKSALRDWMGCPSGGRPDEGTEEETE-VIIIEVDEEGGGAVSAAAVVLPVLLL  549
MT-MMP-2   PITVWK-GIPESPQGAFVHKENGFTYFYKEGVLEIQTTRYSRLEPGHPRSILKDLSGCDGPTDRVKEGHSPPDDVDIVIKLDNTASTVKAIAIVIPCILA  571
Consensus  .I...F.GI....DAVF......--YFF.G.....FD...-......-.....-W..C------------------------------------------  800
                                                                                      ▲IS-3

MMP-1      ---------------------------------  469
MMP-2      ---------------------------------  660
MMP-3      ---------------------------------  477
MMP-7      ---------------------------------  267
MMP-8      ---------------------------------  468
MMP-9      ---------------------------------  708
MMP-10     ---------------------------------  476
MMP-11     ---------------------------------  489
MMP-12     ---------------------------------  470
MT-MMP-1   LLVLAVGLAVFFFRRHGTPRRLLYCQRSLLDKV  582
MT-MMP-2   LCLLVLVYTVFQFKRKGTPRHILYCKRSMQEWV  604
Consensus  ---------------------------------  833
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Fig. 2 **Alignment of MMP aa sequences.** The reported human MMPs are listed and aligned with the deduced MT-MMP-1 and MT-MMP-2 proteins. Specific insertions characteristic to MT-MMPs are indicated by the upward arrows (IS-1 to IS-3)

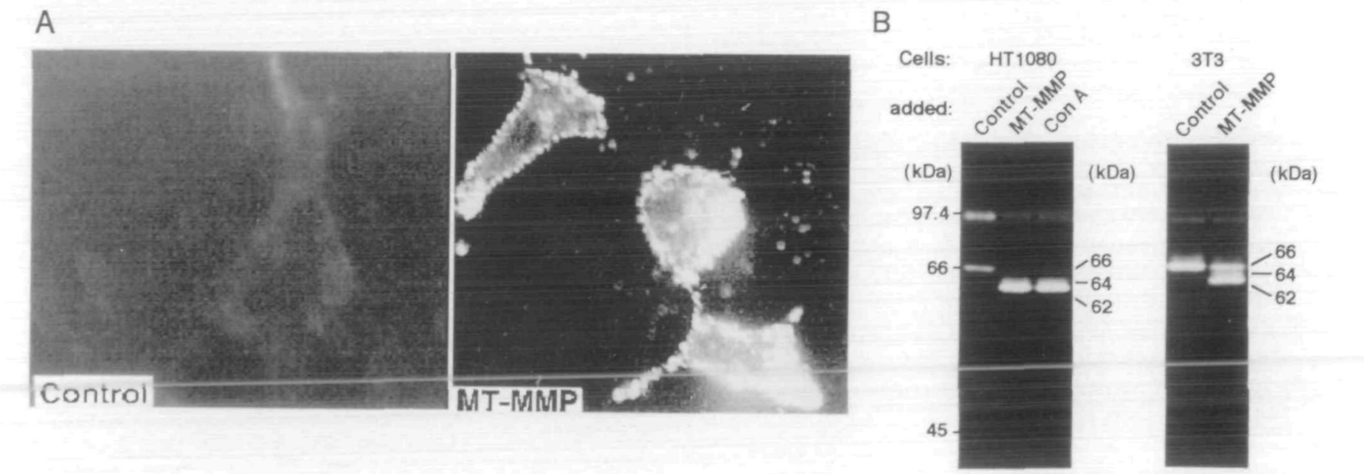

Fig. 3. **Expression of MT-MMP.** A: Cell surface localization of MT-MMP expressed in COS-1 cells. COS-1 cells transfected with control or MT-MMP-1 expression plasmid were stained by an immunofluorescent antibody staining method using a monoclonal antibody against MT-MMP-1. B: Processing of progelatinase A by the expression of MT-MMP-1 Culture supernatants from HT-1080 or NIH3T3 cells transfected with control or MT-MMP-1 expression plasmid or treated with concanavalin A were analyzed by gelatin zymography.

third insertion at the C terminus contains a hydrophobic amino acid stretch which can pass through the plasma membrane and act as a potential transmembrane domain. Therefore, they were named membrane-type MMP (MT-MMP-1 and MT-MMP-2, respectively) (*23, 24*). MT-MMP proteins were localized in the membrane by the immunofluorescent staining of transfected cells with monoclonal antibodies raised against MT-MMP-1 and MT-MMP-2 peptides (Fig. 3A). These antibodies recognized 63 and 64 kDa proteins for MT-MMP-1 and MT-MMP-2, respectively.

Activation of progelatinase A by MT-MMP expression

The expression of MT-MMP on the cell surface fits the requirements for an activator for progelatinase A. The expression of MT-MMP-1 in cells secreting 68 kDa progelatinase A and 92 kDa progelatinase B converted only progelatinase A to the 62 kDa fully active form through the 64 kDa intermediate as demonstrated by gelatin zymography (Fig. 3B) (*24*). MT-MMP-2 expression also induced progelatinase A activation (*23*). The MT-MMP-1 mRNA transcript and protein were expressed in MDA-MB-231 cells stimulated with concanavalin A and HT1080 cells exposed to TPA, indicating that the gelatinase A activator in these cells is MT-MMP-1 (*28, 29*).

Expression of MT-MMP genes in human tissues

MT-MMP-1 and MT-MMP-2 mRNA transcripts (4.5 and 12 kb, respectively) are expressed in various tissues, but they are distributed quite differently (*22, 23*). MT-MMP-1 mRNA expression is predominant in the lungs, kidneys, and placenta where extracellular matrix remodeling is relatively active, and lowest in the brain. In contrast, MT-MMP-2 mRNA expression is high in the heart, brain, and placenta, and is undetectable in the lungs, kidneys, liver, spleen, and muscle.

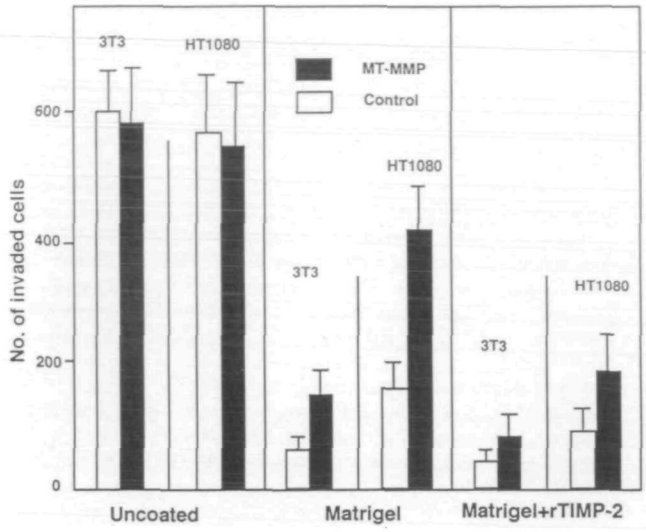

Fig. 4. **Expression of MT-MMP-1 stimulates the invasion of cells *in vitro*.** NIH3T3 and HT1080 cells were transfected with MT-MMP-1 (MT-MMP) or control plasmids, then the invasion of the reconstituted basement membrane in the presence (Matrigel + rTIMP-2) or absence of 10 μg/ml recombinant TIMP-2 (Matrigel) was assayed. Motility was analyzed on uncoated filters (Uncoated).

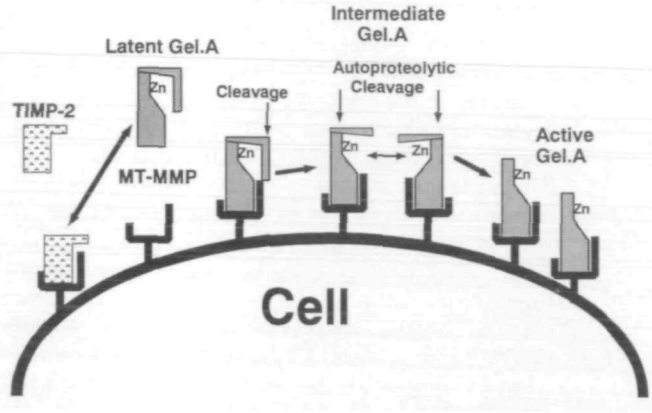

Fig. 5. **Scheme illustration of the binding and activation of progelatinase A by MT-MMP and its inhibition by TIMP-2 on the cell surface.**

Elevated expression of MT-MMP-1 in tumor tissues

A significant statistical association between the expression level of activated gelatinase A in tumor tissue and tumor spread has been reported. Therefore, attempts have been made to identify the gelatinase A activator in tumor tissues (*24, 30, 31*). MT-MMP-1 expression is elevated in various tumor tissues including lung, gastric, colon, and breast cancers, in which activated gelatinase A is expressed. Detailed statistic analyses have demonstrated a close correlation between MT-MMP-1 expression and gelatinase A activation in lung carcinoma, indicating that MT-MMP-1 is involved in the activation of gelatinase A in tumor tissue. Although the MT-MMP-2 cDNA fragment has been obtained from melanoma tissue, elevated expression of MT-MMP-2 is not often observed in tumor tissues.

According to immunohistochemical studies of gastric carcinomas, MT-MMP-1 is predominantly localized in and on carcinoma cells (*30*). On the other hand, gelatinase A is immunolocalized only on the cell membranes of carcinoma cells. Almost all gelatinase A-positive tumors expressed MT-MMP-1 in the cells as well. In most of these, fibroblasts and vascular endothelial cells in the advanced carcinoma tissue were also immunostained for MT-MMP and gelatinase A and the staining was weak or negative in the normal tissue remote from the carcinoma. It seems that MT-MMP-1 is expressed in the stromal cells in pathophysiological conditions, where the extracellular matrix macromolecules are undergoing rapid remodeling. Colocalization of gelatinase A with MT-MMP-1 on the tumor cell membrane raised the question as to how gelatinase A, of which the mRNA transcript was detected in stromal cells around tumor cells, localizes on the tumor cell membrane. This is consistent with the *in vitro* finding that activated gelatinase A is enriched in the plasma membrane of cancer cells. Although the specific binding of gelatinase A to the surface of tumor and normal fibroblast cells has been described, a membrane protein that functions as a gelatinase A receptor has not been identified.

MT-MMP-1 also functions as a receptor of gelatinase A

The fact that activated gelatinase A is enriched in the plasma membrane of cancer cells in which MT-MMP localizes, suggests that MT-MMP is involved not only in the activation but also in the binding of gelatinase A (*32, 33*). The specific binding of gelatinase A to the cells expressing MT-MMP was demonstrated by a study using ^{125}I-labeled progelatinase A that was processed to the intermediate form (Sato, H., Takino, T., Kinoshita, T., Imai, K., Okada, Y., Stetler-Stevenson, W.G., and Seiki, M., manuscript submitted). Complex formation between MT-MMP and gelatinase A was demonstrated by immunoprecipitation using monoclonal antibodies against MT-MMP and gelatinase A. These results indicated that MT-MMP serves not only as an activator but a receptor of gelatinase A. Processing from the intermediate to fully active form was dependent on the gelatinase A concentration. Unlike the wild-type progelatinase A, a mutant defective in catalytic function was processed only to the intermediate form by co-expression with MT-MMP. Thus, the processing of progelatinase A to intermediate form is catalyzed by the function of MT-MMP and the binding with MT-MMP on the cell surface concentrates the intermediate form locally to allow autoproteolytic processing to the fully active form.

Blocking of gelatinase A binding by TIMP-2

TIMP-2 but not TIMP-1 binds to the surface of normal fibroblast cells and inhibits the activation of progelatinase A by these cells (*34*; Sato, H., Takino, T., Kinoshita, T., Imai, K., Okada, Y., Stetler-Stevenson, W.G., and Seiki, M., manuscript submitted). This suggests direct interaction between TIMP-2 and MT-MMP. Indeed, TIMP-2 bound to cells expressing MT-MMP and interfered with the binding and activation of progelatinase A. Immunoprecipitation has shown that TIMP-2 forms a complex with MT-MMP. Therefore, TIMP-2 is thought to competitively inhibit binding of gelatinase A to MT-MMP.

Expression of MT-MMP stimulates the invasion of cells

As gelatinase A degrades various extracellular matrix macromolecules, including type IV collagen and laminin,

we determined whether or not progelatinase A activated by MT-MMP expression enhances the invasiveness of cells producing progelatinase A. We found that MT-MMP expression in HT-1080 and NIH3T3 cells more than doubled the number of invasive cells compared with controls when analysed using a modified Boyden Chamber (Fig. 4) (*24*). The invasion was sensitive to TIMP-2, which inhibits binding and activation of gelatinase A by MT-MMP and also the proteolytic activity of activated gelatinase A.

Implications

Tumor spread reportedly correlates with increased levels of activated gelatinase A (*10, 11*). This activation occurs in association with the plasma membrane and activated gelatinase A binds specifically to the tumor cell surface, although the molecular features of these interactions remain to be elucidated. MT-MMP-1 fulfills the criteria for a plasma membrane associated activator and receptor of gelatinase A. *Via* this mechanism, MT-MMP localizes matrix digestion to the vicinity of the tumor cell surface (Fig. 5). The cell surface binding of gelatinase A not only promotes enzyme activation but also regulates enzyme activity by increasing the rate of substrate cleavage (*35*). The cell surface localization of proteinases is a common cellular strategy for regulating pericellular proteolysis, as exemplified by urinary-type PA (uPA), the activity of which is localized predominantly on the cell surface *via* binding to the specific uPA receptor (*36*). Co-expression of MT-MMP and gelatinase A in osteoblasts during mouse embryonic development and the efficient activation of progelatinase A in the tissue indicate that the MT-MMP-gelatinase A system plays an important role in tissue remodeling during organogenesis (Kinoh, A., Sato, H., Tsunezuka, Y., Okada, Y., Kawashima, A., and Seiki, M., manuscript submitted). Thus, MT-MMP and its family play a central role in the activation and cell surface localization of gelatinase A not only in tumor invasion and metastasis but also during normal development. Identification of the proteinase that triggers the proteolytic cascade of human cancers should be valuable both as an index of prognosis and as a guide in the design of antiproteolytic strategies aimed at controlling the progression of the disease.

Note Added in Proof—While this manuscript was being prepared, the new MT-MMP was reported [Will, H. and Hinzmann, B. (1995) *Eur. J. Biochem.* **231**, 602-608]. Thus, MT-MMP-2 in this paper will be renamed as MT-MMP-3.

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