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Advances in Brief

Molecular Cloning of a Novel Membrane-type Matrix Metalloproteinase from a Human Breast Carcinoma[1]

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Abstract

A new member of the matrix metalloproteinase (MMP) family of enzymes has been cloned from a human breast carcinoma cDNA library. The isolated cDNA contains an open reading frame 1554 bp long, encoding a polypeptide of 518 amino acids. The predicted amino acid sequence displays a similar domain organization as the remaining MMPs, including a prodomain with the activation locus, the zinc-binding site, and the hemopexin domain. In addition, it contains a C-terminal extension, rich in hydrophobic residues and similar in size to those present in the different membrane-type MMPs (MT-MMPs) identified to date. On the basis of these structural characteristics, this novel MMP has been tentatively called MT4-MMP, because it represents the fourth member of this subclass of MMPs characterized mainly by the occurrence of putative transmembrane domain in their amino acid sequences. MT4-MMP also contains a nine-residue insertion between the propeptide and the catalytic domain, which is a common feature of MT-MMPs and stromelysin-3. This amino acid sequence insertion ends with the consensus sequence R-X-R/K-R, which seems to be essential in the activation of these proteinases by furin. Northern blot analysis of polyadenylated RNAs isolated from a variety of human tissues revealed that the MT4-MMP gene (*MMP-17*) is expressed mainly in the brain, leukocytes, the colon, the ovary, and the testis. The expression of MT4-MMP in leukocytes together with its putative membrane localization suggest that this enzyme could be involved in the activation of membrane-bound precursors of growth factors or inflammatory mediators such as tumor necrosis factor-α. In addition, MT4-MMP transcripts were detected in all breast carcinomas, as well as in all breast cancer cell lines analyzed in the present work. On the basis of these expression data in breast tumors, a potential role for human MT4-MMP in the tumoral process is also suggested.

Introduction

The MMPs[3] form a family of structurally related, zinc-dependent endopeptidases that are involved in the degradation of the extracellular matrix and basement membranes. These enzymes play a critical role in normal tissue-remodeling processes, including embryonic growth and development, cell migration, uterine involution, and wound healing. In addition, these proteinases are also involved in a number of pathological processes, such as rheumatoid arthritis, pulmonary emphysema, and tumor cell invasion and metastasis (1, 2).

At present, 13 human MMPs have been isolated and characterized at the amino acid sequence level, including the recently identified collagenase-3 (3) and three MT-MMPs (4–7). According to structural and functional characteristics, these MMPs can be classified into at least four different subfamilies of closely related members: collagenases, stromelysins, gelatinases, and MT-MMPs, although there are some MMPs, such as macrophage metalloelastase (8) and stromelysin-3 (9), that do not belong to these groupings. All MMPs characterized to date are similar in that they are synthesized in an inactive proenzyme form, contain zinc-binding sites, and can be inhibited by tissue-specific inhibitors and chelating agents. However, MMPs demonstrate distinct or partially overlapping substrate specificities toward extracellular matrix components. Thus, the collagenases degrade fibrillar collagens by cleavage of the native helix at a single peptide bond, generating fragments of about three-fourths and one-fourth of the size of the original molecule (10, 11). The gelatinases (type IV collagenases) recognize basement membrane and denatured collagens and may act synergistically with interstitial collagenases in the degradation of fibrillar collagens (12, 13). Finally, stromelysins exhibit the widest range of substrates and are able to degrade many extracellular proteins, such as proteoglycans, fibronectin, and laminin (14).

The expression of these different MMP genes is regulated strictly to maintain the connective tissue homeostasis as well as the rapid and localized tissue remodeling that occurs during many normal physiological processes. However, in a number of pathological situations, including cancer invasion and metastasis, these stringent regulatory mechanisms are lost, and, consequently, different MMPs seem to be overproduced either by the tumor cells themselves or by the surrounding stromal cells to facilitate the invasion of adjacent normal tissues. On these bases, samples of human tumor specimens seem to be appropriate starting materials in which to isolate and characterize putative novel members of the MMP family with potential involvement in the spread of cancer. Recently, in fact, by following this strategy, we have cloned from human breast carcinomas a novel MMP (collagenase-3; Ref. 3), as well as other hydrolytic enzymes or inhibitors, including cathepsin-O (15), biphenyl hydrolase (16), and TIMP-3 (17), which could be important in the development and progression of these tumors. In this work, we have used a PCR-based, homology cloning approach to examine the possibility that additional yet uncharacterized members of the MMP family could be overproduced in breast tumors. We report herein the molecular cloning and nucleotide sequence of a cDNA coding for a novel member of this family of proteolytic enzymes, which belongs to the MT subclass of MMPs and which has been called MT4-MMP tentatively. We also analyze its expression in human tissues and show that the gene encoding MT4-MMP (*MMP-17*) is expressed mainly in the brain, leukocytes, the colon, the ovary, and the testis. Finally, we present evidence that MT4-MMP is expressed in all examined primary breast carcinomas and breast cancer cell lines.

Materials and Methods

Materials. Samples of human breast carcinomas were obtained from women who had undergone surgery for primary breast cancer. The samples were frozen in liquid nitrogen immediately and stored at −70°C until used. A human breast carcinoma cDNA library constructed in λgt11 and two Northern

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[3] The abbreviations used are: MMP, matrix metalloproteinase; MT, membrane-type; RT, reverse transcription; poly(A)$^+$, polyadenylated.

blots containing poly(A)$^+$ RNAs from different human tissues were obtained from Clontech (Palo Alto, CA). Restriction endonucleases and other reagents used for molecular cloning were from Boehringer Mannheim (Mannheim, Germany). Oligonucleotides were synthesized by the phosphoramidite method in an Applied Biosystems (Foster City, CA) model 381 A DNA synthesizer and used directly after synthesis. The RNA PCR kit used for the RT of total RNA and cDNA amplification was purchased from Perkin Elmer/Cetus (Norwalk, CT). Double-stranded DNA probes were radiolabeled with [^{32}P]dCTP (3000 Ci/mmol) using a commercial random-priming kit from Amersham Corp. (Amersham, United Kingdom).

RT and PCR Amplification of Breast Carcinoma RNA. Total RNA was isolated from a breast carcinoma by guanidinium thiocyanate-phenol-chloroform extraction (18) and used for cDNA synthesis with the RNA PCR kit from Perkin-Elmer/Cetus. After RT using 1 μg total RNA and random hexamers as primer according to the instructions of the manufacturer, the whole mixture was used for PCR with two degenerate oligonucleotides corresponding to conserved regions in MMPs [5′-CCNCGNTG(TC)GGNGTNCCNGA and 5′-TGNCC(AG)AA(TC)TC(AG)TGNGCNGCNAC, respectively]. The PCR reaction was carried out in a GeneAmp 2400 PCR system from Perkin-Elmer for 40 cycles of denaturation (94°C, 1 min), annealing (50°C, 1 min), and extension (72°C, 1 min). The PCR products were phosphorylated with T4 polynucleotide kinase, and the DNA band of the expected size (~0.4 kb) was gel purified and ligated in the *Sma*I site of pUC18. DNA from 40 independent clones was isolated and sequenced. RT-PCR experiments were also performed in similar conditions for analysis of MT4-MMP expression in a series of primary breast carcinomas and breast cancer cell lines. In this case, the specific primers for MT4-MMP were 5′-GGCCCTGGTAGTACGGCCGCA and 5′-CTGGAGCGACATTGCGCCCCT.

Screening of a Human Breast Carcinoma cDNA Library. About 1 × 10^6 plaque-forming units of a commercially available human breast carcinoma cDNA library (Clontech) were plated using *Escherichia coli* Y1088 as a host and screened using as a probe the partial cDNA cloned by RT-PCR from a breast cancer specimen following the procedure described above. Hybridization to the radiolabeled probe was carried out at 65°C for 18 h in 5× saline-sodium phosphate-EDTA [1× = 150 mM NaCl, 10 mM NaH_2PO_4, and 1 mM EDTA (pH 7.4)], 10× Denhardt's solution (1× = 0.02% BSA, 0.02% polyvinylpyrrolidone, and 0.02% Ficoll), 0.1% SDS, and denatured herring sperm DNA (100 μg/ml). Subsequently, the filters were washed twice for 1 h at 65°C in 1× SSC [0.15 M NaCl and 0.015 M Na citrate (pH 7.0)] and 0.1% SDS and subjected to autoradiography. Following plaque purification, the cloned insert was excised by *Eco*RI digestion and subcloned in pUC18.

Nucleotide Sequence Analysis. Selected DNA fragments were inserted in the polylinker region of phage vectors M13mp18 and M13mp19 and sequenced by the dideoxy terminator method using either the M13 universal primer or cDNA-specific primers and the Sequenase version 2.0 kit (United States Biochemical Corp., Cleveland, OH). In all cases, both strands were analyzed to confirm the sequence results. Sequence ambiguities were solved by substituting dITP for dGTP in the sequencing reactions. Computer analysis of DNA and protein sequences was performed with the software package of the University of Wisconsin (Madison, WI) Genetics Computer Group.

Northern Blot Hybridization. Northern blots containing 2 μg poly(A)$^+$ RNA of different human tissue specimens were prehybridized at 42°C for 3 h in 50% formamide, 5× saline-sodium phosphate-EDTA, 2× Denhardt's solution, 0.1% SDS, and 100 μg/ml denatured herring sperm DNA and then hybridized for 48 h under the same conditions, using a 2.1-kb fragment (from positions 432–2500) of the MT4-MMP cDNA as a probe. Filters were washed with 0.2× SSC and 0.1% SDS for 2 h at 50°C and subjected to autoradiography. RNA integrity and equal loading were assessed by hybridization with an actin probe as indicated by Clontech.

Results and Discussion

As a previous step to search for novel members of the MMP gene family that could be overexpressed in human mammary carcinomas, two degenerate oligonucleotides with sequences based on conserved domains among the different members of this family of proteinases were synthesized. The first oligonucleotide corresponded to the propeptide region containing the cysteine residue that seems to be essential for maintaining the latency of these enzymes, whereas the antisense primer was selected from the conserved amino acid sequence involved in the coordination of the zinc atom at the active site (1). These primers were used then for RT-PCR experiments with total RNA isolated from a series of independent human breast tumors. After amplification at low-stringency conditions, a band of about 0.4 kb that contained presumably a portion of different MMPs was obtained. This material was cloned in the plasmid vector pUC18, and DNA from many independent clones was isolated and sequenced. Systematic nucleotide sequencing analysis of these clones revealed that many of them corresponded to human MMPs isolated and characterized previously. However, one of the isolated clones (X-24) showed a nucleotide sequence similar to, but distinct from, the reported sequences for human MMPs, thus suggesting that at least one of the selected breast carcinomas expressed a putative novel member of this protein family. Because the limited amount of RNA available from this breast tumor specimen hampered the preparation of the cDNA library required to isolate a complete cDNA clone for this presumably novel MMP, we examined the possible occurrence of these clones in a series of available human breast carcinoma cDNA libraries. For this purpose, two specific primers (5′-CAGGCTC-CAGCCCCCACCAAG and 5′-GCGTCGAAGGGGTAGCCGTCG) corresponding to inner sequences of the nucleotide sequence for clone X-24 obtained in the original RT-PCR experiment were synthesized and used for PCR amplification of DNA isolated from the different breast cancer cDNA libraries. A band of the expected size (229 bp) was amplified at high levels from cDNA corresponding to a commercially available library prepared from ZR-75–1 breast cancer cells (data not shown). According to these results, we used this library as starting material to attempt the isolation of a full-length cDNA for the putative MMP. On screening of ~1 × 10^6 plaque-forming units using the PCR-generated cDNA fragment as the probe, 16 positive clones were identified. One of them, clone 5.4, had an insert of about 1.7 kb, which could be large enough to contain the complete coding information for a MMP. This insert was subcloned in pUC18, and its complete nucleotide sequence was determined as described in "Materials and Methods." Computer analysis of this sequence (Fig. 1) revealed an open reading frame 1554 bp long, starting with an ATG codon at position 432 and ending with a TGA codon at position 1988.

A detailed comparison of the predicted amino acid sequence with those corresponding to the other human MMPs characterized to date showed that the percentage of identities ranges from 38.4% with MT-MMP2 to 26.7% with M_r 92,000 type IV collagenase. In addition, the deduced amino acid sequence from the human cDNA isolated in this work displays a number of features characteristic of the members of the MMP family of proteins (Fig. 2). Thus, it contains a prodomain region with the activation locus containing the essential cysteine residue, a catalytic domain of about 170 residues including the zinc-binding site with the consensus sequence HE*X*GH*XXXXX*H, and a fragment of approximately 200 amino acids with sequence similarity to hemopexin. Interestingly, the identified sequence contains a C-terminal extension, rich in hydrophobic residues and similar in size to those present in the different MT-MMPs characterized to date (4–7). On the basis of these structural features, this novel MMP has been called MT4-MMP tentatively, because it represents the fourth member of this subclass of MMPs characterized mainly by the occurrence of putative transmembrane domains in their amino acid sequences. Furthermore, and following the nomenclature system proposed by Okada *et al.* (19), we would assign number 17 to the novel MMP described here, MMP-15 and MMP-16 corresponding to the two MT-MMPs identified recently by Will and Hinzmann (6) and Takino *et al.* (7). In this regard, we would like to propose a novel nomenclature system for MT-MMPs based on the addition of the identification number to the MT prefix instead of the system used previously for the members of

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1       GAATTCCGGCGGAGTTTTGCTGTTATTGCCCAGGCTAGAGTGCAATGGTGCGATCTTGGCTCACAGCAACCTCCA  75
76      CCTCCCAGAGTCAAGCAATTCTCCTGCCTCAGCCTCCTGAGTAGCTGGGATTACAGGCGTGTGCCACAGCACCCG  150
151     GCCAGGGACATCAGGTTTATTAAGACACTTTTCCGACAGCTGCCCAGGGAAGAGACAAGAGGTGCCTTGTGGGCA  225
226     GATAGGGGGCTGGGAGGGGGCCTGCCCGGAAGCAGTGGTGGCCCGTGGCAGGCTTCTCACTGGGTAGGACCGGGC  300
301     CCTCTGTTGCACCCCCTCACCCTGCTCTCTGCCCTCAGGAGTGGCTAAGCAGGTTCGGTTACCTGCCCCCGGCTG  375
376     ACCCCACAACAGGGCAGCTGCAGACGCAAGAGGAGCTGTCTAAGGCCATCACAGCCATGCAGCAGTTTGGTGGCC  450
                                                          M  Q  Q  F  G  G  L
451     TGGAGGCCACCGGCATCCTGGACGAGGCCACCCTGGCCCTGATGAAAACCCCACGCTGCTCCCTGCCAGACCTCC  525
          E  A  T  G  I  L  D  E  A  T  L  A  L  M  K  T  P  R  C  S  L  P  D  L  P
526     CTGTCCTGACCCAGGCTCGCAGGAGACGCCAGGCTCCAGCCCCCACCAAGTGGAACAAGAGGAACCTGTCGTGGA  600
          V  L  T  Q  A  R  R  R  R  Q  A  P  A  P  T  K  W  N  K  R  N  L  S  W  R
601     GGGTCCGGACGTTCCCACGGGACTCACCACTGGGGCACGACACGGTGCGTGCACTCATGTACTACGCCCTCAAGG  675
          V  R  T  F  P  R  D  S  P  L  G  H  D  T  V  R  A  L  M  Y  Y  A  L  K  V
676     TCTGGAGCGACATTGCGCCCCTGAACTTCCACGAGGTGGCGGGCAGCACCGCCGACATCCAGATCGACTTCTCCA  750
          W  S  D  I  A  P  L  N  F  H  E  V  A  G  S  T  A  D  I  Q  I  D  F  S  K
751     AGGCCGACCATAACGACGGCTACCCCTTCGACGCCCGGCGGCACCGTGCCCACGCCTTCTTCCCCGGCCACCACC  825
          A  D  H  N  D  G  Y  P  F  D  A  R  R  H  R  A  H  A  F  F  P  G  H  H  H
826     ACACCGCCGGGTACACCCACTTTAACGATGACGAGGCCTGGACCTTCCGCTCCTCGGATGCCCACGGGATGGACC  900
          T  A  G  Y  T  H  F  N  D  D  E  A  W  T  F  R  S  S  D  A  H  G  M  D  L
901     TGTTTGCAGTGGCTGTCCACGAGTTTGGCCACGCCATTGGGTTAAGCCATGTGGCCGCTGCACACTCCATCATGC  975
          F  A  V  A  V  H  E  F  G  H  A  I  G  L  S  H  V  A  A  A  H  S  I  M  R
976     GGCCGTACTACCAGGGCCCGGTGGGTGACCCGCTGCGCTACGGGCTCCCCTACGAGGACAAGGTGCGCGTCTGGC 1050
          P  Y  Y  Q  G  P  V  G  D  P  L  R  Y  G  L  P  Y  E  D  K  V  R  V  W  Q
1051    AGCTGTACGGTGTGCGGGAGTCTGTGTCTCCCACGGCGCAGCCCGAGGAGCCTCCCCTGCTGCCGGAGCCCCCAG 1125
          L  Y  G  V  R  E  S  V  S  P  T  A  Q  P  E  E  P  P  L  L  P  E  P  P  D
1126    ACAACCGGTCCAGCGCCCCGCCCAGGAAGGACGTGCCCCACAGATGCAGCACTCACTTTGACGCGGTGGCCCAGA 1200
          N  R  S  S  A  P  P  R  K  D  V  P  H  R  C  S  T  H  F  D  A  V  A  Q  I
1201    TCCGGGGTGAAGCTTTCTTCTTCAAAGGCAAGTACTTCTGGCGGCTGACGCGGGACCGGCACCTGGTGTCCCTGC 1275
          R  G  E  A  F  F  F  K  G  K  Y  F  W  R  L  T  R  D  R  H  L  V  S  L  Q
1276    AGCCGGCACAGATGCACCGCTTCTGGCGGGGCCTGCCGCTGCACCTGGACAGCGTGGACGCCGTGTACGAGCGCA 1350
          P  A  Q  M  H  R  F  W  R  G  L  P  L  H  L  D  S  V  D  A  V  Y  E  R  T
1351    CCAGCGACCACAAGATCGTCTTCTTTAAAGGAGACAGGTACTGGGTGTTCAAGGACAATAACGTAGAGGAAGGAT 1425
          S  D  H  K  I  V  F  F  K  G  D  R  Y  W  V  F  K  D  N  N  V  E  E  G  Y
1426    ACCCGCGCCCCGTCTCCGACTTCAGCCTCCCGCCTGGCGGCATCGACGCTGCCTTCTCCTGGGCCCACAATGACA 1500
          P  R  P  V  S  D  F  S  L  P  P  G  G  I  D  A  A  F  S  W  A  H  N  D  R
1501    GGACTTATTTCTTTAAGGACCAGCTGTACTGGCGCTACGATGACCACACGAGGCACATGGACCCCGGCTACCCCG 1575
          T  Y  F  F  K  D  Q  L  Y  W  R  Y  D  D  H  T  R  H  M  D  P  G  Y  P  A
1576    CCCAGAGCCCCCTGTGGAGGGGTGTCCCCAGCACGCTGGACGACGCCATGCGCTGGTCCGACGGTGCCTCCTACT 1650
          Q  S  P  L  W  R  G  V  P  S  T  L  D  D  A  M  R  W  S  D  G  A  S  Y  F
1651    TCTTCCGTGGCCAGGAGTACTGGAAAGTGCTGGATGGCGAGCTGGAGGTGGCACCCGGGTACCCACAGTCCACGG 1725
          F  R  G  Q  E  Y  W  K  V  L  D  G  E  L  E  V  A  P  G  Y  P  Q  S  T  A
1726    CCCGGGACTGGCTGGTGTGTGGAGACTCACAGGCCGATGGATCTGTGGCTGCGGGCGTGGACGCGGCAGAGGGGC 1800
          R  D  W  L  V  C  G  D  S  Q  A  D  G  S  V  A  A  G  V  D  A  A  E  G  P
1801    CCCGCGCCCCTCCAGGACAACATGACCAGAGCCGCTCGGAGGACGGTTACGAGGTCTGCTCATGCACCTCTGGGG 1875
          R  A  P  P  G  Q  H  D  Q  S  R  S  E  D  G  Y  E  V  C  S  C  T  S  G  A
1876    CATCCTCTCCCCCGGGGGCCCCAGGCCCACTGGTGGCTGCCACCATGCTGCTGCTGCTGCCGCCACTGTCACCAG 1950
          S  S  P  P  G  A  P  G  P  L  V  A  A  T  M  L  L  L  L  P  P  L  S  P  G
1951    GCGCCCTGTGGACAGCGGCCCAGGCCCTGACGCTATGACACACAGCGCGAGCCCATGAGAGGACAGAGGCGGTGG 2025
          A  L  W  T  A  A  Q  A  L  T  L  *
2026    GACAGCCTGGCCACAGAGGGCAAGGACTGTGCCGGAGTCCCTGGGGGAGGTGCTGGCGCGGGATGAGGACGGGCC 2100
2101    ACCCTGGCACCGGAAGGCCAGCAGAGGGCACGGCCCGCCAGGGCTGGGCAGGCTCAGGTGGCAAGGACGGAGCTG 2175
2176    TCCCCTAGTGAGGGACTGTGTTGACTGACGAGCCGAGGGGTGGCCGCTCCAGAAGGGTGCCCAGTCAGGCCGCAC 2250
2251    CGCCGCCAGCCTCCTCCGGCCCTGGAGGGAGCATCTCGGGCTGGGGGCCCACCCCTCTCTGTGCCGGCGCCACCA 2325
2326    ACCCCACCCACACTGCTGCCTGGTGCTCCCGCCGGCCCACAGGGCCTCCGTCCCCAGGTCCCCAGTGGGGCAGCC 2400
2401    CTCCCCACAGACGAGCCCCCCACATGGTGCCGCGGCACGTCCCCCCTGTGACGCGTTCCAGACCAACATGACCTC 2475
2476    TCCCTGCTTTGTAGCGGCCCGGAATTC  2502
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Fig. 1. cDNA and deduced amino acid sequence of MT4-MMP. The deduced amino acid sequence begins with the first ATG codon of the cDNA. The incomplete Alu sequence present in the 5′ end of the cDNA is *underlined*.

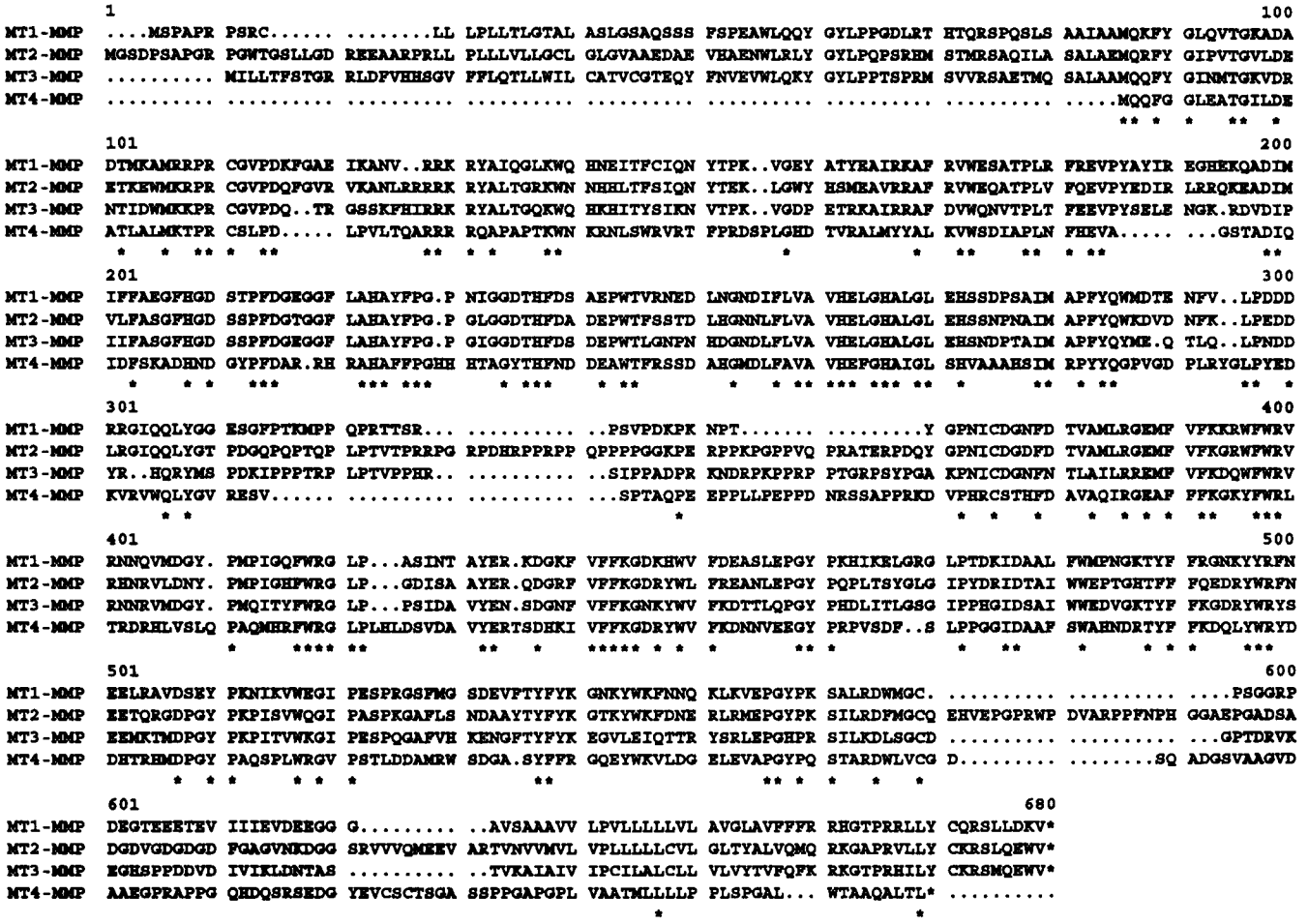

Fig. 2. Alignment of amino acid sequences for MT MMPs. Amino acid sequences of MT1-MMP, MT2-MMP, and MT3-MMP were obtained from the EMBL/Genbank databases, and the multiple alignment was performed with the PILEUP program of the University of Wisconsin Genetics Computer Group package. Identical residues that are common to all sequences are indicated by *asterisks* below the sequences.

this subfamily of MMPs, which adds the identification number to the MMP suffix. In our view, this latter system could generate confusion by suggesting that MT-MMP2 corresponds to the putative membrane-bound form of gelatinase A (MMP-2) rather than to the second identified member of the membrane-bound MMP subfamily.

It is also worth mentioning that MT4-MMP contains a nine-residue insertion between the propeptide and the catalytic domain, which is also a characteristic feature of the remaining MT-MMPs (4–7) as well as stromelysin-3 (9). This amino acid sequence insertion ends with the consensus sequence R-X-R/K-R, which Pei and Weiss (20) found recently to be essential in the activation of stromelysin-3 by furin. Consequently, it is tempting to speculate that MT4-MMP also could be subjected to the same activation mechanism.

Finally, and also in relation to the structural analysis of the nucleotide sequence of the cDNA coding for MT4-MMP, it is remarkable that the largest cDNA clone isolated in the present work contains an Alu repetitive sequence inserted in its 5′ end (Fig. 1). This short, interspersed element is oriented in the same transcriptional direction as the *MT4-MMP* gene and belongs to the Sp subfamily, which is believed to have been disseminated in the human genome about 35 million years ago. The occurrence of this Alu sequence in the 5′ region is very unusual, because despite the fact that this group of short, interspersed elements constitute about the 5% of the human genome, most of them have been found in introns or in spacer DNA, being spliced out during the process of RNA maturation (21). The possibility that its insertion in the MT4-MMP mRNA could be the consequence of a cloning artifact during the construction of the cDNA library must be ruled out, because we have found the same Alu transcript in the cDNAs isolated from a distinct brain cDNA library. Furthermore, nucleotide sequence analysis of genomic clones encoding MT4-MMP has revealed that this Alu sequence is also present in the corresponding region of the isolated clones (data not shown). The biological significance of the presence of this repetitive sequence in the 5′ end of human MT4-MMP mRNA is unknown. In this regard, it should be remembered that Alu sequences have a high degree of similarity with the elongation arrest domain of 7SL RNA, a component of the signal recognition particle involved in protein secretion (22). On the basis of this homology, this Alu element detected here within the MT4-MMP mRNA could have the potential to modulate MT4-MMP expression, as proposed already for Alu transcripts that are transported to the cytoplasmic compartment (23). In relation to this, it is also remarkable that the Alu sequence is inserted in the place of the putative signal sequence that targets all MMPs to the secretory pathway; thus, it could compromise the correct secretion or intracellular trafficking of this novel MMP. In relation to this, it should be mentioned that polyclonal antibodies raised against a synthetic peptide derived from MT4-MMP recognized preferentially a protein of M_r ~70,000 in a Western blot analysis of human brain extracts, suggesting that MT4-MMP is a translated protein (data not shown). Further studies will be required to examine this possibility as well as

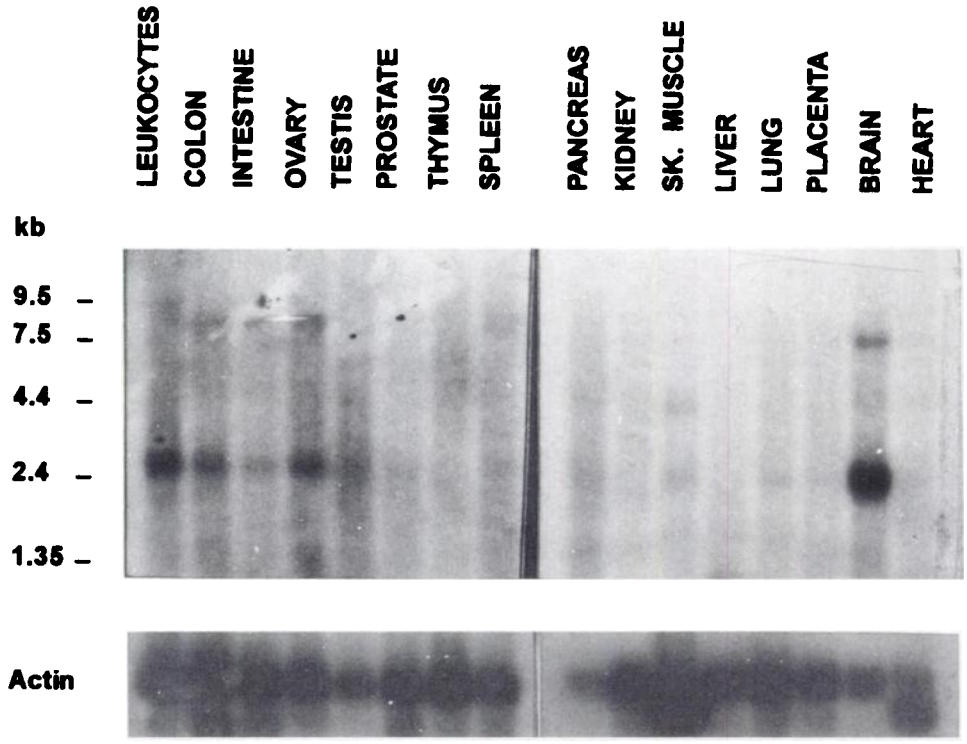

Fig. 3. Northern blot analysis of MT4-MMP mRNA in human tissues. About 2 μg poly(A)$^+$ RNA from the indicated tissues were analyzed by hybridization with a 1660-bp fragment of the cDNA (lacking the Alu repeat region) for MT4-MMP. The positions of RNA size markers are shown. Subsequently, filters were hybridized to a human actin probe to ascertain the differences in RNA loading among the different samples.

the hypothesis that the occurrence of the Alu sequence in the cDNA for MT4-MMP is a consequence of an alternative splicing event resulting in the incorporation of the repetitive element in a significant portion of the mature MT4-MMP mRNAs.

In the present work, we also have performed an analysis of MT4-MMP expression in a variety of normal human tissues, as well as in a series of primary breast carcinomas and breast cancer cell lines. For this purpose, two Northern blots containing poly(A)$^+$ RNAs extracted from a wide variety of human tissues, including leukocytes, colon, small intestine, ovary, testis, prostate, thymus, spleen, pancreas, kidney, skeletal muscle, liver, lung, placenta, brain, and heart, were hybridized with a fragment of the cDNA coding for MT4-MMP, which lacked the 5′-flanking region containing the Alu repetitive sequence. As can be seen in Fig. 3, a clear, hybridizing band of about 2.7 kb was detected in RNA from the brain, leukocytes, the colon, the ovary, and the testis. In addition, a minor band of about 7.5 kb could be detected in the brain and ovary and probably corresponds to an alternative transcript generated by differential posttranscriptional processing of the mRNA for MT4-MMP. A similar situation has been described for other human MMPs, such as collagenase-3, in which the different transcripts are the result of alternative use of distinct polyadenylation sites (3).

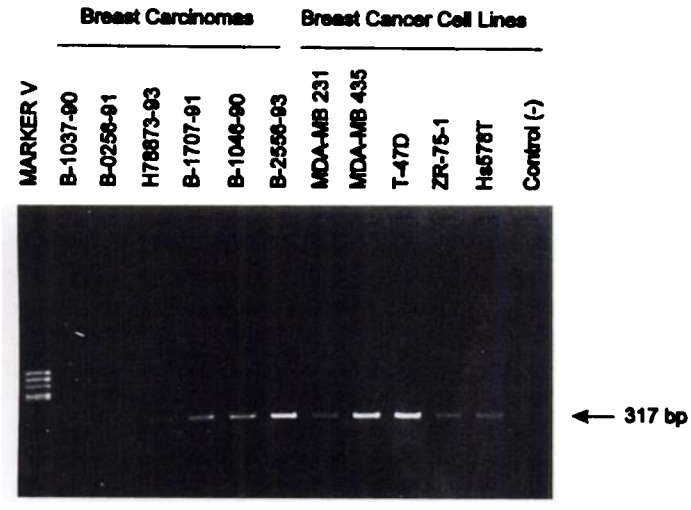

Fig. 4. Expression analysis of MT4-MMP in breast carcinomas and breast cancer cell lines. RT-PCR was performed on 1 μg RNA from the samples. A 317-bp fragment corresponding to a segment of MT4-MMP was amplified in a volume of 100 μl, and 10 μl of the reaction were separated on a 2% agarose gel run in Tris-borate-EDTA. pBR322 digested with *Hae*III (Marker V; Boehringer Mannheim) was used as a size marker.

The high-level expression of human MT4-MMP in brain, leukocyte, or reproductive tissues suggests that this enzyme could play some specialized activity in these tissues or cells. Thus, in the case of the brain, MT4-MMP could be involved in growth cone invasiveness and neurite extension in a fashion similar to that proposed for stromelysin-1 (24). On the other hand, its expression in the ovary could be indicative of the participation of MT4-MMP in some of the extracellular matrix remodeling processes occurring within this tissue during the reproductive cycle, including rupture of the follicular wall, cumulus cell expansion, or corpus luteum formation (25). Furthermore, the expression of MT4-MMP in leukocytes together with its putative membrane localization suggest that it could play some role in the activation of some secreted proteinases, membrane-bound precursors of growth factors, or inflammatory mediators such as tumor necrosis factor-α (26–28). In this regard, it is also of interest that none of the remaining MT-MMPs described to date has been found to be expressed in leukocytes, thus reinforcing the possibility of a potential and specific role of MT4-MMP in these cells. Finally, because MT4-MMP was cloned from a breast cancer cDNA library originally, in this work, we have carried out a preliminary study of its expression in primary breast carcinomas as well as in breast cancer cell lines. To do that, we performed RT-PCR analysis with RNAs obtained from a number of tumors and breast cancer cell lines of different characteristics, including T-47D, ZR-75–1, Hs-578T, MDA-MB231, and MDA-MB435. Oligonucleotides 5′-GGCCCTGGTAGTACGGCCGCA and 5′-CTGGAGCGACATTGCGCCCCT were used as the

primer pair to amplify a 317-bp segment corresponding to nucleotides 677–993 of the MT4-MMP cDNA. As can be seen in Fig. 4, a band of the expected size and confirmed to be MT4-MMP by nucleotide sequencing was amplified from all examined breast carcinomas and breast cancer cell lines, suggesting that this enzyme could be involved somewhat in the lytic processes associated with invasive breast cancer lesions. The availability of the cDNA coding for MT4-MMP isolated in the present work will be very useful for examining this question as well as to elucidate the potential role of this novel MT-MMP in a series of physiological processes, including activation of precursors of cytokines and growth factors.

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References

1. Birkedal-Hansel, H., Moore, W. G. I., Bodden, M. K., Windsor, L. J., Birkedal-Hansen, B., DeCarlo, A., and Engler, J. A. Matrix metalloproteinases: a review. Crit. Rev. Oral Biol. Med., *4:* 197–250, 1993.
2. Stetler-Stevenson, W. G., Aznavoorian, S., and Liotta, L. A. Tumor cell interactions with the extracellular matrix during invasion and metastasis. Annu. Rev. Cell Biol., *9:* 541–573, 1993.
3. Freije, J. M. P., Díez-Itza, I., Balbín, M., Sánchez, L. M., Blasco, R., Tolivia, J., and López-Otín, C. Molecular cloning and expression of collagenase-3, a novel human matrix metalloproteinase produced by breast carcinomas. J. Biol. Chem., *269:* 16766–16773, 1994.
4. Sato, H., Takino, T., Okada, Y., Cao, J., Shinagawa, A., Yamamoto, E., and Seiki, M. A matrix metalloproteinase expressed on the surface of invasive tumour cells. Nature (Lond.), *370:* 61–65, 1994.
5. Okada, A., Bellocq, J. P., Rouyer, N., Chenard, M. P., Rio, M. C., Chambon, P., and Basset, P. Membrane-type matrix metalloproteinase (*MT-MMP*) gene is expressed in stromal cells of human colon, breast, and head and neck carcinomas. Proc. Natl. Acad. Sci. USA, *92:* 2730–2734, 1995.
6. Will, H., and Hinzmann, B. cDNA sequence and mRNA distribution of a novel human matrix metalloproteinase with a potential transmembrane domain. Eur. J. Biochem., *231:* 602–608, 1995.
7. Takino, T., Sato, H., Shinagawa, A., and Seiki, M. Identification of the second membrane-type matrix metalloproteinase (*MT-MMP2*) gene from a human placenta cDNA library. J. Biol. Chem., *270:* 23013–23020, 1995.
8. Belaaouaj, A., Shipley, J. M., Kobayashi, D. K., Zimonjic, D. B., Popescu, N., Silverman, G. A., and Shapiro, S. D. Human macrophage metalloelastase: genomic organization, chromosomal location, gene linkage, and tissue-specific expression. J. Biol. Chem., *270:* 14568–14575, 1995.
9. Basset, P., Bellocq, J. P., Wolf, C., Stoll, I., Huntin, P., Limacher, J. M., Podhajcer, O. L., Chenard, M. P., Rio, M. C., and Chambon, P. A novel metalloproteinase gene specifically expressed in stromal cells of breast carcinomas. Nature (Lond.), *348:* 699–704, 1990.
10. Welgus, H. G., Jeffrey, J. J., and Eisen, A. Z. The collagen substrate specificity of human skin fibroblast collagenase. J. Biol. Chem., *256:* 9511–9515, 1981.
11. Hasty, K. A., Jeffrey, J. J., Hibbs, M. S., and Welgus, H. G. The collagen substrate specificity of human neutrophil collagenase. J. Biol. Chem., *262:* 10048–10052, 1987.
12. Collier, I. E., Wilhelm, S. M., Eisen, A. Z., Marmer, B. L., Grant, G. A., Seltzer, J. L., Kronberger, A., He, C., Bauer, E. A., and Goldberg, G. I. H-*ras* oncogene-transformed human bronchial epithelial cells (TBE-1) secrete a single metalloprotease capable of degrading basement membrane collagen. J. Biol. Chem., *263:* 6579–6587, 1988.
13. Senior, R. M., Griffin, G. L., Fliszar, C. J., Shapiro, S. D., Goldberg, G. I., and Welgus, H. G. Human 92- and 72-kilodalton type IV collagenases are elastases. J. Biol. Chem., *266:* 7870–7875, 1991.
14. Wilhem, S. M., Collier, I. E., Kronberger, A., Eisen, A. Z., Marmer, B. L., Grant, G. A., Bauer, E. A., and Goldberg, G. I. Human skin fibroblast stromelysin: structure, glycosylation, substrate specificity, and differential expression in normal and tumorigenic cells. Proc. Natl. Acad. Sci. USA, *84:* 6725–6729, 1987.
15. Velasco, G., Ferrando, A. A., Puente, X. S., Sánchez, L. M., and López-Otín, C. Human cathepsin O: molecular cloning from a breast carcinoma, production of the active enzyme in *Escherichia coli*, and expression analysis in human tissues. J. Biol. Chem., *269:* 27136–29142, 1994.
16. Puente, X. S., and López-Otín, C. Cloning and expression analysis of a novel serine hydrolase with sequence similarity to prokaryotic enzymes involved in the degradation of aromatic compounds. J. Biol. Chem., *270:* 12926–12932, 1995.
17. Uría, J. A., Ferrando, A. A., Velasco, G., Freije, J. M. P., and López-Otín, C. Structure and expression in breast tumors of human TIMP-3, a new member of the metalloproteinase inhibitor family. Cancer Res., *54:* 2091–2094, 1994.
18. Chomczynski, P., and Sacchi, N. Single-step method of RNA isolation by guanidinium thiocyanate phenol chloroform extraction. Anal. Biochem., *162:* 156–159, 1987.
19. Okada, Y., Nagase, H., and Harris, E. D., Jr. A metalloproteinase from human rheumatoid synovial fibroblast that digests connective tissue matrix components. J. Biol. Chem., *261:* 14245–14255, 1986.
20. Pei, D., and Weiss, S. J. Furin-dependent intracellular activation of the human stromelysin-3 zymogen. Nature (Lond.), *375:* 244–247, 1995.
21. Schmid, C. W., and Jelinek, W. R. The Alu family of dispersed repetitive sequences. Science (Washington DC), *216:* 1065–1070, 1982.
22. Ullu, E., and Tschudi, C. Alu sequences are processed *7SL* RNA genes. Nature (Lond.), *312:* 171–172, 1984.
23. Siegel, V., and Walter, P. Removal of the Alu structural domain from signal recognition particle leaves its protein translocation activity intact. Nature (Lond.), *320:* 81–84, 1986.
24. Nordstrom, L. A., Lochner, J., Yeung, W., and Ciment, G. The metalloproteinase stromelysin-1 (transin) mediates PC12 cell growth cone invasiveness through basal laminae. Mol. Cell. Neurosci., *6:* 56–68, 1995.
25. Curry, T. E., Dean, D. D., Sanders, S. L., Pedigo, N. G., and Jones, P. B. C. The role of ovarian proteases and their inhibitors in ovulation. Steroids, *54:* 501–521, 1989.
26. Massagué, J., and Pandiella, A. Membrane anchored growth factors. Annu. Rev. Biochem., *62:* 515–541, 1993.
27. Gearing, A. J. H., Beckett, P., Christodoulou, M., Churchill, M., Clements, J., Davidson, A. H., Drummond, A. H., Galloway, W. A., Gilbert, R., Gordon, J. L., Leber, T. M., Mangan, M., Miller, K., Nayee, P., Owen, K., Patel, S., Thomas, W., Wells, G., Wodd, L. M., and Woolley, K. Processing of tumor necrosis factor-α precursor by metalloproteinases. Nature (Lond.), *370:* 555–557, 1994.
28. McGeeham, G. M., Becherer, J. D., Bast, R. C., Boyer, C. M., Champion, B., Connolly, K. M., Conway, J. G., Furdon, P., Karp, S., Kidao, S., McElroy, A. B., Nichols, J., Pryzwansky, K. M., Schoenen, F., Sekut, L., Truesdale, A., Verghese, M., Warner, J., and Ways, J. P. Regulation of tumour necrosis factor-α processing by a metalloproteinase inhibitor. Nature (Lond.), *370:* 558–561, 1994.