Membrane type 1 matrix metalloproteinase (MT1-MMP) is expressed on cancer cell membranes and activates the zymogen of MMP-2 (gelatinase A). We have recently isolated MT1-MMP complexed with tissue inhibitor of metalloproteinases 2 (TIMP-2) and demonstrated that MT1-MMP exhibits gelatinolytic activity by gelatin zymography (Imai, K., Ohuchi, E., Aoki, T., Nomura, H., Fujii, Y., Sato, H., Seiki, M., and Okada, Y. (1996) Cancer Res. 56, 2707–2710). In the present study, we have further purified to homogeneity a deletion mutant of MT1-MMP lacking the transmembrane domain (ΔMT1) and native MT1-MMP secreted from a human breast carcinoma cell line (MDA-MB-231 cells) and examined their substrate specificities. Both proteinases are active, without any treatment for activation, and digest type I (guinea pig), II (bovine), and III (human) collagens into characteristic 3/4 and 1/4 fragments. The cleavage sites of type I collagen are the Gly775–Ile776 bond for α1(I) chains and the Gly775–Leu776 and Gly781–Ile782 bonds for α2(I) chains. ΔMT1 hydrolyzes type I collagen 6-5- to 4-fold more preferentially than type II or III collagen, whereas MMP-1 (tissue collagenase) digests type III collagen more efficiently than the other two collagens. Quantitative analyses of the activity of ΔMT1 and MMP-1 indicate that ΔMT1 is 5–7.1-fold less efficient at cleaving type I collagen. On the other hand, gelatinolytic activity of ΔMT1 is 8-fold higher than that of MMP-1. ΔMT1 also digests cartilage proteoglycan, fibronec tin, vitronectin and laminin-1 as well as α1-proteinase inhibitor and α2-macroglobulin. The activity of ΔMT1 on type I collagen is synergistically increased with co-incubation with MMP-2. These results indicate that MT1-MMP is an extracellular matrix-degrading enzyme sharing the substrate specificity with interstitial collagens, and suggest that MT1-MMP plays a dual role in pathophysiological digestion of extracellular matrix through direct cleavage of the substrates and activation of proMMP-2.

Matrix metalloproteinases (MMPs) are zinc endopeptidases consisted of 14 different members and implicated in the extracellular matrix (ECM) degradation under both physiological and pathological conditions (1). Among the MMPs, MMP-2 (gelatinase A) is reported to be most related to invasion and metastasis in various human cancers (2). All these MMPs except for at least MMP-11 (stromelysin-3) are secreted as inactivezymogens (proMMPs), and thus their activation is one of the most important steps for the regulation of MMP activities. Research on the activation mechanisms of proMMP-2 has greatly progressed in recent years, since membrane type 1 MMP (MT1-MMP) has been cloned as an activator of proMMP-2 (3). The expression of MT1-MMP in human lung and gastric carcinomas is well correlated with the activation of proMMP-2 (4, 5), suggesting that the proMMP-2 activation by MT1-MMP is a key step for the cancer cell invasion and metastases. On the other hand, one can expect that MT1-MMP possesses enzymic activity to the ECM macromolecules, since the structure of the catalytic domain of MT1-MMP is similar to that of other MMPs. Actually, we have recently demonstrated that a deletion mutant of MT1-MMP lacking the transmembrane domain (ΔMT1) and native MT1-MMP, both of which were isolated in the complex forms with tissue inhibitor of metalloproteinases 2 (TIMP-2), exhibit gelatinolytic activity after separation from TIMP-2 on gelatin zymography (6). However, information about the substrate specificity of MT1-MMP is still limited, although Pei and Weiss (7) have very recently reported that deletion mutants of MT1-MMP have some ECM-degrading activity.

In the present studies, we have purified to homogeneity both ΔMT1 from the stable transfectants and native MT1-MMP secreted from a human breast carcinoma cell line (MDA-MB-231) and examined the substrate specificity. The results demonstrate that ΔMT1 and MT1-MMP digest fibrillar collagens, i.e. type I, II, and III collagens, into typical 3/4- and 1/4-length fragments like MMP-1 (tissue collagenase) as well as other ECM components including gelatin, proteoglycan, fibronectin, vitronectin, and laminin-1. In addition, ΔMT1 activates proMMP-2, and the activity of type I collagen is synergistically increased by co-incubation of the substrate with MMP-2.

EXPERIMENTAL PROCEDURES

Materials—Materials were obtained as follows: bovine serum albumin, Brij 35, Coomassie Brilliant Blue R250, 2-mercaptopethanol, tris, 3-(2,4-dinitrophenyl)-L-2,3-diaminopropionyl.

The abbreviations used are: MMP, matrix metalloproteinase; 
proMMP, corresponding zymogen form; MT-MMP, membrane-type MMP; ΔMT1, MT1-MMP lacking transmembrane domain; APMA, p-aminophenylmercuric acetate; PAGE, polyacrylamide gel electrophoresis; TIMP-2, tissue inhibitor of metalloproteinases 2; ECM, extracellular matrix; CHO, Chinese hamster ovary; Mca, (7-methoxycoumarin-4-yl)acetyl; Dpa, N-3-(2,4-dinitrophenyl)-1,2,3-diaminopropionyl.
Substrate Specificity of MT1-MMP

Ferrin (human), and tosyl-Phe-CH₂Cl-tresyn from Sigma; EDTA, iodoacetamide, and sodium dodecyl sulfate from Wako Chem., Japan; p-aminohippurinacurric acetate (APMA) from Aldrich; α₁-proteinase inhibitor (human) and α₂-macroglobulin (human) from Calbiochem; Dulbecco's modified Eagle's medium, antibiotics, lactalbumin hydrolysate, and fetal calf serum from Life Technologies, Inc.; and calf skin gelatin from Amicon Corp. (Beverly, MA); gelatin-Sepharose and Sephadex G-10 from Pharmacia Fine Chemicals, Sweden; DEAE-cellulose (DE-52) from Whatman, UK; Affi-gel 10 from Bio-Rad; [³⁵S]Acetic anhydride (20 mCi/mmole), Na₂¹¹¹ (16.4 μCi/mg), and [³H]iodoacetamide (90 mCi/mmole) from Amersham Corp., Japan; a synthetic quencher-containing peptide substrate (human-D-Ala-Ala-Arg-NH₂), from Peptide Institute, Inc., Japan. Pro-MMP-1, -2, -3, and -9, and recombinant TIMPs (rTIMP-1 and rTIMP-2) were purified as described previously (8–11). Laminin-1 and type IV collagen were purified from guinea pig and type III, V, and VI collagens from human placenta as described previously (14, 15). Fibronectin and vitronectin were purified from human plasma (16). Type II collagen from bovine cartilage was purchased from Nitta Gelatin, Japan and insoluble elastin from Elastin Products Co. (Owensville, MO). Bovine nasal cartilage proteoglycan subunit was prepared according to the methods of Nagase and Woessner (17).

Cell Cultures and Stable Transfectants of ΔMT1—The SV40 early promoter was cloned into the plasmid (Stratagene, La Jolla, CA) was used to express ΔMT1, which lacks the COOH-terminal transmembrane and cytoplasmic domain of MT1-MMP (ΔAla⁹⁶–Val⁹⁸) (18). A cell line constitutively expressing ΔMT1 was established by two-step selection of CHO cells lacking dihydrofolate reductase gene co-transfected with ΔMT1 cDNA/pSG5 plasmids and pKg5 plasmid containing neomycin resistance gene and dihydrofolate reductase/pSV2 vector as described previously (18). The established cells were cultured in medium containing 0.2% lactalbumin hydrolysate for 4 days. MDA-MB-231 cells were grown in monolayer cultures in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum and treated with 50 mM Tris-HCl, pH 7.5, 0.15 M NaCl, 10 mM CaCl₂, 0.05% Brij 35, and 0.02% NaN₃ for 2 h at 23°C, and then the buffer was replaced with 50 mM HEPES-KOH buffer, pH 7.5, 0.15 M NaCl, 5 mM CaCl₂, and 0.02% Brij 35 by spin columns (6). Freshly prepared cross-linker (bis(sulfosuccinimidyl) substrate) (Pierce) was added to the samples at a final concentration of 4 mM and incubated for 45 min at 20°C. After termination of the reactions by incubation with 50 mM Tris for 15 min on ice, they were subjected to SDS-PAGE (10% total acrylamide) under reduction and the gels were autoradiographed. M₄ changes of proMMP-2 during activation with ΔMT1 were also examined by autoradiography of the iodinated proMMP-2 after SDS-PAGE (9% total acrylamide).

Reconstitution of Enzyme Complexes—Concentrations of M₁-MMP, M₂-MMP, and ΔMT₁ were determined by titration of their activities against rTIMP-2 (concentration determined by amino acid analysis) in an assay using Mca-Pro-Leu-Glu-Leu-Dpa-Ala-Arg-NH₂ at 37°C for 1 h (22). Residual enzymatic activities were measured and plotted versus TIMP-2 concentrations. A linear plot of activity against the inhibitor molarity was extrapolated to be zero activity at molarity of the enzyme solution.

Digestion of Extracellular Matrix Macromolecules—ΔMT1 was incubated with various ECM components and other protein substrates including carboxymethylated transferrin (23), α₁-proteinase inhibitor and α₂-macroglobulin in 50 mM Tris-HCl, pH 7.5, 0.15 M NaCl, 10 mM CaCl₂, 0.05% Brij 35, and 0.02% NaN₃, at indicated temperatures. The reactions were stopped with 20 mM EDTA and digestion products were analyzed by SDS-PAGE. Specific activity of ΔMT1 and MMP-1 against type I collagen and gelatin was determined using ¹⁴C-acetylated type I collagen and its heat-denatured collagen (gelatin), respectively. The degradation of acid-soluble type I (guinea pig), II (bovine), and III (human) collagens was quantitated using the gel scanning protocol described by Welgus et al. (24). The activity of ΔMT1 to cartilage proteoglycan and insoluble elastin were assayed as described previously (17, 25). Synergistic effects of ΔMT1 (or MMP-1) and MMP-2 on type I collagen digestion were assayed using ¹⁴C-acetylated collagen.

Sequence Analyses of ΔMT1-digested Type I Collagen—Human type I collagen was digested with ΔMT1 at 27°C, and the fragments generated were separated by SDS-PAGE, and then transferred on polyvinylidene difluoride membranes. The band of 1/4-length fragments was sequenced by 492 sequencer (Applied Biosystem, Foster, CA).

RESULTS

Purification of ΔMT1 and Native MT1-MMP

Dissociation of ΔMT1 from ΔMT1-TIMP-2 complex was efficiently performed by a stepwise elution method with EGTA and urea in the anti-TIMP-2-IgG-Sepharose column. ΔMT1 was recovered in the EGTA eluate, and the final material (80.3 μg) purified from the culture medium (500 ml) migrated as a single protein band of M₄ 56,000 under reducing conditions (M₄ 52,000 under nonreducing conditions) (Fig. 1A). Absence of MMP-1 contamination was verified by the sandwich enzyme immunoassay and immunoblotting for MMP-1 (data not shown). Native MT1-MMP was also purified from the MDA-MB-231 cell-derived MT1-MMP/TIMP-2 complex; the final material (5 μg) was purified from 500 ml of the culture medium. Purified MT1-MMP showed a protein band of M₄ 56,000 under reducing conditions and that of M₄ 52,000 under nonreduction (Fig. 1A). ΔMT1 and native MT1-MMP digested type I gelatin and carboxymethylated transferrin into the indistinguishable fragments (Fig. 1B), indicating that the activities of both enzymes are identical. Both proteinases were already active without any treatment and there was no decrease in the activity even after storage at 4°C for 4 months. Since a small amount of native MT1-MMP was purified, ΔMT1 was used mainly for the following studies.

Reconstitution of ΔMT1-TIMP-2 Complex and Activation of Pro-MMP-2 by ΔMT1

To study the interaction of ΔMT1 with TIMP-2, cross-linking experiments were carried out by incubating iodinated ΔMT1 with TIMP-2. Radiiodination did not cause any significant changes in the intrinsic properties of the proteinase. When the
reaction mixture was analyzed by autoradiography after SDS-PAGE, ΔMT1 made a complex with TIMP-2 of ~M47,000, while ΔMT1 alone resulted in a broader band of M45,000 (Fig. 2). In agreement with our previous data showing that ΔMT1-TIMP-2 complex forms a trimolecular complex with proMMP-2 through the COOH termini of TIMP-2 and proMMP-2 (6), ΔMT1 per se did not make a bimolecular complex with proMMP-2 (Fig. 2). Reconstituted ΔMT1-TIMP-2 complex showed no enzymic activity in the assays using either Mca-peptide or [14C]gelatin, as we have previously reported with the original ΔMT1-TIMP-2 complex (6).

The action of ΔMT1 on the processing of proMMP-2 was examined. Radiolabeled proMMP-2 was incubated with ΔMT1 in different molar ratios ranging from 1:1 to 1:10, and Mr changes of proMMP-2 molecule were analyzed by autoradiography and gelatin zymography after SDS-PAGE. As shown in Fig. 3A, ΔMT1 processed proMMP-2 of Mr 72,000 into the Mr 69,000 intermediate species in a dose-dependent manner, while APMA treatment generated fully active form of Mr 66,000. On gelatin zymography under nonreduction proMMP-2 of Mr 68,000 was processed by ΔMT1 to the intermediate species of Mr 64,000 and active form of Mr 62,000, the latter of which showed a very faint proteolytic band (Fig. 3B). This processing of proMMP-2 by ΔMT1 confirmed the previous data showing that MT1-MMP initially cleaves proMMP-2 to the intermediate form, which is then autokatallytically processed to the fully active form only when higher concentrations of proMMP-2 are present (26).

Degradation of Extracellular Matrix Macromolecules

Digestion of Collagens by ΔMT1 and MT1-MMP—ΔMT1 cleaved type I, II, and III collagens under the nondenaturing conditions, i.e. at 27 °C, generating 3/4- and 1/4-length fragments of these collagens (Fig. 4A). On the other hand, the collagens were degraded into multiple fragments when incubated at 35–37 °C, probably because of thermal denaturation of the substrates to gelatins (data not shown). Since the digestion products were similar to those obtained by the action of MMP-1, NH2-terminal sequence analyses on the 1/4 fragments of type I collagen were performed. The NH2 terminus of α1(I) chains was Ile776-Ala-Gly-Gln-X-Gly-Val-Val-Gly-Leu and α2(I) chains had NH2-terminal Leu786-Leu-Gly-Ala-Hyp-Gly-Lle and Ile782-Leu-Gly-Leu-Hyp-Gly-Ser in approximately 1:2 molar ratio. Native MT1-MMP also digested type I, II, and III collagens into typical 3/4 and 1/4 fragments (Fig. 4B). However, no degradation of type IV, V, and VI collagens by ΔMT1 and MT1-MMP were observed at the non-denaturing temperatures under 33 °C, whereas type IV and V collagens, but not type VI collagen, were digested into fragments at 35 and 37 °C (data not shown).

The catalytic efficiency of type I, II, and III collagens by ΔMT1 and MMP-1 was estimated by incubation of the collagens with increasing concentrations of the proteinases. ΔMT1 most preferentially digested type I collagen; the susceptibility of type I collagen was 6.5- and 4-fold higher than that of type II and III collagens, respectively. On the other hand, the activity of MMP-1 to type III collagen was approximately 4.4- and 25.6-fold greater than that to type I and II collagens, respectively. Kinetic analyses of the type I collagen degradation by MMP-1 and ΔMT1 were performed in the samples containing increasing amounts of type I collagen and constant amounts of the proteinases. Lineweaver-Burk plots were constructed from the velocity data, and values of Kcat and kcat/Km were extracted (Table 1). MMP-1 exhibited a Kcat of 1.3 μm and a kcat/Km of 22.2 molecules of collagen degraded/enzyme molecule/h, whereas a Kcat of 2.9 μm and a kcat/Km of 7.1 molecules degraded/enzyme molecule/h were obtained with ΔMT1. Thus, the kcat/Km value of ΔMT1

![Fig. 1. SDS-PAGE of the purified ΔMT1 and native MT1-MMP (A) and of the gelatin digestion products by them (B).](image)

![Fig. 2. Cross-linking experiments of the ΔMT1 with proMMP-2 and TIMP-2.](image)

![Fig. 3. SDS-PAGE (A) and gelatin zymography (B) of proMMP-2 incubated with ΔMT1.](image)
Substrate Specificity of MT1-MMP

To assess the synergistic effect of ΔMT1 and MMP-2 on collagen digestion, 14C-labeled type I collagen was incubated at 35 °C with ΔMT1 (16 nM) in the presence of various amounts of MMP-2 ranging from 0 to 16 nM. As shown in Table II, the collagenolytic activity of ΔMT1 was augmented up to 7.3-fold compared with ΔMT1 alone, although MMP-2 itself showed no collagenolytic activity. Similar experiments were performed with MMP-1 (3 nM) and MMP-2 ranging from 0 to 16 nM. The activity of MMP-1 was also 6.1-fold enhanced in the presence of MMP-2. This effect was ascribed to the accelerated degradation of the collagenolytic fragments (gelatin) by MMP-2, since the collagen digestion products generated by ΔMT1 treatment were completely hydrolyzed into peptides in the presence of MMP-2 (Fig. 6A). Similar data was obtained with type II and III collagens incubated with ΔMT1 and MMP-2 (Fig. 6, B and C). On the other hand, no such effect was found with ΔMT1 and MMP-1; the collagenolytic activity in the presence of ΔMT1 and MMP-1 was equal to the sum of both proteinase activities.

**DISCUSSION**

The present studies have demonstrated for the first time that, like interstitial collagenases, i.e. MMP-1 (tissue collagenase), MMP-8 (neutrophil collagenase), and MMP-13 (collagenase-5), both recombinant and native secreted forms of MT1-MMP digest the native fibrillar collagen types I, II, and III into their typical 3/4 and 1/4 fragments. Although MT1-MMP is structurally assigned in the MMP gene family to the MT-MMP subgroup consisting of MT1, MT2, MT3, and MT4-MMPs, the substrate specificity suggests that MT1-MMP is a member of the interstitial collagenases. ΔMT1 and native MT1-MMP were purified from their complex forms with TIMP-2 by dissociation of the complex due probably to the conformational changes caused by EGTA treatment in the anti-TIMP-2-IgG-Sepharose
by immunoblotting.\textsuperscript{2} Thus, contamination of MMP-1 in the purified preparations was carefully ruled out since it is secreted by both cell lines. During the purification steps, proMMP-1 was eliminated by using anti-MMP-1-IgG-Sepharose and anti-TIMP-2-IgG-Sepharose column chromatographies, and no contamination in the preparations was verified by sandwich enzyme immunoassay and immunoblotting for MMP-1. This was also supported by the data that the final products have a single protein band of \( M_t \) 56,000 and were already active without any treatment for activation. In addition, the collagenolytic activity of \( \Delta \text{MT1} \) was different from that of MMP-1. \( \Delta \text{MT1} \) preferentially cleaved type I collagen over type II and III collagens, whereas MMP-1 cleaved preferentially type III collagen over type I and II collagens. Comparison of the type I collagenolytic activity of \( \Delta \text{MT1} \) with that of MMP-1 revealed that \( \Delta \text{MT1} \) is 5–7.1-fold less efficient than MMP-1. In contrast, gelatinolytic activity of \( \Delta \text{MT1} \) was 8-fold higher than MMP-1. Knäuper et al. \textsuperscript{(28)} recently reported that three collagenases have distinct substrate specificity to collagens and gelatins; MMP-1, -8, and -13 preferentially digest collagen types I, I, and II, respectively, and both MMP-8 and MMP-13 exhibit 4.9- and 41-fold higher activity against type I gelatin than does MMP-1. Thus, the present data suggest that \( \Delta \text{MT1} \) shares the proteolytic characteristics with MMP-8. Higher gelatinolytic efficiency of MMP-8, MMP-13, and gelatinases (MMP-2 and -9) is explained by the presence of key residues specifically conserved in the active sites of these MMPs \textsuperscript{(28)}. \( \Delta \text{MT1} \) also conserves the residues including Ile in the S1 pocket, negatively charged Glu just preceding the third His residue and invariant Pro three amino acids after the His residue.

It has been established that MMP-1 and MMP-8 cleave type I, II, and III collagens at a specific single site after the Gly residue of the partial sequences Gly-(Ile or Leu)-(Ala or Leu) located approximately 3/4 from the NH\(_2\) terminus in these collagens. Unlike MMP-1 and MMP-8, however, MMP-13 hydrolyzes \( \alpha \) chains of type II collagen at the Gly\textsuperscript{306}–Leu\textsuperscript{307} and Gly\textsuperscript{509}–Gln\textsuperscript{510} bonds. The present study also demonstrated that \( \Delta \text{MT1} \) cleaves the Gly\textsuperscript{775}–Leu\textsuperscript{776} and Gly\textsuperscript{781}–Ile\textsuperscript{782} bonds of \( \alpha \)2(I) chains. Since \( \alpha \)1(1) chains were hydrolyzed only at the Gly\textsuperscript{775}–Leu\textsuperscript{776} bond, cleavage of the Gly\textsuperscript{781}–Ile\textsuperscript{782} bond may be a secondary cleavage. This two-site cleavage by \( \Delta \text{MT1} \) may be related with higher gelatinolytic activity of this enzyme, but its biological function remains unclear at the present time. Pei and Weiss \textsuperscript{(7)} have reported that a deletion mutant of MT1-MMP (\( \Delta \text{Pro}\textsuperscript{508}–\text{Val}\textsuperscript{582} \)) (MT1-MMP\textsuperscript{1–508}) digests several ECM molecules including gelatin, fibronectin, laminin, vitronectin, and dermatan sulfate proteoglycan. However, MT1-MMP\textsuperscript{1–508} had no ability to cleave type I collagen. The structural difference between \( \Delta \text{MT1} \) in the present study and MT1-MMP\textsuperscript{1–508} is that \( \Delta \text{MT1} \) is longer with 27 amino acid residues in its COOH terminus. Subsequent to the active site, it has been demonstrated that the activity of MT1-MMP\textsuperscript{1–508} is identical to that of MT1-MMP except for the activity to fibrillar collagens, it seems likely that the COOH-terminal sequence of the 27 amino acid residues is essential to the collagenolytic activity probably because the sequence is necessary for the intact conformation of the hemopexin-like domain of MT1-MMP, which may interact with the collagen molecules. The present data that MT1-MMP derived from MDA-MB-231 cells also possesses collagenolytic activity indicate that collagenase activity of MT1-MMP is not artificial, but natural. Our previous study \textsuperscript{(6)} demonstrated that active \( \Delta \text{MT1} \) has Ala\textsuperscript{112} at the NH\(_2\) terminus, indicating that the Tyr\textsuperscript{112} is lost during the intracellular activation. In MMP-1 and MMP-8, the

\textbf{FIG. 6. Synergistic digestion of type I, II, and III collagens by \( \Delta \text{MT1} \) and MMP-2.} A. Type I collagen (10 \( \mu \)g) was incubated with buffer alone (lane 1), 100 ng of \( \Delta \text{MT1} \) (lane 2), 100 ng of \( \Delta \text{MT1} \) and 35 ng of MMP-2 (lane 3), or 35 ng of MMP-2 (lane 4) at 35 °C for 4 h. After termination of the reaction with 20 mM EDTA, the degradation products were analyzed on SDS-PAGE (10% total acrylamide) under the reducing conditions. B and C, type II and III collagens (10 \( \mu \)g each) were incubated with buffer alone (lane 1), 100 ng of \( \Delta \text{MT1} \) (lane 2), 100 ng of \( \Delta \text{MT1} \) and 35 ng of MMP-2 (lane 3), or 35 ng of MMP-2 (lane 4) at 35 °C for 4 h, respectively. The digestion products were subjected to SDS-PAGE as described in A. Note that the digestion fragments generated by the action of \( \Delta \text{MT1} \) are completely hydrolyzed with MMP-2 (lane 3 in A, B, and C).

\begin{table}[h]
\centering
\caption{Synergism of MMP-2 to the degradation of type I collagen by \( \Delta \text{MT1} \) or MMP-1}
\begin{tabular}{|c|c|c|}
\hline
MMP-2 \(^\text{a}\) & \( \Delta \text{MT1} \) (16 nm) & MMP-1 (3 nm) \\
\hline
(ng) & & \\
0 & 1.0 & 1.0 \\
4 & 2.5 & 2.4 \\
8 & 6.2 & 5.2 \\
16 & 7.3 & 6.1 \\
\hline
\multicolumn{3}{l}{\textsuperscript{a} No collagenolytic activity was detected with MMP-2.}
\end{tabular}
\end{table}
The degradation of the fibrillar collagens is considered to be sequentially performed by gelatinases after the initial cleavage at collagen triple helix by collagenases (1). A synergistic effect of MMP-1 and MMP-2 on the fibrillar collagen digestion has been reported (31) and further confirmed in the present study. Similar accelerated digestion of the collagens was demonstrated with MT1 and MMP-2. The combination of MT1-MMP and MMP-2 may be crucial for the pericellular collagen degradation in cancer invasion and metastasis, because MT1-MMP can activate proMMP-2 on the carcinoma cell surfaces. So-called “aggrecanase” depletion of aggrecan from the articular cartilage, leading to loss of tensile strength of the tissue. So-called “aggrecanase” site only when the enzyme is incubated with the substrate in a very high concentration (34). Previous studies (33), none of MMPs except for MMP-8 cleaves the Glu373–Ala374 bond. MMP-8 can digest aggrecan molecules at the aggrecanase-site only when the enzyme is incubated with the substrate in a very high concentration (34). Previous studies (35) have demonstrated that the aggrecanase activity is a cell-mediated event, suggesting the pericellular proteolysis of aggrecan. In fact, our preliminary studies showed that MT1-MMP is highly co-expressed with MMP-2 in human osteoarthritic chondrocytes. It is, therefore, reasonable to speculate that aggrecanase may be MT1-MMP or combined action of MT1-MMP and MMP-2. This possibility should be elucidated by further studies.

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