Constitutive and Induced CD44 Shedding by ADAM-Like Proteases and Membrane-Type 1 Matrix Metalloproteinase

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ABSTRACT

CD44 is a receptor for hyaluronan and mediates signaling that regulates complex cell behavior including cancer cell migration and invasion. Shedding of the extracellular portion of CD44 is the last step in the regulation of the molecule-releasing interaction between the ligand and cell. However, highly glycosylated forms of CD44 have hampered the identification of the exact cleavage sites for shedding and the responsible proteases. In this study, we found that expression of membrane-type 1 matrix metalloproteinase (MT1-MMP) increased shedding of the 65–70 kDa CD44H (standard form) fragments and generated two additional smaller fragments. We purified the shed fragments and identified the cleaved sites by mass spectrometry. Specific antibodies that recognize the newly exposed COOH terminus by cleavage were prepared and used to analyze shedding at each site. Shedding of the 65–70 kDa fragments was inhibited by tissue inhibitor of metalloproteinase 3 (TIMP-3) but not by TIMP-1 and TIMP-2, suggesting involvement of a disintegrin and metalloproteinase (ADAM)-like proteases, although shedding is affected by MT1-MMP. Conversely, shedding of the two smaller fragments was inhibited by TIMP-2 and TIMP-3 but not TIMP-1, suggesting involvement of MT1-MMP itself. Shed fragments cleaved at these sites were also detected in human tumor tissues. Increased shedding at one of the MT1-MMP-sensitive sites was observed in the tumor compared with the surrounding normal tissue. However, no significant difference was observed with shedding by ADAM-like proteases. Thus, the cleavage sites for the shedding of CD44H were identified for the first time, and the results provide a basis for exploring the unknown biologic roles of shedding at different sites.

INTRODUCTION

The extracellular matrix not only constitutes the framework of tissues but also regulates various cellular functions such as proliferation, differentiation, apoptosis, and migration. CD44 is an adhesion molecule that acts as a major receptor for hyaluronan, an abundant glycosaminoglycan in the extracellular matrix (1). Hyaluronan fills intercellular spaces in various tissues (2) and has been implicated in many biologic processes including inflammation, wound healing, remodeling of tissue, cell migration, and invasion (3). CD44 binds hyaluronan at the globular lectin-like domain and regulates various cell physiologies via mechanisms that are not yet understood. The ligand-binding domain is linked to the cell surface such as growth factors (basic fibroblast growth factor, fibroblast growth factor 8, and heparin-binding epidermal growth factor), cytokines (osteopontin, receptors (ErbB4), and matrix metalloproteinases (MMPs) (1, 5–9). For example, MMP-7 binds to a heparan-sulfate proteoglycan in the v3 region of a variant form of CD44 (7) and cleaves HB-epidermal growth factor, which may activate ErbB4 (7). MMP-7 also induces cell migration through the processing of osteopontin (10). MMP-9 is another soluble MMP that binds to CD44, and the bound form of MMP-9 is reported to activate the latent form of transforming growth factor β, and stimulate tumor invasion and angiogenesis (11).

Proteins that bind CD44 may be regulated in turn through the actin cytoskeleton because CD44 is connected to the structure via the cytoplasmic domain. Membrane-type 1 (MT1)-MMP has a strong invasion-promoting activity that is used frequently by malignant tumors (12). To degrade the extracellular matrix barrier in the direction of cell locomotion, MT1-MMP must attain a polarized localization at the leading edge. CD44, which localizes at the ruffling edge of migrating cells, binds to MT1-MMP and regulates its localization to the edge through the hemopexin-like domain of MT1-MMP and the stem region of CD44H (8).

The shedding of CD44 is an event that is observed frequently in many types of cells (4, 13, 14), and shed CD44 has been detected in culture supernatants (13–15), arthritic synovial fluid (16), and plasma (17, 18). It has been reported that higher levels of shed CD44 were detected in serum from patients with malignant cancer and metastasis (19–21). The proteases responsible for the shedding are mainly metalloproteinases because synthetic metalloproteinase inhibitors with a broad spectrum inhibit the shedding almost completely (15, 22), although some serine proteinases may also participate (19 –21). The proteases responsible for the shedding are mainly metalloproteinases because synthetic metalloproteinase inhibitors with a broad spectrum inhibit the shedding almost completely (15, 22), although some serine proteinases may also participate (19). MT1-MMP was thought to be an enzyme responsible for the shedding because it binds CD44H, has an ability to cleave CD44H at least in vitro, and expression of MT1-MMP increases CD44 shedding in the cells (15). However, it is not necessarily clear whether MT1-MMP directly shed CD44 at a cellular level and whether it is the enzyme that is solely responsible for the shedding.

In this study, we found that expression of MT1-MMP increased the shedding of 65–70 kDa CD44H fragments that are produced commonly by many types of cells and generated two additional smaller fragments using a human melanoma cell line. We purified the three groups of heterogeneously glycosylated fragments and determined the cleavage sites by mass spectrometry. Cleavage site-specific antibodies generate additional diversification by adding variable exon-coded sequences to the stem. Additional variation of CD44 is conferred by extensive glycosylation at multiple sites including variant exon-coded sequences (1). Although the core protein of CD44H is 37 kDa, it usually appears as a molecule of 80–100 kDa as a result of heavy glycosylation.

Other than hyaluronan, ligands in the extracellular space such as type I collagen, fibronectin, fibrin, laminin, and chondroitin sulfate have been reported to bind CD44 (4). However, CD44 is not merely a receptor for the extracellular matrix molecules in that it also acts as a platform for signal transmission by assembling bioactive molecules on the cell surface such as growth factors (basic fibroblast growth factor, fibroblast growth factor 8, and heparin-binding epidermal growth factor), cytokines (osteopontin), receptors (ErbB4), and matrix metalloproteinases (MMPs) (1, 5–9). For example, MMP-7 binds to a heparan-sulfate proteoglycan in the v3 region of a variant form of CD44 (7) and cleaves HB-epidermal growth factor, which may activate ErbB4 (7). MMP-7 also induces cell migration through the processing of osteopontin (10). MMP-9 is another soluble MMP that binds to CD44, and the bound form of MMP-9 is reported to activate the latent form of transforming growth factor β, and stimulate tumor invasion and angiogenesis (11).

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were then generated and used to monitor the shedding at each site. Shedding near the membrane site that generates 65–70 kDa fragments occurs constitutively, and the sensitivity of the shedding to tissue inhibitors of metalloproteinase (TIMPs) suggested ADAM-like proteases for the cleavage. Expression of MT1-MMP increased shedding at all three of the sites including the site of processing by the ADAM-like proteases. MT1-MMP itself cleaved the most NH₂-terminal position that generates 37–40 kDa fragments. CD44 shedding at these sites was detected in human tumors, with increased shedding of the fragments cleaved at the MT1-MMP-sensitive site.

MATERIALS AND METHODS

Cell Culture and Induction of MT1-MMP Expression. A375 human melanoma cells were obtained from the Japanese Cancer Resource Bank. The cells were cultured in RPMI 1640 (Sigma-Aldrich Japan, Tokyo, Japan) supplemented with l-glutamine, 10% FCS, and penicillin/streptomycin (Life Technologies, Inc., Grand Island, NY). Induction of MT1-MMP expression in the cells was accomplished with the Tet-off system (Clontech), in which a combination was used comprising the pTET-off vector encoding a fusion protein consisting of the Tet repressor and the transactivator VP16 and MT1-MMP cDNA cloned into a pTRE vector with multiple Tet-response elements. The expression of MT1-MMP can be induced by removing doxycycline from the culture medium.

Expression of CD44H and TIMPs. The cDNA for CD44H, TIMP-1, TIMP-2, and TIMP-3 were used to express the products in the cells. The cDNA-encoded products were expressed using an adenoviral vector, the Adeno-X Expression System (Clontech). As a control vector, the expression vector for LacZ was used. Adenovirus carrying cDNA was propagated in HEK293 cells, purified by CsCl gradient centrifugation, and titered by serial-dilution end point assay (23) of the WHO International Classification of Human Tumors. Clinical Samples. Fresh tissue and serum samples of human carcinomas were obtained from patients who agreed to provide samples during surgery at the University Hospital, School of Medicine, Keio University. Samples were also provided by Dr. Hiroshi Ueno at the Tokyo Disaster Medical Center, Tokyo, Japan. The specimens were assessed according to the standard criteria of the WHO International Classification of Human Tumors.

Immunohistochemistry. Tissue samples of the carcinoma were fixed with periodate-lysine-parafomaldehyde fixative (27) for 18–24 h at 4°C, and paraffin sections were reacted with polyclonal antibodies to CD44H (30 μg/ml; anti-CS1 IgG, anti-CS2 IgG, and anti-CS3 IgG) or with polyclonal antibodies preincubated with the antigen peptide as a negative control. After reactions with biotinylated horse IgG to mouse IgG (Vector Laboratories) and avidin-biotin-peroxidase complex (Dako), color was developed with 3,3’-diaminobenzidine tetrahydrochloride (Sigma Chemical Co.) as described previously (27). Staining of tested cells with control rabbit IgG and mouse IgG was also negative (data not shown).

RESULTS

CD44H Shedding in a Human Melanoma A375 Cell Line. To analyze the constitutive and induced CD44H shedding, we used a human melanoma A375 cell line in which expression of MT1-MMP can be induced under control of the Tet-off promoter system. CD44H was expressed using an adenovirus vector carrying the cDNA. The shed fragments accumulated in the culture medium were analyzed by Western blotting using two mAbs that recognize different parts of the stem region of CD44 (26). One mAb, 285–2F12 (26), recognizes the NH₂-terminal region of the stem, whereas the other, 268–1F5, recognizes the carbohydrate near the NH₂-terminal region of the stem. The reactivity of mAb 268–1F5 was affected by proteinase inhibitors BB94 (15, 28). A375 cells do not express a significant amount of endogenous CD44 but they have the ability to shed CD44 as 65–70 kDa fragments (Fig. 1B).

Expression of MT1-MMP was induced by removing doxycycline from the culture medium (Fig. 1B, right panel). Activated MT1-MMP appeared as a 60-kDa band and several autodegraded fragments were also observed, as reported previously (29, 30). Expression of MT1-MMP increased the amount of 65–70 kDa bands and also generated additional new bands of 50–60 and 37–40 kDa as detected with mAb...
from smallest to largest. The extracellular portion of MT1-MMP 1D8 was used. The three major fragments (Fr) correspond to the fragments in Fig. 2 that were determined to contain the amino acids 130–285.

Fig. 1. Anti-CD44 antibodies used for purification of the shed fragments. A, ectodomain sequence of CD44H and recognition sites of antibodies. The extracellular portion of CD44H is presented. From the NH2 terminus, this portion contains signal peptide, a ligand-binding domain, and stem sequences as indicated. The sequences recognized by the monoclonal antibodies (mAbs) are boxed. The box for mAb 285–2F12 corresponds to amino acids 130–162 and that for mAb 268–1F5 to amino acids 210–230. Underlined sequences correspond to the fragments in Fig. 2 that were determined to contain the cleavage sites. Cleavage sites for shedding are also indicated as CS1-CS3. Detection of membrane-type 1 matrix metalloproteinase cleavage sites. Cleavage sites for shedding are also indicated as CS1-CS3.

285–2F12 (Fig. 1B, left panel). Conversely, mAb 268–1F5 did not react with the 65–70 kDa and 37–40 kDa bands (Fig. 1B, center panel) despite their reactivity to mAb 285–2F12. This difference presumably is caused by the glycosylation that distorts the reactivity to mAb 285–2F12 or by a lack of the recognition sequence in the fragments (26). Thus, we referred to the bands ranging from 37 to 40 kDa as fragment 1 (Fr.1), 50–60 kDa bands as fragment 2 (Fr.2), and 65–70 kDa bands as fragment 3 (Fr.3), as indicated in Fig. 1B. Smaller fragments than Fr.1 were not detected even when NH2-terminally FLAG (DYKDDDDK)-tagged CD44H was expressed and examined with anti-FLAG antibody (data not shown).

Purification of the Shed CD44H Fragments and Determination of the Cleavage Sites. To purify the shed CD44H fragments, immunoaffinity columns conjugated with either anti-CD44H mAb 285–2F12 or 268–1F5 were prepared. Conditioned medium was collected after MT1-MMP expression was induced in the cells and applied to the columns. Absorbed proteins were then eluted with 0.1% trifluoroacetic acid, and immunoreactive fractions were combined. From the column conjugated with mAb 285–2F12, Fr.1 was obtained as a major band and Fr.3 as a minor band (Fig. 2A, left panel). Conversely, Fr.2 was the major band from the mAb 268–1F5 column, with Fr.3 as a minor band (Fig. 2A, right panel).

The collected samples were then deglycosylated and digested with trypsin for mass spectrometric analysis. Trypsin digestion generates either Arg or Lys at the COOH terminus; therefore, these fragments were captured using an anhydrotrypsin agarose column. The unbound fraction was expected to include the fragments containing the cleavage sites for shedding by the cells and was subjected to reverse-phase chromatography. Four peaks were detected in the sample obtained from the mAb 285–2F12-conjugated column (data not shown), and each of these peaks was analyzed using the matrix-assisted desorption ionization-MS/MS workstation in the single MS mode. Two of the MS spectra (2661.7051 Da and 3045.8093 Da) matched well with the CD44H peptides spanning amino acids (aa) 224–249 (2661.57 Da) and 163–192 (3045.99 Da), respectively (Fig. 1A, underlined sequences). The NH2 terminus of these fragments coincided with one of the possible trypsin-sensitive sites. These two peaks were additionally analyzed in the tandem MS/MS mode to confirm the aa sequence (Fig. 2, B and C). Peaks obtained from the laser-generated fragments coincided completely with the sequences of aa 224–249 (bn ion series for 2–4, 6, and 15; and the yn ion series for 3, 11–15, 17–22, and 25) and 163–192 (bn ion series for 2 and 6 and yn ion series for 7–10, 12–18, 20, 23, and 24), respectively.

Five peaks were obtained using reverse-phase chromatography of the sample obtained from the mAb 268–1F5-conjugated column (data not shown) and were analyzed similarly. Two of the MS spectra (1060.8508 Da and 2661.8606 Da) matched the CD44H peptides spanning aa 224–233 (1060.04 Da) and aa 224–249 (2661.57 Da), respectively. MS/MS spectrums shown in Fig. 2, D and E, clearly matched the sequences of aa 224–233 (bn ion series for 3–8 and yn ion series for 5, 7, and 9) and aa 224–249 (bn ion series for 3, 4, and 15; and yn ion series for 6, 10–15, 18–23, and 25), respectively.

Thus, three cleavage sites were determined corresponding to the three major fragments (Fr.1, Fr.2, and Fr.3). These sites were referred to as Cleavage Site 1 (CS1) for Gly192–Tyr, CS2 for Gly 233–Ser, and CS3 for Ser7249.Gln, as indicated in Fig. 1A.

Three Cleavage Sites Corresponding to the Three Major Fragments: Preparation of Cleavage Site-Specific Antibodies. Specific antibodies for the sequences at the COOH-terminal end that are newly exposed by the cleavage were prepared using synthetic peptides (STSGG192, HPSGG233, and HSHGS249). To confirm the specificity of the antibodies, recombinant CD44H stem fragments with the expected COOH terminus were prepared, ST192 (aa 130–233), ST233 (aa 130–233), and ST249 (aa 130–249). As a negative control, the stem fragment ST268 (aa 130–268) was used. This stem fragment has all of the sites in an uncleaved form. The antibody raised against STSGG192 (anti-CS1 IgG) reacted specifically to ST192 (Fig. 3A). Similarly, anti-CS2 IgG specifically reacted to ST233 and anti-CS3 IgG to ST249, respectively. None of these antibodies reacted to the uncleaved forms.

Using these antibodies, the conditioned medium of A375 cells with or without expression of MT1-MMP was examined by Western blotting. Anti-CS1 IgG recognized Fr.1 of 37–40 kDa and very weakly recognized the 65–70 kDa bands (Fig. 3B). Anti-CS2 IgG reacted specifically to Fr.2 of 50–60 kDa, and anti-CS3 IgG reacted specifically to Fr.3 of 65–70 kDa. Thus, shedding at the three identified cleavage sites generated the three major fragments. Although the 65–70 kDa bands were recognized weakly by anti-CS1 IgG, they may not be identical to those recognized by anti-CS3 IgG because no cross-reactivity of anti-CS1 IgG to ST249 was observed (Fig. 3A).
although they affect the enzymes differently depending on the subgroups to which they belong. TIMP-1 inhibits soluble MMPs but not MT-MMPs and ADAMs. TIMP-2 inhibits all of the MMPs but not ADAMs. TIMP-3 inhibits all of the MMPs and ADAMs. As a synthetic inhibitor, BB94, which inhibits both classes of MMPs and ADAMs, was also used.

Conditioned medium of A375 cells was prepared in the presence or absence of the inhibitors and the amount of each fragment was measured (Fig. 4). Shedding of Fr.1 and Fr.2 was increased by MT1-MMP and inhibited by TIMP-2, TIMP-3, and BB94 but not by TIMP-1. Shedding of Fr.3 was detected even without MT1-MMP, but it was increased by MT1-MMP. Shedding of Fr.3 was inhibited by TIMP-3 and BB94 but not by TIMP-1, and also was inhibited weakly by TIMP-2. Thus, the shedding at CS1 and CS2 is most likely mediated by MT1-MMP itself and that of CS3 by proteases in the ADAM family.

Two peptides spanning aa 187–218 (peptide A) and aa 228–254 (peptide B) were synthesized and used for digestion by MT1-MMP in vitro. Peptide A contains CS1 and peptide B contains CS2 and CS3. These peptides were incubated with the active form of MT1-MMP for 24 h and analyzed by mass spectrometry. MT1-MMP cleaved peptide A exactly at CS1 but did not cleave peptide B (data not shown). We also tested shorter peptides containing CS2, but to no avail (data not shown). These peptides were not digested by MMP-1, -2, -3, -7, or -9 (data not shown).

Detection of the Shed CD44 Fragments in Human Carcinoma Tissue. Because both MT1-MMP and CD44 are expressed frequently in tumors, shed CD44 fragments in tumor tissues were measured using

![Figure 2](https://example.com/fig2.png)

**Fig. 2.** Purification of the shed fragments and determination of the cleavage sites. **A,** purification of the shed fragments. Conditioned medium obtained from the A375 cells (5 x 10⁶) expressing CD44H and membrane-type 1 matrix metalloproteinase was applied to immunoaffinity columns conjugated with monoclonal antibody 285-2F-12 or 268-1F5. The bound CD44H fragments were eluted with a trifluoroacetic acid solution and subjected to SDS-PAGE under reducing conditions. The gel was stained with Sypro Ruby. **B–E,** matrix-assisted desorption ionization-MS/MS spectra obtained for the tryptic fragments that are expected to contain the cleavage sites. Amino acid sequences determined from the laser-fragmented peaks are indicated below.
the sandwich EIA system to confirm whether cleavage at the determined sites actually occurs in vivo. Tissue homogenates were prepared from 21 oral carcinoma specimens and corresponding normal tissues and were subjected to the assay (Fig. 5). In normal tissues, there was more of the fragment cleaved at CS3 than those at the other two sites. In carcinomas, the amount of each shed fragment was 0.144\(\pm\)0.06 (CS1), 0.027\(\pm\)0.015 (CS2), and 0.402\(\pm\)0.262 ng/mg protein (CS3; mean\(\pm\)SD), respectively. Shedding at CS1 significantly increased in carcinomas (2.4 folds; \(P<0.05\)), although the difference was not significant with the fragment cleaved at CS3. In contrast, the amount of fragment cleaved at CS2 was very small and did not differ significantly between normal and carcinoma tissue samples. Thus, CD44 shedding at CS1 and CS3 represents the normal physiologic process and increased shedding at CS1 is associated with malignant tumors.

**Detection of the Shed Fragments in Situ.** Cleaved CD44 fragments are released from the cell surface. However, this may not occur for some time because hyaluronan and other ligands form a large complex and have multiple sites for receptor binding. Therefore, we tried to localize the shed fragments in carcinoma tissues using the specific antibodies to CS1 and CS3. Representative immunostaining samples for five cases of oral, gastric, and hepatic carcinomas are presented in Fig. 6. MT1-MMP was detected mainly in carcinoma cells with some weakly positive cells in the stroma as reported previously (31). The fragment cleaved at CS1 showed a similar staining pattern to MT1-MMP. The fragment cleaved at CS3 was also observed in carcinomas, but strong signals were also detected in neutrophil-like cells in the stroma, where expression of MT1-MMP is not so evident. The staining was specific to the antigens used for immunization because all of the signals were absorbed completely by the antigen peptides.

**DISCUSSION**

Shedding is the last event in the regulation of CD44 function, although information about the cleavage sites, responsible proteases, and the regulation of the shedding events has been limited. In this study, we purified the shed CD44 fragments from a melanoma A375 cell line and determined the cleavage sites using matrix-assisted desorption ionization-MS/MS. Because CD44 is highly glycosylated...
in a heterogeneous manner, identification of the cleavage sites is essential for obtaining information about the responsible proteases.

Three cleavage sites (CS1, CS2, and CS3) were identified and specific antibodies that recognize the new epitopes exposed at the cleaved COOH terminus were generated. Using these antibodies, cleavage at the sites was found to generate three major fragments, 37–40 kDa (Fr.1), 50–60 kDa (Fr.2), and 65–70 kDa (Fr.3). Sandwich EIA systems were also developed and were used to monitor the specific cleavage event at each site. Because the cleavage at CS1 and CS2 was inhibited by TIMP-2 but not by TIMP-1, these two sites were expected to be cleaved by MT1-MMP. However, only the peptide containing CS1 was cleaved by recombinant MT1-MMP in vitro.

In a previous study, we digested the CD44 stem fragment (ST268) with recombinant MT1-MMP in vitro and identified one major and two minor cleavage sites (15). The major site coincided with CS1. Thus, the previous result from the in vitro study was confirmed at the cellular level. However, cleavage at the two minor sites was not detected in the present study, so these minor sites may exist only in vitro. The G-X sequence in CS1 and CS2 appears frequently at MMP-sensitive sites in proteins (32). However, these sites were not cleaved by MMP-1, -2, -3, -7, or -9, at least under the in vitro conditions in which CS1 was cleaved by MT1-MMP. However, it is possible that MT1-MMP-related enzymes such as MT2-MMP, MT3-MMP, and MT5-MMP may cleave CS1 and CS2.

The cleavage at CS3 was inhibited by TIMP-3 and BB94 but not by TIMP-1 and TIMP-2. This profile best fits the enzymes in the ADAM family (33, 34). Almost all of the cell lines that express CD44 constitutively shed it as 65–70 kDa fragments. Although we do not know which ADAM members are responsible for the shedding, it

whereas the peptide containing CS2 was not cleaved by recombinant MT1-MMP in vitro. In a previous study, we digested the CD44 stem fragment (ST268) with recombinant MT1-MMP in vitro and identified one major and two minor cleavage sites (15). The major site coincided with CS1. Thus, the previous result from the in vitro study was confirmed at the cellular level. However, cleavage at the two minor sites was not detected in the present study, so these minor sites may exist only in vitro. The G-X sequence in CS1 and CS2 appears frequently at MMP-sensitive sites in proteins (32). However, these sites were not cleaved by MMP-1, -2, -3, -7, or -9, at least under the in vitro conditions in which CS1 was cleaved by MT1-MMP. However, it is possible that MT1-MMP-related enzymes such as MT2-MMP, MT3-MMP, and MT5-MMP may cleave CS1 and CS2.

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must be those constitutively expressed in a wide array of cell lines. Interestingly, the cleavage at CS3 was enhanced by expression of MT1-MMP. Thus, some regulatory link may exist between the responsible ADAMs and MT1-MMP. Down-regulation of MT1-MMP expression in HT1080 cells using RNA interference (RNAi) also reduced the shedding of the 65–70 kDa fragments (28).

The shedding sites determined for CD44H in this study are not merely the ones observed in cell culture in vitro, but rather represent physiologic events in vivo. A significant amount of the fragments cleaved at CS1 and CS3 was detected by sandwich EIA in tumors. Conversely, the amount of the fragment cleaved at CS2 was very small. Interestingly, the amount of the fragment cleaved at CS1 was significantly larger in tumors than in normal tissue (P < 0.05). However, the cleavage at CS3 did not differ greatly between tumor and normal tissues. Immunostaining of the tumor tissue using the cleavage site-specific antibodies identified the location of the fragment cleaved at CS1 as being MT1-MMP-expressing tumor cells, whereas the location of the fragment cleaved at CS3 was identified as being tumor cells and neutrophil-like cells with strong intensity in the stroma. Thus, CD44 shedding at CS1 in tumors is most likely cleaved by MT1-MMP.

It has been reported that CD44 fragments circulate in the body and their level in serum is increased in patients with cancer (19–21). Because fragments in serum are highly heterogeneous in forms of glycosylation, sandwich EIA systems used to measure the amount of fragment cleaved at a specific site would be a powerful tool with which to monitor fragments that may reflect certain disease conditions like cancer. Unfortunately, however, the present system is not sufficiently sensitive for this purpose and we are trying to improve the assay.

Cleavage at the three sites all disrupts cell-ligand interaction through CD44H. However, the stem region where the cleavage occurs has multiple functional elements such as the site for insertion of the fragment cleaved at CS1 as being MT1-MMP-expressing tumor cells, whereas the location of the fragment cleaved at CS3 was identified as being tumor cells and neutrophil-like cells with strong intensity in the stroma. Thus, CD44 shedding at CS1 in tumors is most likely cleaved by MT1-MMP.

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