Matrilysin-2, a New Matrix Metalloproteinase Expressed in Human Tumors and Showing the Minimal Domain Organization Required for Secretion, Latency, and Activity

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Abstract

We have identified a human placenta cDNA coding for a new member of the matrix metalloproteinase (MMP) family. The isolated cDNA encodes a polypeptide of 261 amino acids, the smallest MMP identified to date, which contains several structural features of MMPs including the signal sequence, the prodomain involved in enzyme latency, and the catalytic domain with the zinc-binding site. However, it lacks the hinge region and hemopexin-domain present in most MMPs. According to these structural characteristics, the human MMP described herein has been called matrilysin-2 (MMP-26), because it exclusively shares with matrilysin this minimal domain organization required for secretion, latency, and activity. The amino acid sequence of matrilysin-2 also contains a threonine residue adjacent to the Zn-binding site that has been defined as a specific feature of matrilysin. Chromosomal location of the matrilysin-2 gene showed that it maps to the short arm of chromosome 11, a location distinct to that of other MMP genes. Matrilysin-2 was expressed in Escherichia coli, and, after purification and refolding, the recombinant protein was found to degrade synthetic substrates commonly used for assaying MMPs. Furthermore, this protein hydrolyzed type IV collagen, fibronectin, fibrinogen, and gelatin, which indicated that matrilysin-2 is a potent enzyme with a wide substrate specificity. In addition, it was found that matrilysin-2 is able to activate progelatinase B. Proteolytic activity of matrilysin-2 against all of these substrates was abolished by synthetic inhibitors and by tissue inhibitors of metalloproteinases. Expression analysis revealed that matrilysin-2 is detected not only in placenta and uterus but is widely expressed in malignant tumors from different sources as well as in diverse tumor cell lines. These data together with its broad spectrum of proteolytic activity, suggest that matrilysin-2 may play a role in some of the tissue-remodeling events associated with tumor progression.

Introduction

It is well established that metastatic spread of tumor cells is responsible for most cancer deaths. Consequently, the elucidation of the molecular mechanisms involved in the metastatic process is the focus of much current cancer research. A large body of evidence indicates that this process is largely mediated by the combined action of proteolytic enzymes able to degrade extracellular matrix and basement membranes, thus facilitating tumor invasion and metastasis. Among the variety of proteinases potentially implicated in tumor progression, the MMPs have attracted considerable interest because of their ability to collectively degrade essentially all protein constituents of connective tissues. MMPs form a family of endopeptidases whose complexity has considerably increased after the finding of a series of new family members identified in both normal and pathological conditions, including tumor biopsies. Thus, 19 different human MMPs have been cloned and characterized at the amino acid sequence level. On the basis of their substrate specificity, primary structures, and cellular localization, MMPs can be divided into at least five subfamilies: the collagenases, gelatinases, stromelysins, MT-MMPs, and other MMPs. The structure of most members of the MMP family is organized into four well-defined domains: a signal peptide to direct secretion from the cell, a propeptide with a conserved cysteine residue involved in maintaining enzyme latency; a catalytic domain with a Zn-binding site; and a hemopexin-like domain at the COOH-terminal region. However, some enzymes like gelatinases and MT-MMPs contain additional domains, whereas other MMPs such as matrilysin and MMP-23 are devoid of the hemopexin domain present in all of the remaining human MMPs identified to date.

The association of MMPs with malignant tumors is well documented both in vitro and in vivo. In general, MMP levels, as compared with nonmetastatic counterparts, are abnormally elevated in metastatic tumor cell lines. On the other hand, high MMP levels are associated with invasive lesions and poor clinical prognosis in cancer patients. However, the finding of new members of the MMP family and their subsequent functional characterization at both genetic and biochemical levels has opened new views on the role of these enzymes in tumor progression. Thus, evidence is accumulating that MMPs are not exclusively involved in the proteolytic breakdown of the tissue barriers for metastatic spread. In fact, MMPs have been reported to play direct roles in other critical events in tumor evolution that occur at earlier stages, events such as tumor promotion, modulation of the growth of the primary tumor, and angiogenesis.

The finding that MMPs may be involved in such diverse biological processes during tumor progression has stimulated the search for new family members that could participate in some of the events associated with malignant tumors. In this work, we describe the molecular cloning and complete nucleotide sequence of a cDNA coding for matrilysin-2, a new MMP that shares a number of structural and functional properties with matrilysin (MMP-7). We also report the expression of the gene in Escherichia coli and perform an analysis of the enzymatic activity of the recombinant enzyme with the finding that matrilysin-2 is a potent enzyme with a wide substrate specificity on extracellular matrix and basement membrane proteins. Finally, we establish the chromosomal location of the matrilysin-2 gene in the human genome and analyze its expression in human tumors from different sources.
Materials and Methods

**Materials.** Specimens of human tumors were obtained from patients who had undergone surgery for diverse malignancies. Chemicals and reagents were obtained from Sigma (Poole, United Kingdom). Restriction endonucleases were from New England Biolabs (Ipswich, MA) and Roche Molecular Biochemical (Mannheim, Germany), respectively. Synthetic oligonucleotides were prepared in an Applied Biosystems (Foster City, CA) model 392A DNA synthesizer. Double-stranded DNA probes were radiolabeled with \(^{32}P\)dCTP (3000 Ci/mmol) purchased from Amersham International (Little Chalfont, Buckinghamshire, United Kingdom) using a commercial random priming kit from the same company. Panels of monochromosomal somatic cell hybrid DNAs and radiation hybrid DNAs were provided by the Human Genome Mapping Resource Center (Hinxton, Cambridge, United Kingdom).

**Screening of EST Database.** A search of the GenBank database of human ESTs for sequences with homology to MMPs, allowed us to identify a sequence (AI 743415; deposited by National Cancer Institute, Cancer Genome Anatomy Project) that was derived from a pool of cDNA libraries and that showed similarity with sequences of known human MMPs. To obtain this DNA fragment, we performed PCR amplification of a human cDNA from placenta (Clontech, Palo Alto, CA) with two specific primers 5'-CCATTTT-GACAGAAGTAGCA-3' (mat2-1) and 5'-CATTAGGTTGTCCAGATGAA-3' (mat2-2) derived from the AI 743415 sequence. The PCR reaction was carried out in a GeneAmp 2400 PCR system (Perkin-Elmer/Cetus) for 40 cycles of denaturation (94°C, 15 s), annealing (54°C, 30 s), and extension (72°C, 20 s).

The 232-bp PCR product that was amplified from human placenta cDNA was cloned, and its identity was verified by nucleotide sequencing.

**5'-Extension of Isolated cDNAs.** The 5'-ends of cloned cDNAs were extended by successive cycles of RACE using RNA from human placenta and the Marathon cDNA amplification kit (Clontech), essentially as described by the manufacturer. Finally, the full-length cDNA was obtained by PCR amplification using the Expand Long PCR kit (Boehringer-Mannheim). The PCR reactions were performed for 35 cycles of denaturation (15 s at 94°C), annealing (15 s at 64°C), and extension (2 min at 68°C), with primers 5'-ATGCACGTGCATCATTAAAGAGTT-3' (mat2-14) and 5'-CAGGGTGAATAAGTGCTCTTGCTC-3' (mat2-15). After gel purification, the amplification product was cloned and sequenced by standard procedures.

**Chromosomal Mapping.** DNA from a panel of 24 monochromosomal somatic cell hybrids containing a single human chromosome in a mouse or hamster cell line background was PCR-screened for the presence of the genomic sequence flanked by the primers: 5'-GAATAAGTCCTCTGTGCT-3' (mat2-1) and 5'-ATGCAGCTCGTCATCTTAAGAGTT-3' (mat2-14) and 5'-CAGGGTGAATAAGTGCTCTTGCTC-3' (mat2-15). After gel purification, the amplification product was cloned and sequenced by standard procedures.

**Expression Analysis.** Analysis of matrilysin-2 expression in human tissues was first performed by PCR amplification of a panel of cDNAs from commercially available libraries (Clontech). To do that, total RNA-phage DNA from the diverse human cDNA libraries was amplified with two primers (mat2-1, and mat2-2) specific for matrilysin-2, and the PCR reaction was performed as described above. To study matrilysin-2 expression in human tumors, we performed RT-PCR amplification with RNA from tumor biopsies using the RNA PCR kit from Perkin-Elmer. After RT using 1 μg of total RNA and random hexamers as primer according to the instructions of the manufacturer, the whole mixture was used for PCR with primers mat2-6 (5'-ACCTACTT-GACAGTGCAGTC-3') and mat2-9 (5'-GAGTGCTCTAAAGGTTCTTTA-3') for β-actin amplification, 2-μl aliquots were amplified in the same way with the oligonucleotides 5'-GTTGGGCGCGGCTCTAGGC-3' and 5'-TTT-GATGTCAGGCAATTT. Negative controls were performed using all of the reagents with the exception of random hexamers. Amplification products were analyzed by agarose gel electrophoresis.

**Enzymatic Assays.** Enzymatic activity of purified recombinant matrilysin-2 was detected using the synthetic fluorogenic substrates Mca-Pro-Leu-Glu-Leu-Dpa-Ala-Arg-NH\(_2\) (QF-24), Mca-Pro-Leu-Ala-Nva-Dpa-Ala-Arg-NH\(_2\) (QF-35), and Mca-Pro-Cha-Gly-Nva-His-Ala-Dpa-NH\(_2\) (QF-41; provided by C. G. Knight, University of Cambridge, United Kingdom). Routine assays were performed at 37°C at substrate concentrations of 1 μM in an assay buffer of 50 mM Tris/HCl, 5 mM CaCl\(_2\), 150 mM NaCl, 0.05% (w/v) Brij 35 and 150 mM imidazole. Fractions containing purified protein were combined and dialyzed against 50 mM Tris/HCl (pH 7.6), 5 mM CaCl\(_2\), 200 mM NaCl, and 0.05% Brij 35.

**Results**

**Identification, Molecular Cloning, and Characterization of a Human Placenta cDNA Encoding a New Member of the MMP Family.** To identify novel MMPs, we performed an exhaustive screening of the GenBank database of ESTs looking for entries with sequence similarity to previously described enzymes of this family. This analysis allowed us to identify a 593-bp EST that, when translated, generated an open reading frame with sequence similarity to the catalytic domain of MMPs. A cDNA containing part of this EST was 734-bp fragment of the matrilysin-2 cDNA containing the pro- and catalytic domains, was generated by PCR amplification with primers 5'-GAATCATTCCGTCATCATTAAAGAGTT-3' (EcoRI-promat2) and 5'-CGGAATTCGACGGTATGTCATAGTA-3' (EcoRI-promat2) using the full-length matrilysin-2 cDNA in pUC18. The PCR amplification was performed for 30 cycles of denaturation (95°C, 10 s), annealing (46°C, 10 s), and extension (68°C, 1 min), using the Expand High Fidelity PCR system. Because of the design of the oligonucleotides, the amplified fragment could be cleaved at both ends with EcoRI and BglII and ligated in frame into the pRSETB E. coli expression vector (Invitrogen), thereby adding a NH\(_2\)-terminal His\(_6\) tag to the product. The resulting pRSET-proM2 vector was transformed into BL21(DE3)lys8 competent E. coli cells and expression was induced by the addition of IPTG (0.5 mM final concentration) followed by further incubation for 3–20 h at 30°C. Recombinant protein obtained in inclusion bodies was solubilized using 20 mM Tris buffer (pH 7.6) containing 6 M GdnHCl, and 5 mM DTT, and purified in a Superdex-75 column (Amersham-Pharmacia, Uppsala, Sweden) equilibrated with 20 mM Tris buffer (pH 7.6) containing 3 mM GdnHCl, and 5 mM DTT. After SDS-PAGE analysis, peak fractions with the recombinant protein were pooled and the GdnHCl concentration adjusted to 6 M. Refolding was achieved by dilution (1:50) into a 50 mM Tris buffer (pH 7.6) containing 5 mM CaCl\(_2\), 200 mM NaCl, 50 μM ZnSO\(_4\), 0.05% Brij 35, 2.5 mM oxidized glutathione, 2.5 mM reduced glutathione, 20% glycerol, and 2.3 μM urea, and stirring overnight at 4°C. The refolding mixture was applied to a Ni-chelated nitriolactic acid agarose column (Qiagen) equilibrated with the above buffer. The protein bound via the NH\(_2\)-terminal His\(_6\) tag was eluted with a 50 mM Tris buffer (pH 7.6) containing 5 mM CaCl\(_2\), 200 mM NaCl, 0.05% Brij 35, and 150 mM imidazole. Fractions containing purified protein were combined and dialyzed against 50 mM Tris/HCl (pH 7.6), 5 mM CaCl\(_2\), 200 mM NaCl, and 0.05% Brij 35.

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obtained by PCR amplification of total λ-phage DNA prepared from a human placenta cDNA library.

A detailed comparative analysis of the sequence, obtained for the identified EST with the sequence of MMPs, clearly indicated that it was not long enough to encode a functional MMP. To extend the partial cDNA sequence toward the 5' end, we performed 5'-RACE experiments using RNA from human placenta as a template. Successful 5'-RACE experiments led us finally to obtain a fragment long enough to contain the entire coding information for the identified MMP. Computer analysis of the obtained sequence (European Molecular Biology Laboratory accession number AJ251531) showed the presence of an open reading frame coding for a protein of 261 amino acids with a predicted molecular mass of 29.7 kDa.

This sequence contains two potential sites of N-glycosylation (N-G-T and N-V-T, at positions 64 and 133, respectively) and exhibits overall sequence similarity with other MMPs, the maximum percentage of identities being with MMP-12 (about 50%). The identified amino acid sequence also shows the presence of a series of structural features characteristic of MMPs (Fig. 1). Thus, the finding of a NH2-terminal hydrophobic sequence adjacent to the initiator methionine suggests the presence of the signal peptide that targets these enzymes to the secretory pathway. This sequence is followed by a prodomain region with the activation locus containing the essential cysteine residue (at position 82) for maintaining enzyme latency. Finally, the identified sequence contains a catalytic domain of about 170 residues including the consensus sequence HEEXXXGXXHS (at positions 208–219) involved in the coordination of the catalytic zinc atom at the active site of MMPs. Similarly, the structural zinc-ligands (His-158, Asp-160, His-173, and His-186), and the structural calcium-ligands (Asp-165, Asp-188, and Glu-191) strictly conserved in MMPs, are also present in this domain of the identified protein. This catalytic domain also shows a methionine residue located seven amino acids COOH-terminal to the zinc-binding site, that is absolutely conserved in all of the MMPs. The presence of a Thr residue immediately before the first His of the zinc-binding site (Fig. 1) is also remarkable. This residue is exclusively found in human, mouse, rat, and cat matrilysin among all vertebrate MMPs and has been defined as a specific structural signature of this enzyme (15, 16). More interestingly, the predicted amino acid sequence lacks the hinge sequence and the COOH-terminal hemopexin-like domain characteristic of most MMPs. In fact, these domains are present in all human MMPs characterized to date with the exception of matrilysin (MMP-7) and MMP-23 (17, 18). However, MMP-23 does exhibit a unique COOH-terminal domain rich in cysteine and proline residues and lacks the cleavable signal sequence and cysteine-switch region generally present in all MMPs, thus being very distinct from the novel MMP described herein.

When all of these structural comparisons are taken together, it seems that the newly identified human MMP is closely related to matrilysin in its modular design and markedly different from all of the

Fig. 1. Amino acid sequence alignment of human matrilysin-2 with matrilysin from different species. The amino acid sequences of previously described matrilysins (human (Hs), cat (Fd), rat (Rn), and mouse (Mm)) were extracted from the SwissProt database, and the multiple alignment was performed with the PILEUP program of the Genetics Computer Group package (University of Wisconsin). The threonine residue characteristic of matrilysins is boxed. Common residues to all sequences are shadowed. Numbering corresponds to the sequence of human matrilysin-2.

Fig. 2. Chromosomal mapping of the human matrilysin-2 gene. Total DNA (100 ng) from the 24 monochromosomal somatic cell lines was PCR-amplified with primers 5'-AAATTCTGGAAATCCTGGAGTTGTCC and 5'-GTGGGGTACATTATGGAGCTCTG as described in “Materials and Methods.” ADNA digested with EcoRI and HindIII (Marker III, Boehringer Mannheim) was used as a size marker.
remaining human MMPs described to date. According to these data, we propose to give the name matrilysin-2 to the novel MMP family member identified in this work. According to the nomenclature system for vertebrate MMPs, matrilysin-2 would be MMP-26, inasmuch as MMP-25 corresponds to MT6-MMP, the last family member recently identified in fetal liver and brain tumors (6).

**Chromosomal Location of the Human Matrilysin-2 Gene.** To establish the chromosomal location of the human matrilysin-2 gene, we first used a PCR-based strategy to screen a panel of somatic cell hybrid lines containing a single human chromosome in a rodent background. The sequence-tagged site specific for the matrilysin-2 gene was generated by using two specific oligonucleotides presumably corresponding to different exons of the gene. As can be seen in Fig. 2, positive amplification results were obtained only in the hybrid containing the autosome number 11. Because no amplification products were observed in the hybrids containing the remaining human chromosomes, we can conclude that the matrilysin-2 gene maps to chromosome 11. To determine more precisely the chromosomal location of the gene, we next performed a PCR-based screening of a panel of radiation hybrids containing human chromosome fragments in a rodent background. Computer analysis of positive amplification results indicated that the matrilysin-2 gene is located at 3.2 cR from the top of chromosome 11 linkage group. This location at the short arm of chromosome 11, around 11p15, differs from that reported for other MMP genes that have been previously found to map at the long arm of this chromosome and clustered at 11q22 (19).

**Production of Recombinant Matrilysin-2 in Bacterial Cells and Analysis of the Enzymatic Activity.** To analyze the enzymatic properties of matrilysin-2, we first expressed the cloned cDNA in *E. coli*. A cDNA coding for the pro- and catalytic domains of this protein was subcloned into the expression vector pSETB, and the resulting plasmid was transformed into *E. coli* BL21 (DE3)pLysS. Transformed bacteria were induced with IPTG, and a band of the expected size (M, 29,000) was detected when protein extracts were analyzed by SDS-PAGE (Fig. 3A). This recombinant protein was purified using size-exclusion and Ni-NTA-agarose chromatography, and then was subjected to a refolding procedure as described previously (18). However, and similarly to other MMPs, all of the proenzyme was autoactivated after performing the last step of dialysis, which resulted in the generation of a putative active enzyme with a molecular mass of about 19 kDa (Fig. 3A). This size is identical to that obtained after SDS-PAGE analysis of the recombinant protein produced by using a cDNA construct containing the catalytic domain of matrilysin-2 exclusively (data not shown). To assess the substrate specificity of the recombinant matrilysin-2, a series of synthetic quenched fluorescent peptides that is commonly used for assaying MMPs were used. As shown in Fig. 3B, the general MMP substrate Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH2 (QF-24), the collagenase/gelatinase substrate Mca-Pro-Cha-Gly-Nva-His-Ala-Dpa-NH2 (QF-41), and the stromelysin substrate Mca-Pro-Leu-Ala-Nva-Dpa-Ala-Arg-NH2 (QF-35) were hydrolyzed by matrilysin-2. These hydrolytic activities were completely abolished in the presence of EDTA (50 mM) or the hydroxamic-acid-based inhibitor BB-94 (Fig. 3B). Finally, we examined the potential inhibition of active matrilysin-2 by different available TIMPs. To this purpose, we used a constant enzyme concentration of 20 nM in the fluorometric measurements were made at excitation and emission. The experiments were also performed in the presence of the MMP inhibitor BB-94 (25 nM). In C, synthetic peptide QF-41 was incubated with active matrilysin-2 in the presence or absence of 20 nM of the indicated TIMPs, and fluorescence at the indicated times was monitored as above.

Fig. 3. Production and purification of recombinant matrilysin-2 in *E. coli* and analysis of its enzymatic activity on synthetic peptides. A, SDS-PAGE analysis of soluble and insoluble fractions of bacterial extracts (pSETB and pPRO-M2), and of fractions obtained after solubilization and purification by size-exclusion (Superdex-75) and affinity (Ni-NTA) chromatography of the insoluble pPRO-M2 extracts (promat-2). The processed form of the enzyme generated after dialysis of the purified promatrilysin-2 is indicated as active mat-2. The sizes of the molecular weight markers (MWM) are shown to the left. In B, synthetic fluorescent peptides QF-24, QF-35, and QF-41 (1 μM) were incubated with active matrilysin-2 (20 nM) in 50 mM Tris/HCl, 5 mM CaCl2, 150 mM NaCl, and 0.05% (v/v) Brij 35 (pH 7.6) with a final concentration of DMSO of 1%, for the indicated times at 37°C. The fluorometric measurements were made at λex = 328 nm, and λem = 393 nm, where ex stands for excitation and em stands for emission. The experiments were also performed in the presence of the MMP inhibitor BB-94 (25 nM). In C, synthetic peptide QF-41 was incubated with active matrilysin-2 in the presence or absence of 20 nM of the indicated TIMPs, and fluorescence at the indicated times was monitored as above.
to process to a significant extent any of the other tested proMMPs, which suggests that both matrilysin-2 and progelatinase B could act coordinately as part of a proteolytic cascade. Taken together, these results provide evidence that matrilysin-2 is a potent enzyme with a wide range of substrates and with the inhibitory profile characteristic of members of the MMP family of endopeptidases.

**Analysis of Matrilysin-2 Expression in Normal and Tumor Tissues.** To investigate the presence of matrilysin-2 transcripts in human tissues, Northern blots containing poly(A)^+^ RNAs prepared from a variety of normal tissues (leukocytes, colon, small intestine, ovary, testis, prostate, thymus, spleen, pancreas, kidney, skeletal muscle, liver, lung, placenta, brain, and heart) were hybridized with the full-length cDNA isolated for this enzyme. However, matrilysin-2 transcripts were virtually undetectable in all of the analyzed tissues (data not shown). Nevertheless, PCR amplification experiments with cDNAs from different human tissues allowed us to confirm that matrilysin-2 is expressed in placenta, the tissue originally used for cloning this enzyme (Fig. 5A). In addition, these PCR amplification experiments allowed us to detect the expression of matrilysin-2 in a sample from a uterus (Fig. 5A). Additional studies confirmed that expression in human uterus is only circumscribed to very specific phases of the menstrual cycle. According to these data, we can conclude that matrilysin-2 exhibits a very restricted expression pattern in normal tissues. To evaluate the possibility that this enzyme was produced by human tumors, we performed a preliminary survey of the expression of this gene in a series of tumor cell lines and malignant tumors of diverse sources. These analyses revealed that matrilysin-2 expression could be detected in tumor cells of different origin such as promyelocytic leukemia (HL-60), colorectal adenocarcinoma (SW480), and lung carcinoma (A549), as well as in a variety of tumors, including endometrial, lung, and prostate adenocarcinomas (Fig. 5B and data not shown). These results, together with the above data showing that matrilysin-2 is active against a variety of extracellular matrix and basement membrane proteins, indicate that this novel enzyme may be added to the growing list of proteases potentially implicated in tumor progression.

**Discussion**

Because MMPs are emerging targets for therapeutic intervention in cancer, there has been considerable interest in identifying novel members of this proteinase family that could be involved in any of the different steps mediated by MMPs during tumor progression. In this work, we report the identification and structural and enzymatic characterization of a new member of this family, which we have called matrilysin-2, mainly because it shares with matrilysin the minimal domain organization required for secretion, latency, and catalytic activity of these enzymes. Furthermore, matrilysin-2 also shares with matrilysin other structural features as well as a wide substrate specificity against protein components of extracellular matrix and basement membranes. This broad degrading activity is consistent with a putative role for this novel protease in some of the tissue-remodeling processes associated with tumor progression (7, 9–13).

The approach used to identify matrilysin-2 involved a search of the EST databases, followed by the screening of a human placenta cDNA library using as probe a EST fragment amplified from this tissue and showing sequence similarity to the catalytic domain of MMPs. After identification of positive clones and successive RACE-S' experiments, we could isolate and clone a full-length cDNA coding for matrilysin-2, a protein composed of 261 amino acids.

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Unpublished results.

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Fig. 4. Degradation of extracellular matrix compounds and activation of proMMPs by recombinant matrilysin-2. A, fibrinogen; B, fibronectin; C, laminin; D, type I, II, and IV-collagens; and E, gelatin (10 μg each) were incubated with buffer alone (c Lanes) or with 1 μg of matrilysin-2 (Mat-2 Lanes) as described under “Materials and Methods.” F, proMMP-2, proMMP-3, proMMP-9, and proMMP-13 (2 μg each) were incubated at 37°C for 16 h, either with buffer alone or with 2 μg of recombinant matrilysin-2 (c and Mat-2 Lanes, respectively). The digestion products were analyzed by SDS-PAGE (10% acrylamide) under reducing conditions and stained with Coomassie blue after electrophoresis. The sizes (in thousands) of the molecular weight markers (MWM) are shown to the left.
 Marker V (Boehringer-Mannheim) was used as a size marker. c ascertainment RNA integrity and equal loading.

MMPs could be grouped on the basis of the presence or absence of additional subfamilies were created to accommodate the membrane-anchored MT-MMPs as well as other MMPs, such as matrilysins (including matrilysin), and gelatinases. More recently, two groups according to substrate specificity: collagenases, stromelysins, and gelatinases. However, similarly to matrilysin, matrilysin-2 lacks the hinge region and the COOH-terminal hemopexin-like domain present in other MMPs. An additional structural feature exclusively shared by matrilysin and matrilysin-2 among all vertebrate MMPs is the presence of a Thr residue immediately adjacent to the Zn-binding site. This residue is present in matrilysin from different species and has been defined as a specific structural signature of this enzyme, although its functional relevance has not been clarified (15, 16). The identification and cloning of a second human MMP with the same structural design as vertebrate matrilysins (21).

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In addition to all of these structural features, matrilysin-2 also shares with matrilysin a wide degrading activity against proteins present in extracellular matrix and basement membranes. Thus, the refolded catalytic domain that is produced in E. coli exhibits strong proteolytic activity against synthetic peptides used for analyzing the enzymatic properties of MMPs. Furthermore, it efficiently degrades gelatin, fibronecint, fibrinogen, and type IV collagen. By contrast, and consistent with a series of structural properties of matrilysin-2 including lack of both hemopexin-domain and specific residues present in the catalytic domain of collagenases, this enzyme does not cleave triple-helical collagens such as type I and type II collagens. It is remarkable that matrilysin-2 may digest fibrinogen in a very similar manner to that recently reported for MT1-MMP (22). In fact, both are able to degrade the Aα and Bβ chains of this protein whereas the γ chain remains essentially intact. MT1-MMP fibrinolytic activity has been proposed to be essential in neovascularization processes. Similarly, the observation that matrilysin-2 may act as a fibrinolysin opens the possibility that this protease may participate in processes involving fibrin formation and degradation, such as angiogenesis, that are necessary for tumor progression (13). It is also of interest that matrilysin-2 was able to activate progelatinase B, one of the MMPs more frequently associated with malignant tumors. This observation suggests that both enzymes could form part of an activation cascade similar to those operating for activating other MMPs (23). Additional studies will be required to provide more definitive evidence of the putative collaborative role of both enzymes in some of the tissue-remodeling processes in which these enzymes are produced, including tumor progression.

In summary, we have established the chromosomal location of the human matrilysin-2 gene. According to radiation hybrid mapping, this gene localizes to the short arm of chromosome 11, within a region that does not contain any other known MMP gene. These results provide additional evidence that MMP genes have been very dynamic in their evolutionary pathways, and with the exception of several MMP genes clustered in the long arm of chromosome 11, all of the remaining MMP genes are widely dispersed in different human chromosomes. Interestingly, it has been reported that the region containing the matrilysin-2 gene
(11p15) is frequently altered in several human tumors (27). Consequently, it will be of interest to analyze the possibility that matrilysin-2 may be a direct target of these genetic abnormalities.

In conclusion, we have identified and characterized matrilysin-2, the smallest MMP described thus far. This enzyme exhibits significant structural similarities with matrilysin, as well as with a previously described plant enzyme, which suggests that all of them may form part of a specific class of MMPs characterized by exclusively possessing the minimal domains required for the catalytic activity of these endopeptidases. Matrilysin-2 has a wide substrate specificity on extracellular matrix and basement membrane proteins, which is consistent with its potential role in tumor progression. Also in agreement with this possibility, matrilysin-2 has a very restricted expression pattern in human tissues, but is widely found in primary tumors and tumor cell lines from diverse sources. Additional studies will be required to elucidate the precise role of this protease in any of the connective-tissue turnover and remodeling processes occurring during tumor progression.

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