Expression and Tissue Localization of Membrane-Types 1, 2, and 3 Matrix Metalloproteinases in Human Invasive Breast Carcinomas

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ABSTRACT

Activation of the zymogen of matrix metalloproteinase 2 (proMMP-2, gelatinase A) possibly is one of the key steps in invasion and metastasis of various human carcinomas. Three different membrane-type MMPs (MT-MMPs), MT1-, MT2-, and MT3-MMPs are thought to be activators of proMMP-2 in the tissues. MT4-MMP is structurally different from the other three enzymes, and its function as proMMP-2 activator is uncertain.

In the present study of human invasive breast carcinomas, we examined a correlation between the expression of MT1-, MT2-, and MT3-MMPs, immunolocalization of MT1- and MT2-MMPs, and proMMP-2 activation. Northern blot analysis demonstrated the predominant expression of MT1-MMP mRNA in carcino tumors (20 of 20 cases), whereas MT2-MMP was detected in only 25% of the cases (5 of 20 cases), and no detectable expression of MT3-MMP was observed. The expression levels of MT1-MMP but not MT2-MMP correlated well with the presence of lymph node and distant metastases, clinical stages, and size of tumors. Immunohistochemically, MT1-MMP was localized predominantly in the carcinoma cells in all of the samples (32 of 32 cases). Immunostaining of MT2-MMP in the carcinoma cells was observed in only 38% of the cases (12 of 32 cases). Immunoblot analysis of tumor homogenates confirmed the presence of these MT-MMPs. Activation of proMMP-2 was significantly higher in the carcinoma samples with lymph node or distant metastasis compared to carcinoma without metastasis, normal control, or fibrocystic disease (P < 0.05). An increase in the activation ratio of proMMP-2 correlated directly with the expression of MT1-MMP but not MT2-MMP, as measured by either Northern blot analysis or immunostaining. These results suggest that MT1-MMP may play a key role in human breast carcinoma invasion and metastasis by being predominantly responsible for activation of proMMP-2.

INTRODUCTION

Degradation of the ECM surrounding cancer cells and blood vessels is an essential step for cancer invasion and metastasis. Previous studies have demonstrated that MMPs are highly expressed in various cancers (1). Among the MMPs, MMP-2 (gelatinase A) is considered to be especially important in the malignant behavior of the tumor cells (1, 2). However, overexpression is not sufficient for the in vivo action of MMP-2. Because the MMPs, except for MMP-11 (stromelysin 3), are secreted as inactive zymogens (proMMPs), an activation step is required for their function in vivo. proMMPs can be activated by various factors such as organoneroesterases, serine proteinases, hyphochlorous acid, and acid exposure (3). Serine proteinases including trypsin, plasmin, plasma kallikrein, and neutrophil elastase may be generally important as in vivo activators of proMMPs (3), proMMP-2 is unique in that it is activated by membrane-associated metalloproteinases (4, 5) but not by serine proteinases (6). Our previous studies demonstrated that a new MMP with a transmembrane domain, i.e., MT-MMP, is an activator of proMMP-2 (7). After discovery of the MT-MMP, three more members belonging to the MT-MMP subgroup were cloned, and they are now named MT1-, MT2-, MT3, and MT4-MMPs (7—10). Among these MT-MMPs, MT4-MMP is distinguished from others because of its low amino acid sequence homology (less than 30% homology to MT1-MMP; Ref. 10). In addition, it is uncertain whether MT4-MMP can activate proMMP-2, whereas all three MT1-, MT2-, and MT3-MMPs have the ability to activate proMMP-2.

In human breast carcinomas, active MMP-2 species had been detected in the culture media of the carcinoma tissues (11, 12), indicating the presence of proMMP-2 activator(s). In fact, MT1-MMP mRNA expression has been reported in the breast carcinoma tissues (13—15). However, these studies did not show the relationship between the expression and proMMP-2 activation. In addition, the expression of MT2- and MT3-MMPs in human carcinoma tissues has not been examined. Thus, the question of which of the MT-MMPs is responsible for proMMP-2 activation in breast carcinomas also remains unanswered. We, therefore, examined the expression and tissue localization of MT1-, MT2-, and MT3-MMPs and studied correlation between their expression and proMMP-2 activation in human invasive breast carcinomas.

MATERIALS AND METHODS

Clinical Samples and Histology. Fresh tissue samples were obtained from 32 patients with primary invasive breast carcinoma and 19 patients with fibrocystic disease who underwent surgery in the University Hospital, School of Medicine, Kanazawa University, and were used for histology and immunostaining. The patients with carcinoma ranged from 25 to 84 years of age (mean ± SD, 56.8 ± 11.9), and those with fibrocystic disease ranged from 20 to 72 years of age (54.2 ± 13.5). Surgical specimens were fixed with 10% buffered formalin, and paraffin sections were stained with H&E. The carcinomas were classified according to the WHO International Classification of Breast Tumors. Thirty-two cases of invasive carcinomas consisted of infiltrating ductal (28 cases), mucinous (3 cases), and squamous cell carcinomas (1 case). They were carcinomas of grade I (6 cases), grade II (22 cases), and grade III (4 cases). Control normal breast tissues (32 cases) were also obtained from sites remote from the tumor. Foci of fibroadenoma were observed in 4 cases of the 19 fibrocystic disease samples.

Northern Blot Analysis. Total RNA was extracted by the acid guanidinium-phenol-chloroform method (7). Because some samples were not sufficient for RNA extraction, 20 specimens of carcinoma (17 infiltrating ductal and 3 mucinous) and control normal breast tissues obtained from sites remote from the carcinoma and 10 specimens of fibrocystic breast disease were used for RNA preparation. Fibrocystic breast disease tissues included foci of fibroadenoma in 4 cases. Of 20 carcinoma case patients, 10 had lymph node metastasis, and 5 patients showed distant metastasis. The RNA samples (10 μg/tane) were electrophoresed on 1% agarose gels containing 2 M formaldehyde and transferred onto Hybond-N* membranes (Amersham International, Tokyo, Japan). The membranes were hybridized with 32P-labeled probes for MT1-MMP (1.2-kb cDNA fragment corresponding to nucleotides 1647—2889; 2055)
Fig. 1. Characterization of the monoclonal antibodies (clones 114-6G6 and 162-22G5) by immunoblotting and immunostaining of COS-1 cells transfected with cDNA for MT1-, MT2-, or MT3-MMP. A, lysates of the COS-1 cells transfected with pSG5 vector alone (Lane 1) or cDNA for MT1-MMP (Lane 2), MT2-MMP (Lane 3), and MT3-MMP (Lane 4) were resolved by SDS-PAGE under reduction and transferred onto nitrocellulose filters. The filters were immunostained with the antibodies to MT1-MMP (114-6G6) and MT2-MMP (162-22G5) or nonimmune mouse IgG as described in "Materials and Methods." The molecular weights of the protein standards are phosphorylase b (M, 94,000), transferrin (M, 77,000), BSA (M, 68,000), heavy chain of IgG (M, 55,000), ovalbumin (M, 43,000), and carbonic anhydrase (M, 29,000). B, cells transfected with cDNA for MT1-MMP (MT1), MT2-MMP (MT2), or MT3-MMP (MT3) were immunostained with the antibodies to MT1-MMP (114-6G6) and MT2-MMP (162-22G5) as described in "Materials and Methods." Hematoxylin counterstain. Bar, 50 μm.
4 cases of fibroadenoma were fixed with periodate-lysine-paraformaldehyde (PLP) fixative (2) for 18–24 h at 4°C, and the paraffin sections were immunostained using the monoclonal antibodies to MT1-MMP (20 μg/ml; clone 114-G6G) and MT2-MMP (20 μg/ml; clone 162-22G5) or nonimmune mouse IgG (20 μg/ml) as described above. The ratio (%) of immunoreactive cells to total carcinoma cells was measured by counting cells in five different fields at ×200 without knowledge of the clinical data.

Tissue homogenates were prepared from the samples obtained for immunostaining. They were weighed and homogenized in 50 mM Tris-HCl buffer (pH 7.5) containing 0.15 M NaCl, 10 mM CaCl₂, and 0.05% Brij35 on ice. The supernatants were obtained by centrifugation at 4°C for 20 min at 10,000 × g. The protein concentrations were determined by the dye-binding method according to the manufacturer's instructions (Bio-Rad, Hercules, CA). The homogenate samples (20 μg/lane) from five cases were resolved by SDS-PAGE under reduction and transferred onto nitrocellulose filters. The filters were immunostained as described above.

Gelatin Zymography. Gelatinolytic activity in the above-mentioned tissue homogenates was examined by gelatin zymography. The supernatants (20 μg protein/lane) were subjected to SDS-PAGE using gelatin-containing gels as described previously (16). Ratios of proMMP-2 activation were estimated by computer-assisted densitometric scanning of M₆ 62,000 and M₆ 68,000 proteolytic bands, which correspond to active and latent species of MMP-2, respectively (16).

Statistics. Statistical analyses were performed using the χ² test and the two-tailed Mann-Whitney U test. P ≤ 0.05 was considered significant.

RESULTS

mRNA Expression of MT1-, MT2-, and MT3-MMPs. Northern blot analyses of MT1-, MT2-, and MT3-MMPs were performed with total RNA extracted from breast carcinoma, control normal breast, and fibrocystic breast disease tissues. For quantitative analysis of the expression levels of the MT-MMPs, the intensity of the hybridization signal obtained with proteinase probes was normalized to that obtained with GAPDH-specific probe using the Bioimage Analyzer BAS 1000 according to the method reported by Yamamoto et al. (17). Samples with the MT-MMP/GAPDH signal ratio of greater than 0.1 were scored as positive. A strong signal at 4.5 kb was seen in all of the carcinoma samples (20 of 20 cases), whereas no hybridization was detected in the normal breast counterparts (Fig. 2). In fibrocystic disease samples, none or negligible hybridization signal for MT1-MMP was recognized (Fig. 2). The MT2-MMP mRNA of 3.6 kb was also found only in carcinomas, but the expression was confined to lymph node metastasis tissues, respectively (16).

Fig. 2. mRNA expression of MT1-, MT2-, and MT3-MMPs in human breast carcinomas. Total RNA was extracted from surgically resected specimens, and Northern blotting for MT1-, MT2-, and MT3-MMPs was carried out as described in "Materials and Methods." Representative six carcinoma samples (three cases each with or without lymph node metastasis) and two benign breast samples (one fibroadenoma and one fibrocystic disease) are shown. Molecular sizes of the transcripts are 4.5 kb for MT1-MMP, 3.6 kb for MT2-MMP, 12 kb for MT3-MMP, and 1.2 kb for GAPDH. T, N, FA, and FC are the tissue samples from carcinoma, normal control, fibroadenoma, and fibrocystic breast disease, respectively. LN(+) and LN(−) cases with or without lymph node metastasis. RNA from OSC-19 cells was used as a positive control for Northern blotting.

Ref. 7, MT2-MMP (1.2-kb fragment to nucleotides 273-1526; Ref. 8), MT3-MMP (2.1-kb fragment to nucleotides 1—2107; Ref. 9), and GAPDH as described by us previously (7, 9). As for a control, total RNA was extracted from OSC-19 cells (a highly metastatic oral squamous cell carcinoma cell line), which are known to express MT-MMPs (9), and the samples were processed in a similar way. The blotted membranes were scanned by Bioimage Analyzer BAS 1000 (Fuji Photo Film, Tokyo, Japan).

Antibodies, Immunoblotting, and Immunohistochemistry. The monoclonal antibodies to MT1-MMP and MT2-MMP were developed by use of the synthetic peptides REVPAYAIREGHKE (corresponding to the amino acids at positions 160—173 in the human MT1-MMP) and DTDNFQLPEDDLRG (corresponding to the amino acids at positions 281—294 in the mouse MT2-MMP), respectively, and were provided by Dr. Kazushi Iwata at Fuji Chemical Industries, Ltd. (Takaoka, Japan). After screening of the clones by ELISA using these peptides, two clones, 114-G6G and 162-22G5, were selected as a candidate for the antibodies against MT1-MMP and MT2-MMP, respectively. The monospecific reactivity of the antibodies and their applicability to immunohistochemistry were further determined by immunoblotting and immunostaining analyses of COS-1 cells transfected with cDNA for human MT1-, MT2-, or MT3-MMP (7—9) by calcium phosphate coprecipitation methods (9). For immunoblotting, cell lysates were subjected to SDS-PAGE under reduction and transferred onto nitrocellulose filters (Amersham International, Buckinghamshire, United Kingdom). The filters were reacted with 8 μg/ml monoclonal antibodies (IgG) to MT1-MMP (clone 114-G6G) and MT2-MMP (clone 162-22G5) or 8 μg/ml nonimmune mouse IgG. After reactions with biotinylated horse IgG to mouse IgG (Vector Laboratories, Burlingame, CA) and an avidin-biotin-peroxidase complex (Dako, Glostrup, Denmark), the color was developed with 3,3′-diaminobenzidine tetrahydrochloride (Sigma Chemical Co., St. Louis, MO) as described previously (2). Both antibodies specifically recognized corresponding MT1-MMP (M₆ 66,000 proMT1-MMP and M₆ 60,000 MT1-MMP) and MT2-MMP (M₆ 68,000 proMT2-MMP and M₆ 62,000 MT2-MMP) proteins produced by the transfected cells, and no cross-reactivity of the antibodies with MT3-MMP was seen (Fig. 1A). Immunohistochemistry was performed with the transfected cells cultured on Lab-Tek chamber slides (Nunc, Inc., Naperville, IL). The cells were reacted with 20 μg/ml monoclonal antibodies to MT1-MMP (clone 114-G6G) and MT2-MMP (clone 162-22G5) or 20 μg/ml nonimmune mouse IgG and followed by the avidin-biotin-peroxidase-complex methods as described above. As shown in Fig. 1B, the antibodies of 114-G6G and 162-22G5 specifically stained the cells transfected with cDNA for MT1-MMP and MT2-MMP, respectively. No immunostaining was obtained with nonimmune IgG (data not shown).

Tissue samples of the carcinoma (32 cases), control normal breast remote from the tumor (32 cases), and fibrocystic breast disease (19 cases) including 4 cases of fibroadenoma were fixed with periodate-lysine-paraformaldehyde (PLP) fixative (2) for 18–24 h at 4°C, and the paraffin sections were immunostained using the monoclonal antibodies to MT1-MMP (20 μg/ml; clone 114-G6G) and MT2-MMP (20 μg/ml; clone 162-22G5) or nonimmune mouse IgG (20 μg/ml) as described above. The ratio (%) of immunoreactive cells to total carcinoma cells was measured by counting cells in five different fields at ×200 without knowledge of the clinical data.

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Gelatin Zymography. Gelatinolytic activity in the above-mentioned tissue homogenates was examined by gelatin zymography. The supernatants (20 μg protein/lane) were subjected to SDS-PAGE using gelatin-containing gels as described previously (16). Ratios of proMMP-2 activation were estimated by computer-assisted densitometric scanning of M₆ 62,000 and M₆ 68,000 proteolytic bands, which correspond to active and latent species of MMP-2, respectively (16).

Statistics. Statistical analyses were performed using the χ² test and the two-tailed Mann-Whitney U test. P ≤ 0.05 was considered significant.
Fig. 4. Immunolocalization of MT1-MMP and MT2-MMP in the breast carcinoma tissues. Paraffin sections were immunostained with monoclonal antibodies against MT1-MMP (MT1) and MT2-MMP (MT2) or with nonimmune mouse IgG (Cont) as described in “Materials and Methods.” Note that MT1-MMP and MT2-MMP are immunostained within the carcinoma cells, whereas no staining is observed with nonimmune IgG. MT1-MMP also appeared to be localized on the cell membranes of some carcinoma cells (arrows). Hematoxylin counterstain. Bar, 50 μm.

25% of the cases (5 of 20 cases; Fig. 2). MT3-MMP mRNA was undetectable in all of the specimens examined (Fig. 2).

The expression of MT1-MMP and MT2-MMP in the carcinoma samples was compared with clinicopathological factors. The data indicated that expression levels of MT1-MMP, but not MT2-MMP, were significantly higher in the carcinoma samples from patients with lymph node metastasis (0.76 ± 0.15, n = 10) than in those without metastasis (0.42 ± 0.20, n = 10; P < 0.01; Fig. 3 and data not shown for MT2-MMP). In addition, the expression levels were significantly enhanced in the carcinoma group with distant metastasis (0.81 ± 0.18, n = 5) as compared to that without metastasis (0.54 ± 0.24, n = 15; P < 0.05; Fig. 3). Significantly higher expression of MT1-MMP was also observed with the carcinoma group in the advanced stages (stages II-IV; 0.75 ± 0.14, n = 12) than those in the early stage (stage I; 0.35 ± 0.16, n = 8; P < 0.01; data not shown). In addition, larger carcinomas (>2 cm in diameter) showed a significantly higher (2-fold) level of MT1-MMP (0.75 ± 0.14, n = 12) than small carcinomas with <2 cm in diameter (0.35 ± 0.16, n = 8; P < 0.01; data not shown). Although there were no correlations between the MT1-MMP expression and histopathological types of the carcinomas, the expression was remarkably higher in the carcinoma group of grades II and III (0.87 ± 0.21, n = 16) than in grade I carcinomas (0.36 ± 0.24, n = 4; P < 0.05; data not shown). This was mainly because carcinomas with moderate and severe atypia showed higher MT1-MMP expression (0.69 ± 0.19, n = 14) than those with mild atypia (0.36 ± 0.22, n = 6; P < 0.01). No definite correlations were observed between the MT2-MMP expression and clinicopathological factors (data not shown). However, it is notable that three of five cases with distant metastasis, all of which belonged to stage IV, showed expression of MT2-MMP as well as MT1-MMP. No correlations between the MT-MMP expression and menopausal factor or estrogen receptor were observed (data not shown).

Immunohistochemistry and Immunoblotting. MT1-MMP was predominantly immunolocalized to the carcinoma cells in all of the carcinoma cases (32 of 32 cases), in some of which the staining appeared to be present on the cell membranes (Fig. 4). On average, 40% of the total carcinoma cells (41.5 ± 23.8%) were positively immunostained in each case. Stromal cells and endothelial cells of blood vessels in the carcinoma tissues were occasionally immunostained for MT1-MMP (data not shown). Immunoblotting for MT1-MMP and MT2-MMP. The tissue homogenates from carcinoma (T) and control normal tissues (N) resolved by SDS-PAGE were transferred onto nitrocellulose filters, and the filters were immunostained with the antibodies to MT1-MMP and MT2-MMP. Note that two bands corresponding to latent and active forms of MT1-MMP (M, 66,000 and M, 60,000) or MT2-MMP (M, 68,000 and M, 62,000) are found in the carcinoma samples, whereas no such species are recognized in the control normal tissues. The molecular weights of the protein standards are as shown in Fig. 1A.

Fig. 5. Gelatin zymography of the tissue homogenates from breast carcinomas. The supernatants of tissue homogenates from carcinoma (C) and control normal tissues (N) were subjected to gelatin zymography as described in “Materials and Methods.” Representative six carcinoma samples with or without lymph node metastasis (LN(+) and LN(–), respectively) and two benign breast samples (one fibroadenoma and one fibrocystic disease) are shown. Major gelatinolytic activities with Mr 68,000 (arrowhead) and Mr 62,000 (arrow), which correspond to proMMP-2 and MMP-2, respectively, are indicated. The molecular weights of the protein standards are as shown in Fig. 1A.
stromal fibroblasts were weakly immunostained in 26% of the cases with active fibrotic changes (5 of 19 cases; data not shown). Weak immunostaining in some ductal cells was also observed in 32% of the cases (data not shown). However, no staining was seen in normal control samples (data not shown). MT2-MMP was almost exclusively immunolocalized in the carcinoma cells (Fig. 4), but the staining was observed in only 38% of the cases (12 of 32 cases). Approximately one-half of the total carcinoma cells (48.6 ± 25.3%) were immunoreactive for MT1-MMP in the positive cases. No staining was observed with nonimmune mouse IgG (Fig. 4).

Both latent and active forms of MT1-MMP (M₆, 66,000 and M₆, 60,000) and MT2-MMP (M₆, 68,000 and M₆, 62,000) were identified by Western blot in the tissue homogenates of carcinoma samples with positive immunostaining. Immunoblots of the normal or fibrocystic disease samples were negative (Fig. 5).

Activation of proMMP-2 and Correlation with Expression of MT1-, MT2-, and MT3-MMPs. proMMP-2 activation in the supernatants of the tissue homogenates was analyzed by gelatin zymography. The proMMP-2 of M₆, 68,000 was detected in all of the samples examined (Fig. 6). On the other hand, the active species of M₆, 62,000 was found in 97% of the carcinoma samples (31 of 32 cases) compared to 19% of the normal control samples (6 of 32 cases; Fig. 6). Computer-assisted image analyses of the proteolytic bands intensity showed that an activation ratio of proMMP-2 (the ratio of the M₆, 62,000 active form to proMMP-2 and active forms) was significantly higher in the carcinoma samples (0.52 ± 0.13, n = 32) than in the normal control (0.06 ± 0.05, n = 32) or fibrocystic disease samples (0.13 ± 0.09, n = 19; P < 0.01; Fig. 7). In addition, the activation ratio in the carcinoma samples with lymph node metastasis (0.59 ± 0.10, n = 13) was significantly higher than in those without metastasis (0.44 ± 0.16, n = 19; P < 0.05; Fig. 7). Furthermore, the activation was significantly enhanced in the carcinomas with distant metastasis (0.61 ± 0.15, n = 5) compared to those without metastasis (0.48 ± 0.15, n = 27; P < 0.05; data not shown).

The activation ratio of proMMP-2 showed a positive correlation with the expression of MT1-MMP (r = 0.797, P < 0.01; Fig. 8A) but not with MT2-MMP (data not shown). Similar correlation was obtained between the activation ratio and degree of MT1-MMP immunostaining in the carcinoma cases (r = 0.686, P < 0.01; Fig. 8B).

DISCUSSION

The present studies have demonstrated that among three different MT-MMPs, MT1-MMP is consistently expressed in all of the breast carcinoma samples. Northern blot analysis demonstrated that MT1-MMP mRNA is undetectable or present in negligible amounts in the specimens of normal breast and fibrocystic breast disease. Thus, the expression is considered to be predominant in the breast carcinomas, as we have described previously in the gastric carcinomas (2). Immunohistochemically, MT1-MMP was localized in the breast carcinoma cells, although some staining was also observed in the tumor stromal cells. The localization pattern observed here differs from the one obtained using in situ hybridization detection (13—15). The latter showed predominant distribution of the MT1-MMP mRNA in the stromal cells of breast carcinomas. The reason for this discrepancy is not clear. One possible explanation may be absorption of the stromal cell-derived MT1-MMP by carcinoma cells as proposed previously (13). Our recent studies (18) showing the presence of soluble MT1-MMP under some culture conditions appear to support this hypothesis. Another possibility is that the steady-state levels of MT1-MMP protein are higher in carcinoma cells as compared to the stromal cells because of differences in the posttranscriptional regulation. In recent studies on human invasive cervical carcinomas (19) and pulmonary squamous cell carcinomas (14), MT1-MMP expression was detected in carcinoma cells at both mRNA and protein levels.

MT2-MMP was also expressed in the breast carcinoma tissues and localized by immunohistochemistry in the carcinoma cells. However, the expression was found in only 25 and 38% of the cases at the mRNA and protein levels, respectively. Although reverse transcription-PCR showed an amplification of MT3-MMP-specific RNA frag-
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iment in less than 30% of the carcinoma cases (data not shown), no signal for MT3-MMP was observed by Northern blotting. Thus, MT3-MMP expression in the human breast carcinomas appears to be negligible, if any. Taken together, these data indicate that of the three MT-MMPs, MT1-MMP is the predominant MT-MMP expressed in human invasive breast carcinomas.

The activation ratio of proMMP-2 is reported to be enhanced in various human carcinomas such as lung (20, 21), stomach (2), and breast carcinomas (11, 12) in comparison to their normal counterparts, and correlates with the tumor grades of breast carcinomas (12). In the present studies, we have further shown that the activation ratio is remarkably higher in the breast carcinomas with lymph node or distant metastasis than in those without metastasis. In addition, our data have demonstrated for the first time that the activation ratio in the carcinomas directly correlates with MT1-MMP expression levels determined by Northern blotting and immunohistochemistry but not with MT2-MMP or MT3-MMP expression. Therefore, it seems likely that MT1-MMP plays a major role in the activation of proMMP-2 in these tumors. The absence of correlation between MT2-MMP expression and proMMP-2 activation suggests that MT2-MMP may have only a minor role in activation of this enzyme in the breast carcinomas. However, coexpression of MT1-MMP and MT2-MMP in the carcinomas of advanced stages with distant metastasis (three of five cases) suggests possible involvement of MT2-MMP in the metastasis. Further studies are necessary to clarify the biochemical properties and biological function of MT2-MMP.

MT1-MMP was originally characterized as an activator of proMMP-2 (7). However, previous studies including ours (18, 22) demonstrated that MT1-MMP has proteolytic activities against ECM macromolecules such as gelatins, fibronectin, and laminin. In addition, we have recently demonstrated that MT1-MMP also digests fibrillar types I, II, and III collagens generating three-fourths and one-fourth fragments typical for interstitial collagens such as MMP-1 (tissue collagenase). The collagenolytic activity of MT1-MMP is synergistically increased in the presence of MMP-2 (23). MT1-MMP expressed on the cell membranes captures proMMP-2 and activates it into active MMP-2 on the cell surfaces (24). Because MT1-MMP and MMP-2 are colocalized in the gastric (2) and breast carcinoma cells (data not shown), the combination of these MMPs may be a powerful machinery for the pericellular digestion of ECM by carcinoma cells, which facilitates invasion and metastasis.

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