Identification and Characterization of the Fifth Membrane-type Matrix Metalloproteinase MT5-MMP*

(Received for publication, November 9, 1998, and in revised form, December 18, 1998)

Duanqing Pei‡

From the Department of Pharmacology, University of Minnesota, Minneapolis, Minnesota 55455

A new member of the membrane-type matrix metalloproteinase (MT-MMP) subfamily tentatively named MT5-MMP was isolated from mouse brain cDNA library. It is predicted to contain (i) a candidate signal sequence, (ii) a propeptide region with the highly conserved PRCGVPD sequence, (iii) a potential furin recognition motif RRRRNKR, (iv) a zinc-binding catalytic domain, (v) a hemepoxin-like domain, (vi) a 24-residue hydrophobic domain as a potential transmembrane domain, and (vii) a short cytosolic domain. Reverse transcriptase-polymerase chain reaction analysis of its transcripts indicates that MT5-MMP is expressed in a brain-specific manner consistent with the origin of its EST clone from cerebellum. It is also highly expressed during embryonic development at stages day 11 and 15. Like other MT-MMPs, MT5-MMP specifically activates progelatinase A when co-expressed in Madin-Darby canine kidney cells. Its ability to activate progelatinase A is dependent on its proteolytic activity since a mutation converting Glu to Ala in the zinc binding motif HEHEHEGH renders MT5-MMP inactive against progelatinase A. In contrast to other MT-MMPs, MT5-MMP tends to shed from cell surface as soluble proteinases, thus offering flexibility as both a cell bound and soluble proteinase for extracellular matrix remodeling processes. Taken together, these properties serve to distinguish MT5-MMP as a versatile MT-MMP playing an important role in extracellular matrix remodeling events in the brain and during embryonic development.

Members of the matrix metalloproteinase (MMP) family have been well documented as critical players in the breakdown of extracellular matrix (ECM) under both physiological as well as diseased conditions ranging from embryo implantation to cancer progression (1–4). Highly modular in design, all MMPs share three basic functional domains found in the smallest MMP matrilysin: (i) a signal peptide for extracellular targeting, (ii) a prodomain with the cysteine-switch for latency, and (iii) a conserved catalytic domain built around a zinc-binding site HEXXH as the catalytic core (5, 6). The addition of a hemopexin-like domain to this basic design eventually led to the evolution of the rest of the MMP family and confers them with specificity in substrate as well as inhibitor bindings (5, 6). While the majority of the MMPs are secreted as soluble enzymes into extracellular milieu, a subset of MMPs have been identified in recent years to contain additional sequences downstream of the hemopexin-like domain capable of anchoring the MMPs on plasma membrane (7–10). Named after the putative transmembrane domains as membrane-type matrix metalloproteinase 1 to 4 (MT1-, MT2-, MT3-, and MT4-MMPs), these enzymes have been proposed to be the master switches of ECM turnover based on the purported ability of MT-MMPs to activate other MMPs such as progelatinase A and collagenase 3: two degradative enzymes widely implicated in tumor invasion and metastasis (7, 11, 12). However, MT-MMPs themselves are synthesized in latent forms and activation is required for them to exert any proteolytic function (13–16). The mechanism responsible for MT-MMP activation appears to be mediated by members of the proprotein convertase family which can specifically cleave off the prodomain at the carboxyl side of the conserved RXRXXKR motif sandwiched between the pro- and catalytic domains of all MT-MMPs, a mechanism first demonstrated in stromelysin-3 (13–18). Thus, a proprotein convertase/MT-MMP/MMP cascade could be potentially responsible for the regulation of ECM turnover at the level of zymogen activation.

Despite the extensive sequence homology and functional overlap among MT-MMPs, little is known about any functional cooperation among themselves in executing ECM remodeling. Their patterns of expression suggest a complex picture with overlapping expression in both normal and tumor tissues (7–10, 19). For example, breast cancer tissues are known to express MT1-, MT2-, MT3-, and MT4-MMPs individually or together as detected by Northern blotting and in situ hybridization (19–21). In addition, MT1-MMP has been investigated extensively and found to be expressed in other malignant tumors such as those from human brain, colon, pancreas, liver, gastrointestinal organs, ovary, and cervix (18, 22–27). Among tissues and cell examined, expression of MT1-, MT2-, and MT3-MMPs seems to correlate well with the activation of progelatinase A, suggesting that MT-MMPs may act cooperatively toward progelatinase A in vivo (7, 22–27). With the expansion of the MT-MMP family, it becomes apparent that the function of MT-MMPs may not be only restricted to progelatinase A and collagenase 3 activation. In fact, purified MT1-MMP and MT2-MMP can degrade fibronectin, laminin, type I and III collagens, nidogen, tenascin, aggrecan, and perlecan (13, 14, 16). MT3-MMP appears to be able to degrade denatured type I collagen (gelatin), native type III collagen, and fibronectin based on limited studies (28, 29). Taken together,
MT-MMPs are a subgroup of versatile proteases involved in ECM remodeling by both activating other MMPs as well as directly degrading ECM components.

In contrast to secreted MMPs, MT-MMPs may express their proteolytic activities more efficiently by anchoring on cell membrane and enjoying two distinct advantageous properties, which are highly focused on ECM substrates and more resistant to protease inhibitors present in the extracellular milieu (11, 30). Recently, Nakahara and colleagues (31) demonstrated that MT1-MMP is localized in the invadopodia of malignant melanoma via the transmembrane/cytosplasmic domain, responsible for the efficient degradation of subjacent substrates and invasion into ECM in vitro. MT1-MMP can also confer mouse lung carcinoma cells metastatic phenotype upon transfection when analyzed in a tail-vein injection assay in vivo (32).

Therefore, recent attention has been shifting toward the characterization of membrane-bound MMPs and their biochemical properties (11). In this report, the identification and characterization of MT5-MMP, the fifth member of the MT-MMP subfamily, is described.

MATERIALS AND METHODS

Cell Lines and Reagents—MDCK cells and COS 7 were obtained and maintained as described previously (17, 33). DNA restriction and modification enzymes were purchased from Promega (Madison, WI). Oligonucleotide primers were made by the University of Minnesota microchemical core facility. COS cells are used for transient gene expression in cells lacking T antigen such as MDCK cells. For progelatinase A activation, MDCK is preferred because it expresses higher levels of furin, a putative MT5-MMP activator, than COS (17).

cDNA Cloning and Sequence Analysis—The original EST clone EST27028 was obtained from American Type Culture Cell (ATCC, MD). The rest of EST27028 was sequenced by primer-walking using an ABI371 automatic sequencer. The resulting sequence was blasted against Genbank data base and aligned to 321GNFDT of MT1-MMP. The rest of EST27028 contains basically the entire hemopexin-like domain, followed by a putative transmembrane domain and cytosolic domain. Since this gene is homologous, but not identical to known MT-MMPs, it was named MT5-MMP as the fifth member of the MT-MMP subgroup. An EcoRI fragment from this clone was then isolated and used as a probe to isolate the missing part of MT5-MMP from both human and mouse brain cDNA libraries (Stratagene, CLONTECH, CA). From the human cDNA library, at least 10 clones have been isolated and sequenced to find the longest cDNA starting immediately upstream of the 5’ end. A parallel amplification was performed to give rise to the full-length sequence of mouse MT5-MMP. Sequence alignment was performed via Internet using program Multalin version 5.3.3 at http://www.expasy.ch/www/tools.html using blosum62 with Gap weight: 12; Gap length weight: 2. The dendrogram was constructed using ClustalW program.

The Distribution of MT5-MMP as Determined by RT-PCR—Pre-made cDNA panels from mice tissue was purchased from CLONTECH (Palo Alto, CA) and amplified with two primers located at the 3’ portion of MT5-MMP cDNA (5’TGGTCCATCGACTCCGAG-3’ and 5’TATGCGCTGC-CTGCACCG-3’); 2 min at 94°C for denaturation, 33 cycles of 10 s at 94°C, 30 s at 50°C for annealing and 30 s at 72°C for extension, followed by 10 min extension at 72°C. To control for the amount of cDNA used in each reaction, a parallel amplification using primers designed from the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase was performed under the same experimental conditions.

Construction of MT5-MMP Expression Vectors—The cDNA clone number 17 (missing the last 6 residues) was engineered by high fidelity PCR to give rise to MT5-MMPΔ6 which includes a FLAG tag at its COOH terminus for detection using monoclonal antibody (33). A chimeric primer, 5’CTCACTTGTCATCGCTCCGTCTGGATC-3’, was designed to cover part of the MT5-MMP sequence at the carboxyl end as well as the FLAG sequence. This primer was paired with T3 primer from vector to amplify MT5-MMPΔ6 from template clone number 17. The resulting fragment was cloned into pCR3.1uni and confirmed by sequencing as described (17). Full-length MT5-MMP was constructed as another primer, 5’TCTATAC- CACAATCGGACTGCGCGTTAAGTGATGAC-3’, containing the missing 6 residues at the COOH terminus to amplify the entire open reading frame with T3 primer from clone 17. The PCR fragment was cloned and characterized as described above. MT5-MMP(E252A) was made by sequential PCR as described previously using the following primers: 5’GGCTGCTGATCTGCAGCTCAT-3’ and 5’ATGCCGACATGTCGACGGC-3’. The mutant was cloned into the same pCR3.1uni vector and confirmed by DNA sequencing as described (17).

Analysis of MT5-MMP Products and Progelatinase A Activation—The antibody against human MT5-MMP was raised in rabbit using GST-hMT5-MMP (Try135→Cys139) as described previously (34). DNA constructs (1.5 μg each) pCR3.1uniMT5-MMPΔ6, pCR3.1uniMT5-MMP, and pCR3.1uniMT5-MMP(E252A) were transfected into COS 7 cells and their protein products were analyzed by immunoprecipitation as described previously (13, 17). To isolate cytosolic as well as membrane fractions, cells were disrupted initially by repeated freeze-thaw cycles and fractionated by centrifugation to partition into supernatant and cytosolic fractions and pellets as membrane fractions. The pellets were washed extensively and extracted with Triton X-100 (1%) in Tris-buffered saline as described (8). Both cytosolic as well as membrane fractions were analyzed by Western blot as described (33). For progelatinase A activation, pCR3.1GelA (0.1 μg) was transfected either alone or with pCR3.1uniMT5-MMPΔ6, pCR3.1uniMT5-MMP, or pCR3.1 uniMT5-MMP(E252A) into MDCK cells and the conditioned media were analyzed for gelatinase activity by zymography as described previously (13).

Generation of Stable MT5-MMP Transfectants—Expression vector for MT5-MMP was transfected into MDCK cells and stable clones were selected and characterized as described previously (13, 17). The expressed MT5-MMP products were analyzed by immunoprecipitations for both cell and secreted forms as described above. Synthetic metalloproteinase inhibitor BB-94 (5 μm, British Biotech, United Kingdom) was added to the serum-free Dulbeco’s modified Eagle’s medium and allowed to incubate with MT5-MMP cells. The conditioned media were analyzed for MT5-MMP activity by zymography directly, by Western blot after 10-fold concentration using Millipore YM10 membrane filtration as described (13, 34).

RESULTS

Molecular Cloning of MT5-MMP—A search of the public EST data base maintained in the National Center for Biotechnology Information produced a few MMP candidate genes. One such clone, EST27028 from human cerebellum with homology to stromelysin-3, actually resembles closely the hemopexin-like domain of MT1-MMP in a BLAST search of sequence data bases. To prove that this EST clone is part of a novel MT-MMP gene, the remaining portion of the open reading frame was sequenced to reveal the presence of a putative transmembrane domain and cytosolic domain. The resulting sequence shows strong homology to human MT3-MMP, thus named MT5-MMP according to the current terminology of this subgroup (7–10). A 1.5-kilobase fragment from this EST clone was isolated and used as a probe to screen for full-length clones from both human and mouse brain cDNA library (from CLONTECH and Stratagene). So far, a cumulative sequence for the human MT5-MMP covers the entire predicted open reading frame except the pro-domain upstream of the conserved PCRGVPD cysteine switch. A 5’ rapid amplification of cDNA ends strategy was currently under way to recover the missing 5’ end. However, the sequencing for mouse MT5-MMP yielded the full-length open reading frame with both 5’- and 3’-untranslated region (Fig. 1).

The mouse and human MT5-MMP are over 95% homologous, thus, representing indeed a novel gene of the MT-MMP subgroup.

Structural Features of MT5-MMP and Its Relationship to Other MMPs—As shown in Figs. 1 and 2, MT5-MMP possesses...
MT5-MMP and Its Shedding from Cell Surfaces

Characterization of MT5-MMP Protein Products and Their Activities—Since MT5-MMP is highly homologous to other known MT-MMPs capable of activating progelatinase A (7–9), MT5-MMP is hypothesized to be a cell membrane-associated activator of progelatinase A. To test this possibility, MT5-MMPΔ6FLAG, an expression vector derived from the clone 17 of MT5-MMP (missing the last 6 residues at the COOH terminus) was transfected into COS cells and the products were analyzed by immunoprecipitation and immunoblotting as described (17). Consistent with the presence of a putative transmembrane domain at its COOH terminus, MT5-MMP is detected by immunoprecipitation as a 63-kDa major species only in the lysates, not the conditioned media, of cells transfected with MT5-MMPΔ6FLAG (Fig. 4A, lanes 2 and 4), while mock transfected cells are negative (Fig. 4A, lanes 1 and 3). As reported for MT1-MMP (7, 13), this species may represent the proenzyme of MT5-MMP. In addition, there are minor species slightly above the 63-kDa main species which may represent the minor glycosylated form. Interestingly, a visible protein species around 130 kDa was also detected from the MT5-MMPΔ6FLAG activated transfected cells (Fig. 4A, lanes 3). As shown by immunoblotting in Fig. 4B, MT5-MMP is mainly associated with the membrane fraction, not the cytosol (Fig. 4B). MT5-MMPΔ6FLAG activated progelatinase A was tested in co-transfection experiment in MDCK cells. As shown in Fig. 4C, MT5-MMPΔ6FLAG activated co-expressed progelatinase A specifically, whereas gelatini-
nase B was not activated (Fig. 4c, lanes 1–5). In the same experiment, similar amounts of MT1- and MT3-MMPs were also transfected to serve as positive controls. MT5-MMP D6-FLAG appears to be slightly less efficient than MT1- and MT3-MMPs in mediating progelatinase A activation.

Catalytically Inactive MT5-MMP Fails to Activate Progelatinase A—Full-length MT5-MMP was constructed by adding back the missing 6 residues to the carboxyl terminus of clone 17. This full-length MT5-MMP construct is functionally indistinguishable from MT5-MMP D6 in progelatinase A activation (data not shown), with similar efficiency, thus suggesting the last 6 residues are not required for the gelatinase A activation. In an initial attempt to characterize the mechanism responsible for MT5-MMP-mediated gelatinase A activation, a full-length mutant named MT5-MMP(E252A) was generated by substituting Glu252 with an Ala residue within the catalytic motif HE252LGH. A similar mutation has been shown to render progelatinase A catalytically inactive while maintaining overall structural integrity (33, 35). As shown in Fig. 5A, MT5-MMP(E252A) can be expressed in COS cells as protein species indistinguishable from wild-type MT5-MMP (Fig. 5, lanes 2 and 3). In fact, MT5-MMP(E252A) appears to accumulate to a higher level than the wild type enzyme. In a parallel experiment, MT5-MMP(E252A) fails to activate co-transfected progelatinase A in MDCK cells, while the full-length wild type MT5-MMP activated the co-transfected progelatinase A at similar degree as MT5-MMP D6 (Fig. 5B). Thus, the proteolytic activity of MT5-MMP is required for its ability to mediate gelatinase A activation.

Secretion of MT5-MMP Ectodomain into Conditioned Me-
dia—Full-length MT5-MMP as well as MT5-MMPΔ6 were introduced into MDCK cells and stable clones were selected in the presence of G418. A panel of positive clones for both constructs were obtained and shown to be able to activate progelatinase A added exogenously (data not shown). Surprisingly, in addition to the endogenous gelatinase B on the zymograms, additional gelatinolytic species were observed in MT5-MMP positive clones only, suggesting that some MT5-MMP products be secreted into the conditioned media. To examine this possibility, a stable clone F591 expressing full-length MT5-MMP was labeled with [35S]Met under serum-free conditions and processed into cytosolic (Fig. 6A, lanes 2 and 3). However, there is a general shift of MT5-MMP products from the full-length 63-kDa species to smaller fragments around 29, 40–46 kDa in cell lysates of MT5-MMP stable transfectants (Fig. 6A, lanes 2 and 3). In the conditioned media, a major 46-kDa and a minor 44-kDa species were also detected during the course of 3–8-h incubation (Fig. 6A, lanes 5 and 6). To analyze the proteolytic activity of the shed MT5-MMP species, serum-free media conditioned for 48 h were collected from F591 and analyzed on gelatin-zymogram. The major gelatinolytic MT5-MMP species migrates around 28 kDa instead of 44–46 kDa, indicating that the 44–46-kDa MT5-MMP species may be unstable in the culture media (Fig. 6B, lane 2), while the control transfected MDCK cells are negative for this activity (Fig. 6B, lane 1). The discrepancy between the zymogram and immunoprecipitation may be the result of further processing of the 44–46-kDa species into smaller ones during the 48-h incubation period. Since many MMPs undergo autocatalytic processing into smaller fragments, a synthetic MMP inhibitor BB94 was included in the conditioned media to inhibit further fragmentation (34). As a result, more gelatinolytic species were observed in the presence of BB94 including a pair of 48–50-kDa species on zymogram (5 μM, Fig. 6B, lane 3), similar to the 44–46-kDa species identified by immunoprecipitation (Fig. 6A, lane 6). This apparent migratory difference is probably due to the non-reduced and reduced conditions employed by these two electrophoresis procedures. Interestingly, BB94 appears to have enhanced the amount of the 28-kDa species in addition to the 48–50- and 29–33-kDa species (Fig. 6B, lane 3), suggesting the catalytic domain alone (28-kDa species) may decay autocatalytically. To confirm the identity of these gelatinolytic species in lanes 1–3 of Fig. 6B, the same conditioned media were concentrated 10-fold, analyzed by Western blotting using anti-MT5-MMP antisera and shown in lanes 4–6. The gelatinolytic species observed on zymography in lanes 2 and 3 of Fig. 6B are immunoreactive on immunoblots (Fig. 6B, lanes 4–6), albeit with varying degrees of intensity presumably due to the differences in structural integrity of the catalytic domain. MT5-MMP products which are immunoreactive, but negative on zymography, may contain the hemopexin domain only, lacking a functional catalytic domain (Fig. 6B, lane 2 versus 5). A

**Fig. 3. Tissue distribution of mouse MT5-MMP.** Reverse transcribed cDNA (0.5-ng aliquot each) from a multiple tissue panel (CLONTECH) including heart, brain, spleen, lung, liver, skeleton muscle, kidney, and testis (lanes 2–9) were amplified to give rise to the 400-base pair fragment of MT5-MMP. Similar cDNA preparations from whole embryos aged days 7, 11, 15, and 17 (E7 to 17 in lanes 10–13) were analyzed as the adult tissues. To control for the amount of cDNA in each reaction, a parallel PCR reaction using the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was carried out as suggested by the supplier.

**Fig. 4. Mouse MT5-MMP activates progelatinase.** A, MT5-MMP is cell-bound, not secreted. Control (lanes 1 and 3) and mouse MT5-MMPΔ6 (lanes 2 and 4) expression vector were transfected into COS cells and the MT5-MMP products were labeled with [35S]Met (100 μCi/ml, Amersham) for 3 h. The supernatants (lanes 2 and 3) and cell lysates (lanes 3 and 4) were harvested and analyzed by immunoprecipitation using anti-MT5-MMP antisera as described under “Materials and Methods.” The arrowhead indicates the main MT5-MMP species while the line above the arrowhead marks the putative dimer of MT5-MMP. B, MT5-MMP is in the membrane fraction, not the cytosol. COS cells were transfected with control (lanes 1 and 3) or MT5-MMPΔ6 (lanes 2 and 4) as in A and processed into cytosolic (lanes 1 and 2) and membrane fractions (lanes 3 and 4) as described under “Materials and Methods.” Both fractions were analyzed by immunoblotting using anti-MT5-MMP antisera. The MT5-MMP-specific species is indicated by an arrowhead. C, MT5-MMP activates progelatinase A. MDCK cells (lane 5) were transfected with gelatinase A expression vector (0.1 μg, lanes 1–4) and control vector (1.5 μg, lane 1). MT1-MMP (lane 2), MT3-MMP (lane 3), or MT5-MMPΔ6 (lane 4). The conditioned media were analyzed by zymography. Gel B, gelatinase B/MMP9; Gel A, gelatinase A/MMP2; MT1, MT1-MMP; MT3, MT3-MMP; MT5, MT5-MMP; MT5Δ6, MT5-MMP with a deletion of the last 6 residues.
expression vector (0.1 μg, lane 2) or full-length wild type MT5-MMP (1.5 μg, lane 3) or full-length mutant MT5-MMP(E252A) (1.5 μg, lane 4). Conditioned media (48 h) were analyzed by zymography as described in the legend to Fig. 4. MT5, MT5-MMP, MT5EA, MT5-MMP(E252A) mutant.

similar pattern of MT5-MMP products are secreted by MCF7 and T47D cells stably transfected with MT5-MMP. Thus, it is concluded that MT5-MMP is shed into the culture media efficiently.

**DISCUSSION**

The MT-MMPs have been recognized as key regulators for ECM remodeling under both physiological and pathological conditions (11). Much of the attention has been focused on the ability of MT1-, MT2-, and MT3-MMPs to activate progelatinase A or procollagenase 3 on cell surface (7–10, 12). Synthesized as zymogens themselves, activation of proMT-MMPs is a pre-requisite for their proteolytic functions. The mechanism responsible for MT-MMP activation may rely on a basic motif RXKR found in all MT-MMPs, which is recognized and cleaved at the carboxyend by members of the proprotein convertase family such as furin localized intracellularly in the trans-Golgi network (13, 17). Thus, MT-MMPs could potentially be the cell surface anchor of a proteolytic cascade from PC to the soluble MMPs dedicated to the proteolysis of ECM components. The central role of MT-MMPs as key regulators in ECM degradation is further strengthened by the discovery that MT1-, MT2-, and MT3-MMPs can cleave ECM components directly, making MT-MMPs the most versatile MMPs discovered so far (13, 14, 16, 28, 29). The identification of MT5-MMP enlarges the MT-MMP subgroup to include 5 distinct members. This addition ensures that the MT-MMPs overtake the collagensases as the largest subgroup of the MMP superfamily. This finding is likely to reinforce the notion that membrane-associated proteolysis plays a critical role in ECM degradation necessary for many important biological and pathological processes (11, 36, 37).

Sequence alignment indicates that MT5-MMP is closely related to MT3-MMP, followed by MT1- and MT2-MMPs. MT4-MMP appears to be only marginally related to MT5-MMP. The domain structure of MT5-MMP follows the general design of other MT-MMPs with signal peptide, prodomain, RXKR activation motif, catalytic domain, hinge region hemopexin-like domain, and the stem/transmembrane/cytosolic domains. Despite a high degree of homology, MT5-MMP contains two short segments with divergent sequences: the hinge and stem region with unique dibasic motifs potentially recognizable by proprotein convertases (Figs. 1 and 2, Ref. 18). These two segments may confer unique function to MT5-MMP.

Unlike other MT-MMPs, MT5-MMP’s expression is highly restricted. In general, the level of expression for MT5-MMP tends to be much lower than the other MT-MMPs since conventional Northern blot fails to detect any meaningful transcript. RT-PCR analysis of various mouse tissues and developing embryos identified brain as the primary site for MT5-MMP expression and embryos also express this gene at day 11–15 stage. This pattern of expression suggests an unique role for MT5-MMP in mediating ECM turnover during normal biological process. More detailed studies are under way to define the precise location of MT5-MMP expression in mouse brain and developing embryos, especially in comparison with the other MT-MMP. The expression profile of MT5-MMP in human normal or cancer tissues is also under investigation.

The biochemical properties of MT5-MMP mirror those of MT1-, MT2-, and MT3-MMPs closely (7–9). MT5-MMP is able to activate progelatinase A when co-expressed or added exogenously, albeit slightly less efficient than MT1- and MT3-MMPs. However, the ECM degrading activity of MT5-MMP remains to be defined. In an effort to produce and purify active enzyme for substrate studies, stable clones of MT5-MMP have been generated to express full-length MT5-MMP. The 63-kDa species is a major product of MT5-MMP expressed both transiently and stably (Figs. 4 and 6). It is not clear whether this species represents the fully activated form of MT5-MMP, or simply the proform of MT5-MMP. Protein species with similar molecular weight have also been identified for at least MT1-

---

2 D. Pei, unpublished data.
MT5-MMP and Its Shedding from Cell Surfaces

and MT3-MMPs (7, 9). The fact that gelatinase A is activated by MT5-MMP in a proteolysis-dependent pathway suggests that at least a portion of MT5-MMP must have been activated by the cells, presumably in the trans-Golgi network by furin (13).

The most striking feature of MT5-MMP may be its tendency to be shed from the cell surface efficiently (see Fig. 6). A summary model is presented in Fig. 7. The MT5-MMP protein is synthesized, packaged, and delivered to cell surface where proteolytic cleavage on the stem region releases soluble MT5-MMP species into the extracellular space (Fig. 7). For example, MDCK cells stably expressing wild type MT5-MMP secrete and accumulate a pair of immunoreactive proteins at 44–46 kDa in culture media, a molecular mass range consistent with the predicted molecular mass of mature MT5-MMP without the 3–8 h in culture media, a molecular mass range consistent with the predicted molecular mass of mature MT5-MMP without the

and MT3-MMPs (7, 9). The fact that gelatinase A is activated by MT5-MMP in a proteolysis-dependent pathway suggests that at least a portion of MT5-MMP must have been activated by the cells, presumably in the trans-Golgi network by furin (13).

The most striking feature of MT5-MMP may be its tendency to be shed from the cell surface efficiently (see Fig. 6). A summary model is presented in Fig. 7. The MT5-MMP protein is synthesized, packaged, and delivered to cell surface where proteolytic cleavage on the stem region releases soluble MT5-MMP species into the extracellular space (Fig. 7). For example, MDCK cells stably expressing wild type MT5-MMP secrete and accumulate a pair of immunoreactive proteins at 44–46 kDa in 3–8 h in culture media, a molecular mass range consistent with the predicted molecular mass of mature MT5-MMP without the transmembrane/cytosolic domain (Fig. 6A). On zymography, media conditioned for 48 h contain only a 28-kDa gelatinolytic activity, which is likely to be shed MT5-MMP secreted from the cell surface (Fig. 6B). The inclusion of BB94 in the conditioned media actually enhanced the accumulation of the 28-kDa species as well as additional gelatinolytic species including the putative 48–50-kDa mature transmembraneless MT5-MMP species (Fig. 6B). Since BB94 fails to inhibit the shedding process, it is unlikely that MT5-MMP is secreted autocatalytically (Fig. 7). The fact that both MCF7 and T47D breast cancer cells can also shed MT5-MMP from cell surface suggests that the shedding of MT5-MMP can be generalized. It is noteworthy that the two most divergent segments in MT5-MMP, i.e. the hinge region immediately downstream of the catalytic domain and the stem region just NH2-terminal to the transmembrane domain, contain multiple dibasic motifs which could be targets for the members of the proprotein convertase family (see Figs. 1 and 7, Ref. 18). It is of interest to note that MT5-MMP is processed into smaller fragments, including the 44–46-kDa species observed in the conditioned media.
MT5-MMP and Its Shedding from Cell Surfaces

25. Imamura, T., Ohshio, G., Mise, M., Harada, T., Suwa, H., Okada, N., Wang, Z., Yoshitomi, S., Tanaka, T., Sato, H., Arii, S., Seiki, M., and Imamura, M. (1998) *J. Cancer Res. Clin. Oncol.* 124, 65–72
26. Ohtani, H., Motohashi, H., Sato, H., Seiki, M., and Nagura, H. (1996) *Int. J. Cancer* 68, 565–570
27. Gilles, C., Puette, M., Piette, J., Munauf, C., Thompson, E. W., Birembaut, P., and Foidart, J. M. (1996) *Int. J. Cancer* 65, 209–213
28. Matsumoto, S., Katoh, M., Saito, S., Watanabe, T., and Masuho, Y. (1997) *Biochim. Biophys. Acta* 1354, 159–170
29. Shofuda, K., Yasumitsu, H., Nishihashi, A., Miki, K., and Miyazaki, K. (1997) *J. Biol. Chem.* 272, 9749–9754
30. d’Ortho, M. P., Stanton, H., Butler, M., Atkinson, S. J., Murphy, G., and Hembry, R. M. (1998) *FEBS Lett.* 421, 159–164
31. Nakahara, H., Howard, L., Thompson, E. W., Sato, H., Seiki, M., Yeh, Y., and Chen, W. T. (1997) *Proc. Natl. Acad. Sci. U. S. A.* 94, 7959–7964
32. Tsunetzuka, Y., Kinoh, H., Takino, T., Watanabe, Y., Okada, Y., Shinagawa, A., Sato, H., and Seiki, M. (1996) *Cancer Res.* 56, 5678–5683
33. Pei, D., and Yi, J. (1998) *Protein Expression Purif.* 13, 277–281
34. Pei, D., Majmudar, G., and Weiss, S. J. (1994) *J. Biol. Chem.* 269, 25849–25855
35. Crabbe, T., Zucker, S., Cockett, M. I., Willenbrock, F., Tickle, S., O’Connell, J. P., Scorthern, J. M., Murphy, G., and Docherty, A. J. P. (1994) *Biochemistry* 33, 6684–6689
36. Brooks, P. C., Stremblad, S., Sanders, L. C., von Schales, T. L., Aimes, R. T., Stetler-Stevenson, W. G., Quigley, J. P., and Cheresh, D. A. (1996) *Cell* 83, 683–693
37. Nagase, H. (1998) *Cell Res.* 8, 179–186
38. Matsumoto, S., Katoh, M., Saito, S., Watanabe, T., and Masuho, Y. (1997) *Biochim. Biophys. Acta* 1354, 159–170
39. Imai, K., Ohuchi, E., Aoki, T., Nomura, H., Fujii, Y., Sato, H., Seiki, M., and Okada, Y. (1996) *Cancer Res.* 56, 2707–2710