A cDNA encoding a new human matrix metalloproteinase (MMP), tentatively called MMP-23, has been cloned from an ovary cDNA library. This protein exhibits sequence similarity with MMPs, but displays a different domain structure. Thus, MMP-23 lacks a recognizable signal sequence and has a short prodomain, although it contains a single cysteine residue that can be part of the cysteine-switch mechanism operating for maintaining enzyme latency. The C-terminal domain is considerably shortened and shows no sequence similarity to hemopexin, whereas all human MMPs, with the exception of matrilysin, contain four hemopexin-like repeats. Furthermore, MMP-23 is devoid of structural features distinctive of the diverse MMP subclasses, including the specific residues located close to the zinc-binding site in collagenases, the transmembrane domain of membrane-type MMPs, or the fibronectin-like domain of gelatinases. Fluorescent *in situ* hybridization experiments showed that the human MMP-23 gene maps to 1p36, a location which differs from all MMP genes mapped to date. Recombinant MMP-23 produced in *Escherichia coli* exhibits low, but significant proteolytic activity against a synthetic substrate commonly used for assaying MMPs. Northern blot analysis demonstrated that MMP-23 is predominantly expressed in ovary, testis, and prostate, suggesting that this new MMP may play a specialized role in reproductive processes.

Matrix metalloproteinases (MMPs) or matrixins are a family of zinc-dependent endopeptidases that degrade the different extracellular matrix proteins at a neutral pH. These enzymes are produced by many cell types, usually in response to disease processes associated with inflammation or tumor progression. In addition, they have been implicated in the connective tissue remodeling occurring in normal processes such as embryonic development, bone growth, or wound healing (1–4). In recent years, the number of known members of the family has grown after the discovery of a series of new family members identified in both normal or pathological conditions. To date, 16 human MMPs have been cloned and characterized at the amino acid sequence level (3, 5). They can be classified into at least four subfamilies, according to their substrate specificity, primary structures, and cellular localization: the collagenases, gelatinases, stromelysins, and membrane-type MMPs (MT-MMPs). However, there are some recently described enzymes like macrophage metalloelastase (6), stromelysin-3 (7), MMP-19 (8), and enamelysin (5), which do not appear to fall into any of these subfamilies. In addition to all these MMPs identified in human tissues, distinct MMPs have been also cloned from *Xenopus laevis* (9, 10), embryonic sea urchin (11), green alga (12), soybean leaves (13), chicken (14), and *Caenorhabditis elegans* (15). Biochemical characterization of the diverse MMPs has opened new views on the role of these enzymes in connective tissue remodeling processes, and evidence is accumulating that MMPs are not exclusively involved in the proteolytic degradation of extracellular matrix components. Thus, MMPs have been reported to play direct roles in other essential cellular processes such as differentiation, proliferation, angiogenesis, or apoptosis (16). Some of these functions are mediated by the ability of MMPs to catalyze hydrolysis of a variety of substrates including membrane-bound precursors of cytokines, growth factors, or hormone receptors (17, 18), serum-amyloid A (19), insulin-like growth factor-binding proteins (20, 21), proteinase inhibitors (22–25), or interleukin-1p (26).

The identification of expanding roles for MMPs in a wide variety of biological processes has stimulated the search for new family members by using improved cloning strategies. Recently, we have utilized PCR-based methods with degenerate oligonucleotides, and expressed sequence tag (EST)-based approaches for cloning different human MMPs from both normal or tumor tissues (5, 8, 27, 28). In this work, we have examined the possibility that additional yet uncharacterized MMPs could be produced by human tissues, with the finding of a novel family member tentatively called MMP-23. We describe the molecular cloning and complete nucleotide sequence of a cDNA encoding for this proteolytic enzyme. We also report the expression of the gene in *Escherichia coli* and perform a preliminary analysis of the enzymatic activity of the recombinant enzyme. Finally, we report the chromosomal location of the
MMP-23 gene in the human genome and analyze its expression in human tissues.

**EXPERIMENTAL PROCEDURES**

**Materials**—Restriction endonucleases and other reagents used for molecular cloning were from Boehringer Mannheim (Mannheim, Germany). Synthetic oligonucleotides were prepared in an Applied Biosystems (Foster City, CA) model 392A DNA synthesizer. Double-stranded DNA probes were radiolabeled with [32P]dUTP (3000 Ci/mmol) purchased from Amersham International (Buckinghamshire, UK) using a commercial random-primer kit from the same company. A human ovary cDNA library constructed in λDR2 and two Northern blots containing polyadenylated RNAs from different human tissues were from CLONTECH. A panel of monochromosomal somatic cell hybrid DNAs and a human P1 artificial chromosome (PAC) genomic library were provided by the Human Genome Mapping Resource Center (Cambridge, UK).

**Probe Preparation and Screening of a Human Ovary cDNA Library**—A search of the GenBank™ data base of human ESTs for sequences with homology to proteases of the MMP family, allowed us to identify a sequence (W76631; deposited by R. K. Wilson, Washington University-Merck EST Project) derived from a fetal heart cDNA clone, and showing significant similarity with sequences of known human MMPs. To obtain this DNA fragment, we performed PCR amplification of a panel of cDNAs (Quick Screen, CLONTECH) with two specific primers 5'-CTGGGTCTGTCGGCCACCAAG (primer 1) and 5'-CTCTGGGGACACCTGACAG TG (primer 2) derived from the W76631 sequence. The PCR reaction was carried out in a GeneAmp 2400 PCR system from Perkin-Elmer/Cetus for 40 cycles of denaturation (94°C, 15 s), annealing (61°C, 20 s), and extension (72°C, 20 s). The 384-bp PCR product, amplified from human ovary cDNA, was phosphorylated with T4 polynucleotide kinase and cloned into a Smal-cut pUC18 vector. The cloned cDNA was sequenced and found to be virtually identical (97% identities) to the W76631 sequence. This cDNA was then excised from the vector, radiolabeled, and used to screen a human ovary cDNA library according to standard procedures (29). Following plaque purification, the insert was excised by BstHI/XhoI digestion and the resulting fragments subcloned into pUC19.

**5'-Extension of Isolated cDNAs**—The 5'-ends of cloned cDNAs were extended by successive cycles of rapid amplification of cDNA ends (RACE) using RNA from human ovary and the Marathon™ cDNA amplification kit (CLONTECH), essentially as described by the manufacturer. Each cycle of RACE allowed the extension of approximately 60–80 bp of cDNA toward the 5'-end. After cloning and sequencing the amplified products, new specific oligonucleotides were synthesized and used for the next RACE experiment. 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 72°C using the outermost primers. The PCR product, amplified from human ovary cDNA, was phosphorylated with T4 polynucleotide kinase and cloned into a Smal-cut pUC18 vector. The cloned cDNA was sequenced and found to be virtually identical (97% identities) to the W76631 sequence. This cDNA was then excised from the vector, radiolabeled, and used to screen a human ovary cDNA library according to standard procedures (29). Following plaque purification, the insert was excised by BstHI/XhoI digestion and the resulting fragments subcloned into pUC19.

**Nucleotide Sequence Analysis**—DNA fragments of interest were cloned in the polylinker region of phage vector M13mp19 and sequenced by the dideoxy chain termination method, using either M13 universal primer or cDNA specific primers and the Sequenase version 2.0 kit (US Biochemical Corp.). All nucleotides were identified in both strands. Computer analysis of DNA and protein sequences was performed with the GCG software package of the University of Wisconsin Genetics Computer Group (30).

**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'-CGAAACACGACAG-3' and 5'-CTCTGGGGACACCTGACAG TG-3'. Amplification conditions were as follows: 35 cycles of denaturation (94°C, 15 s), annealing (51°C, 15 s), and extension (68°C, 20 s) using the Expand Long PCR kit. Fluorescent in situ hybridization (FISH) mapping of genomic DNA clones for MMP-23 was performed as described previously (31). Genomic clones for MMP-23 were obtained by probing the human PAC genomic library, screened by filter hybridization with the full-length MMP-23 cDNA as probe. DNA from isolated PAC clones was obtained with the standard alkaline lysis method using QIAGEN columns (QIAGEN Inc., Chatsworth, CA), and nick-translated with biotin-16-dUTP. Then, labeled probes were hybridized to normal male metaphase chromosomes obtained from phytohemagglutinin-stimulated cultured lymphocytes, and detected using two avidin-fluorescein layers (32). Chromosomes were diaminid-2-phenylindole dihydrochloride (DAPI)-labeled, and images were captured in a Zeiss Axioskop fluorescent microscope equipped with a CCD camera (Photometrics).

**Northern Blot Analysis**—Nylon filters containing 2 μg of poly(A) RNA of a wide variety of human tissues were prehybridized at 42°C for 3 h in 6× standard saline citrate, 5× SSPE, 0.1% SDS, 1 mM NaH2PO4, 1 mM EDTA, pH 7.4, 10× Denhardt’s solution, 2% SDS, and 100 μg/ml denatured herring sperm DNA, and then hybridized with radiolabeled MMP-23 full-length cDNA for 20 h under the same conditions. Filters were washed with 0.1× SSC, 0.1% SDS for 2 h at 50°C and exposed to autoradiography. RNA integrity and equal loading was assessed by hybridization with an actin probe.

**Construction of Expression Vectors for MMP-23 and Expression in Escherichia coli**—A 973-bp fragment of the MMP-23 cDNA containing the catalytic and C-terminal domains was generated by PCR amplification with primers 5'-AGACAAGCTTCAGCTGACCTCCAG and 5'-AGAGAAAAA GCTTTTACCGGCG. The PCR amplification was performed for 20 cycles of denaturation (95°C, 15 s), annealing (54°C, 15 s), and extension (65°C, 2 min), followed by 10 additional cycles of denaturation (95°C, 15 s), annealing (62°C, 15 s), and extension (68°C, 2 min) using the Expand Long PCR kit and the GeneAmp 9700 PCR system. Due to the design of the oligonucleotides, the amplified fragment could be cleaved at both ends with HindIII and ligated in frame into the pSETB E. coli expression vector (Invitrogen) previously cleaved with the same restriction enzyme. In addition, an expression vector containing a chimera consisting of the MMP-23 catalytic domain and the MMP-23 catalytic domain was constructed by overlap extension mutagenesis using the following strategy: the propeptide domain of MMP-19 was amplified by PCR using a coding primer 5'-GCGCCGCTTGCAGACTACCTGC (primer 1) and a noncoding primer 5'-ACGCGGTGTATTTAAGGGTCTTCGGTTGAAG (primer 2). The amplified fragment was cloned into the pSETB E. coli expression vector (Invitrogen) and sequenced. Recombinant proteins obtained in inclusion bodies were solubilized with 35 μg/ml chloramphenicol and 50 μg/ml ampicillin. 50 μl of the corresponding culture was used to inoculate 200 ml of 2YT medium containing 35 μg/ml chloramphenicol and 50 μg/ml ampicillin. 50 μl of the corresponding culture was used to inoculate 200 ml of 2YT medium containing the above antibiotics. After culture reached an A600 of 0.6, expression was induced by addition of isopropyl-1-thio-β-D-galactopyranoside (IPTG) (0.5 mM final concentration) followed by further incubation for 3–20 h at 30°C. Recombinant proteins obtained in inclusion bodies were solubilized using 20 mM Tris buffer, pH 8.0, containing 0.1 M NaCl and 5 mM dithiothreitol. The solubilized proteins were purified using Ni-NTA agarose. Fractions containing purified recombinant proteins were combined and refolded by dilution (1:10) into refolding buffer 20 mM Tris/ HCl, pH 8.0, 5 mM CaCl2, and 50 μM ZnCl2. Precipitated proteins were removed by centrifugation.

**Enzyme Assays**—Enzymatic activity of purified recombinant MMP-23 or chimeric MMP19/MMP23 was detected using the synthetic fluorescent substrates McaPLGLDpAARHNL, McaPlCyaGnaVaHDpaNH2, and McaPLANvHaDpaARNH2. Routine assays were performed at 37°C at substrate concentrations of 0.5 and 1.5 μM in an assay buffer of 0.1 M Tris/HCl, 10 mM CaCl2, 150 mM NaCl, 0.05% (v/v) Brij 35, pH 7.5 (33). Inhibition of enzymatic activity by recombinant TIMP-1 was evaluated using the above assay.

**RESULTS**

**Identification and Characterization of a Human Ovary cDNA Encoding a New Member of the Matrix Metalloproteinase Family**—To identify putative novel members of the MMP family expressed in human tissues, we screened the GenBank™ data base of ESTs looking for entries with sequence similarity to previously described family members. This analysis allowed us to identify a 419-bp EST that, when translated, generated an open reading frame with significant amino acid sequence sim-
Similarity to the C-terminal region of the catalytic domain characteristic of MMPs. A cDNA containing part of this EST was obtained by PCR amplification of total λ-phage DNA prepared from a human ovary cDNA library. The 384-bp PCR-amplified product was cloned and its identity was confirmed by nucleotide sequence analysis. Then, the cloned fragment was radio-labeled and used as a probe to screen the same ovarian cDNA library used for the previous PCR amplification experiment. Upon screening of approximately 1 \times 10^6 plaque forming units, two positive clones named 1.1 and 1.2 were identified and characterized. DNA was isolated from both positive clones, and their nucleotide sequence was determined by standard procedures. This sequence analysis revealed that one of these clones (1.2) had an insert of 750 bp, which was entirely contained in the 810-bp sequence determined for clone 1.1. A detailed comparative analysis of the sequence obtained for the largest clone with those corresponding to other MMPs suggested that it was incomplete at the N-terminus. To extend the partial cDNA sequence toward the N-terminus, we performed 5'-RACE experiments using a specific oligonucleotide deduced from the end of the 1.1 clone and RNA from human ovary as a template. Successive 5'-RACE experiments performed in similar conditions 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 (Fig. 1) revealed an open reading frame coding for a protein of 390 amino acids with a predicted molecular mass of 43.9 kDa. This sequence contains four potential sites of N-glycosylation (N-L-T, N-H-T, N-A-T, and N-G).
Human MMP-23

Table 1. Prodomain and Catalytic Site of Human MMP-23

| MMP-23 | Prodomain | Catalytic site |
|--------|-----------|---------------|
| M-23   | 27 ALCLLP   | 206 VAHEI     |
| M-1    | 100 PRGVP  | 192 GLH     |
| M-2    | 90 PRGVP   | 186 QM     |
| M-3    | 90 PRGVP   | 182 DFP     |
| M-7    | 85 PRGVP   | 178 G    |
| M-8    | 85 PRGVP   | 176 S     |
| M-9    | 97 PRGVP   | 171 G     |
| M-10   | 95 PRGVP   | 165 G     |
| M-11   | 90 PRGVP   | 163 D     |
| M-12   | 78 PRGVP   | 157 G     |
| M-13   | 94 PRGVP   | 153 G     |
| M-14   | 57 PRGVP   | 149 G     |
| M-15   | 129 PRGVP  | 141 G     |
| M-16   | 87 PRGVP   | 137 G     |
| M-17   | 24 PRGVP   | 133 G     |
| M-18   | 150 PRGVP  | 129 G     |
| M-20   | 96 PRGVP   | 125 G     |

Fig. 2. Partial comparison of the amino acid sequence of MMP-23 with other human MMPs. The amino acid sequences of human MMPs were extracted from the SwissProt data base and the multiple alignment was performed with the PILEUP program of the GCG package (30). Conserved residues around the cysteine switch region and the zinc-binding site characteristic of MMPs are shown in bold. For comparison purposes, numbering in each protein starts in the initiator methionine.

V-T, at positions 92, 148, 232, and 316, respectively). However, computer analysis using the algorithm developed by Nielsen et al. (34) revealed that the deduced amino acid sequence lacks a recognizable signal sequence at its N-terminal end, which is in contrast to all the remaining MMPs. Further analysis of the identified amino acid sequence revealed a significant similarity with other human MMPs, the maximum percentage of identities (35%) being with stromelysin-3. Most of these identities are concentrated in the putative catalytic domain of the novel sequence (Figs. 1 and 2). This domain contains 176 residues including the consensus sequence HEXGHXXXIXIXHS (at positions 211–222) involved in the coordination of the zinc atom at the active site of MMPs. The catalytic domain also shows a methionine residue, located seven amino acids C-terminal to the zinc-binding site, that is absolutely conserved in all MMPs (Fig. 2). This residue has been proposed to play an essential role in the structure of the active site of these enzymes (35). The predicted protein sequence also contains a putative prodomain with a single cysteine residue, which can be part of the activation locus characteristic of MMPs. However, it is noteworthy that this residue is located in a sequence context (A-L-L-P-A) that does not resemble the consensus P-R-C-G-V-P-D motif involved in maintaining the latency of MMPs (Fig. 2). Similarly, the C-terminal domain of the identified sequence is shorter than those of other MMPs, with the exception of matrilysin, and does not exhibit significant sequence similarity to hemopexin. Finally, the putative prodomain and catalytic domains of the identified sequence are separated by a R-X-R furin activation consensus sequence. This sequence has been shown to mediate the intracellular activation of a number of MMPs, including MT-MMPs and stromelysin-3. Taking together all these structural comparisons, we suggest that the isolated cDNA codes for a novel human MMP that we propose to call MMP-23; MMP-22 (also known as C-MMP) corresponds to the last family member recently identified in chicken embryos (14). Finally, it must be mentioned that during revision of this manuscript, we have been aware of the release by GenBank of a series of nucleotide sequences related to that reported herein for MMP-23 isolated from an ovarian cDNA library. Gururajan et al. (36) have also provided evidence that the MMP-23 gene is duplicated, but the gene product resulting of this duplication would be virtually identical in amino acid sequence to that of MMP-23 gene. Furthermore, the sequences for the putative rat and mouse homologs of human MMP-23 have also been recently released by GenBank (accession numbers AB010960 and AF085742, deposited by Onnishi et al. and D. Pei, respectively). These sequences are about 84% identical to the human enzyme and maintain all specific features of this MMP, including the lack of a recognizable signal sequence at the N-terminal end, a short prodomain with a cysteine residue located in a sequence unrelated to the consensus P-R-C-G-V-P-D activation locus of MMPs, as well as a short C-terminal domain with no sequence similarity to hemopexin. It is also of interest that both rat and mouse sequences have a conserved methionine residue at exactly the same position than that proposed in the present work as the starting residue for the human enzyme. Furthermore, both cDNA sequences have an in-frame stop codon closely upstream of the ATG codon encoding this methionine residue. Taken together, these data strongly suggest that the first methionine residue shown in Fig. 1 is the true translation start site of human MMP-23.

Chromosomal Mapping of the Human MMP-23 Gene—To determine the chromosomal location of the human MMP-23 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 MMP-23 gene was generated by using two specific oligonucleotides whose sequence was derived from a noncoding sequence flanking the second exon of the gene, and from a coding sequence of this same exon. As can be seen in Fig. 3, positive amplification results were only obtained in the hybrid containing the autosome number 1. Since no amplification products were observed in the hybrids containing the remaining human chromosomes, we can conclude that the MMP-23 gene maps to chromosome 1. To localize more precisely the MMP-23 gene within chromosome 1, we first isolated MMP-23 gene maps to chromosome 1. To localize more precisely the MMP-23 gene within chromosome 1, we first isolated MMP-23 genomic clones from a human PAC genomic library and used these clones for FISH experiments on human metaphase spreads. As shown in Fig. 4, and in complete agreement with the human-rat somatic hybrid studies, fluorescent signals corresponding to biotinylated MMP-23 clones were located on chromosome 1, and no other chromosome site was labeled above background. After DAPI banding of 60 metaphases showing hybridization in both chromosomes 1, the MMP-23 fluorescent signal was as-
mapped to this region, including that encoding p73, a recently identified p53-related protein (37). However, no other MMP genes have been previously found to map at this chromosome site (38–44).

Production of Recombinant MMP-23 in Bacterial Cells and Analysis of Its Enzymatic Activity—According to the above structural data, MMP-23 has a number of structural features characteristic of previously identified MMP family members. However, its deduced amino acid sequence also shows some unique features that could affect its putative role as a proteolytic enzyme. As a preliminary step to elucidate whether the isolated MMP-23 cDNA codes for a biologically active proteinase, we expressed the cloned cDNA in *E. coli*. A partial cDNA coding for the catalytic and C-terminal domains of human MMP-23 was subcloned into the expression vector pRSETB, and the resulting plasmid was transformed into *E. coli* BL21(DE3)pLysS. Transformed bacteria were induced with IPTG, and the resulting recombinant protein was purified and refolded as described under “Experimental Procedures.” How- ever, all attempts to detect any proteolytic activity of this recombinant protein against substrates commonly used for analyzing MMPs were unsuccessful. Similar results were obtained when other constructs containing the prodomain of MMP-23 or lacking the C-terminal domain of this enzyme were used (data not shown). A possibility to explain these negative results could be based on the inappropriate folding of the recombinant enzymes. We then decided to prepare an expression vector for a chimeric enzyme consisting of the propeptide domain of MMP-19 and the catalytic domain of MMP-23. This chimeric construct was chosen because it would allow autoactivation to occur, thus facilitating the subsequent analysis of the enzymatic activity of the recombinant protein. Transformed bacteria with this construct were induced with IPTG and protein extracts analyzed by SDS-PAGE. According to the obtained results, insoluble fraction of the bacteria transformed with the recombinant plasmid contained a protein of the expected size (31 kDa), which was not present in the control extracts (Fig. 5). This recombinant protein was purified and refolded as described under “Experimental Procedures,” and its degrading activity against specific substrates for MMPs was examined. The chimeric MMP-23 only displayed a low proteolytic activity on the synthetic peptide McaPLGLDpaARNH₂, insufficient to perform accurate kinetic studies. We were, however, able to demonstrate that autoproteolytic degradation during incubation of the proenzyme preparation at 37 °C was abolished in the presence of TIMP-1, confirming that the activity seen with our enzyme preparation was due to a matrix metalloproteinase (Fig. 5, and data not shown). Since the MMP-23 catalytic domain contains two cysteine residues, which are unique among MMPs, we speculate that either the refolding is not very efficient for this particular enzyme, or it might have very different enzymatic properties when compared with the classical MMPs. Consistent with this, no apparent activity of the recombinant protein was detected against other synthetic quenched fluorescent peptide substrates such as McaPLANvaDpaARNH₂ and McaPChaGNvaHADpaNH₂, which are good stromelysin and collagenase substrates, respectively (45, 46). Similarly, no apparent activity was detected against gelatin. Taken together, these preliminary functional analyses suggest that the cloned cDNA encodes for a MMP whose substrate is likely to be distinct to those corresponding to well defined family members such as collagenases, stromelysins or gelatinases. Nevertheless, the possibility that the majority of the recombinant protein is not correctly folded cannot be definitively ruled out.

Analysis of MMP-23 Expression in Human Tissues—To investigate the presence of MMP-23 mRNA transcripts in human tissues, Northern blots containing poly(A)⁺ RNAs prepared from a variety of 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 MMP-23. As shown in Fig. 6, a transcript of about 1.35 kilobase pairs was predominantly detected in ovary, testis, prostate, and heart. A transcript of the same size was also weakly detected in intest-
In this work, we describe the finding of a new human proteinase belonging to the MMP family, which we have tentatively called MMP-23. The strategy followed to identify MMP-23 was first based on a computer search of the EST database, looking for sequences with similarity to previously characterized MMP family members. A single sequence presumably characterized MMP family members. A single sequence presumably described for stromelysin-3 and MT-MMPs (49, 50).

An additional distinctive feature of the structure determined for MMP-23 derives from its unique C-terminal domain. All human MMPs characterized to date, with the exception of matrilysin, contain an hemopexin-like region of about 200 amino acids organized into four recognizable repeats (51, 52). In contrast, the C-terminal domain of MMP-23 contains a domain of only 100 residues that lacks any significant similarity with hemopexin. Because this domain has been reported to be important in defining the substrate specificity of MMPs, and in mediating interactions with inhibitors (53, 54), the occurrence of a shortened domain in MMP-23 could be relevant in determining the substrate specificity and catalytic properties of this novel enzyme. In this regard, it is also worth mentioning that MMP-23 also lacks a series of structural features distinctive of the diverse MMP subclasses, including the Asp, Tyr, and Gly residues located close to the zinc-binding site of collagenases, the fibronectin-like domain of gelatinases, and the transmembrane domain of MT-MMPs (55–59).

Taking all these structural data collectively, it seems clear that MMP-23 cannot be classified in any of the previously defined MMP subfamilies. Consequently, it must be placed into the growing group of “other MMPs,” which includes enzymes such as macrophage metalloelastase, stromelysin-3, MMP-19, or enamelyn, all of them having distinctive structural and/or functional properties (5–8). In this work, we have also exam-

discussion

In this work, we describe the finding of a new human proteinase. The predominant expression of MMP-23 in human reproductive tissues such as ovary, testis, and prostate suggests that this novel MMP could participate in some of the tissue remodeling processes taking place in these tissues during physiological conditions. Nevertheless, the finding of MMP-23 transcripts in other tissues, such as heart, strongly suggests that the function of this enzyme is not restricted to reproductive tissues.

gene and further 5′-RACE experiments, a full-length cDNA coding for MMP-23 was finally isolated and characterized. Structural analysis of the identified sequence for MMP-23 shows that it exhibits a series of protein domains characteristic of MMPs, including a prodomain, a catalytic domain, a hinge region, and a C-terminal domain. However, a more detailed analysis of the sequence deduced for MMP-23 reveals a number of specific structural features for this novel human proteinase. Thus, the identified sequence lacks the recognizable signal sequence present at the N-terminal end of all the remaining MMPs, suggesting that this novel enzyme could function in an intracellular compartment. However, the use of alternative secretory mechanisms as proposed for other proteins such as basic fibroblast growth factor, which are secreted despite lacking signal peptide, cannot be excluded (47). In addition, the prodomain identified for MMP-23 is unusually short although it contains a single Cys residue that can be part of the activation locus characteristic of MMPs. In this regard, it is well known that MMPs are synthesized as inactive precursors with an N-terminal propeptide that maintains the latency of the enzymes through a Cys-switch mechanism (48). According to this mechanism, coordination of the unpaired cysteine residue in the propeptide with the zinc ion in the active site leads to inactivation of the enzyme. Disruption of the Cys-zinc bond by limited proteolysis or conformational perturbations leads to opening of the switch and subsequent autocatalytic cleavages, finally resulting in the generation of a catalytically competent enzyme. The single Cys residue in the propeptide region of human MMP-23, which is conserved in the putative mouse and rat homologs of this protein (GenBank accession numbers AF085742 and AB010960), could participate in maintaining the latency of this enzyme. Nevertheless, the absence of conserved residues around this Cys residue in MMP-23, when compared with other MMPs (Fig. 2), could suggest that these consensus residues (P-R-C-G-V-P-D) are not essential for the appropriate functioning of the Cys-switch mechanism in all MMPs. Additionally, in relation with the activation mechanism of proMMP-23, the presence of a stretch of basic residues linking the pro- and the catalytic domains is indicative of a furin-mediated activation for this enzyme, in a similar fashion to that described for stromelysin-3 and MT-MMPs (49, 50).

Of interest, the C-terminal domain of MMP-23 contains a domain of only 100 residues that lacks any significant similarity with hemopexin. Because this domain has been reported to be important in defining the substrate specificity of MMPs, and in mediating interactions with inhibitors (53, 54), the occurrence of a shortened domain in MMP-23 could be relevant in determining the substrate specificity and catalytic properties of this novel enzyme. In this regard, it is also worth mentioning that MMP-23 also lacks a series of structural features distinctive of the diverse MMP subclasses, including the Asp, Tyr, and Gly residues located close to the zinc-binding site of collagenases, the fibronectin-like domain of gelatinases, and the transmembrane domain of MT-MMPs (55–59).

Taking all these structural data collectively, it seems clear that MMP-23 cannot be classified in any of the previously defined MMP subfamilies. Consequently, it must be placed into the growing group of “other MMPs,” which includes enzymes such as macrophage metalloelastase, stromelysin-3, MMP-19, or enamelyn, all of them having distinctive structural and/or functional properties (5–8). In this work, we have also exam-
The activity of recombinant MMP-23 produced in E. coli as a fusion with the MMP-19 prodomain, and purified and refolded following the same procedure previously used for producing other active MMPs (5, 8, 60). This recombinant protein shows low proteolytic activity against a synthetic peptide commonly used for analyzing the enzymatic properties of MMPs. Nevertheless, the fact that the detected proteolytic activity is inhabitable by TIMP-1 suggests that it corresponds to a bona fide MMP. A likely possibility to explain this low activity may be that the majority of the recombinant protein is not correctly folded. However, the possibility that this novel enzyme may have specific substrates whose nature is currently unknown, cannot be definitely ruled out. Also consistent with the above discussed distant relationship between MMP-23 and other human MMPs, chromosomal mapping of the MMP-23 gene has shown that it is located at chromosome 1, a unique position among all MMP genes mapped to date (38–44). Furthermore, the pattern of MMP-23 expression in human tissues is also somewhat unusual. Thus, in this work, we have provided evidence that this gene is abundantly expressed in a number of normal tissues, which is in marked contrast with the highly restricted expression of most MMPs in adult tissues under normal quiescent conditions. It is also of interest that MMP-23 is predominantly expressed in reproductive tissues, under normal quiescent conditions. It is also somewhat unusual. Thus, in this work, we have provided evidence that this gene is abundantly expressed in a number of normal tissues, which is in marked contrast with the highly restricted expression of most MMPs in adult tissues under normal quiescent conditions.

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REFERENCES

1. Woese, F. J. (1991) FASEB J. 5, 2145–2154
2. Matrisian, L. M. (1992) BioEssays 14, 455–463
3. Birkedal-Hansen, H., Moore W. G. I., Bodden, M. K., Windsor, L. J., Birkedal-Hansen, B., DeCarlo, A., and Engler, J. A. (1993) Crit. Rev. Oral Biol. Med. 4, 197–250
4. Steter-Stevenson, W. G., Aznaravirian, S., and Liotta, L. A. (1993) Annu. Rev. Cell. Biol. 9, 541–573
5. Llanes, E., Pendás, A. M., Knauer, V., Sorsa, T., Salo, T., Salido, E., Murphy, G., Simmer, J. P., Bartlett, J. D., and López-Otín, C. (1997) Biochemistry 36, 15101–15108
6. Belaoaouj, A., Shipley, J. M., Kobayashi, D. K., Zimonjic, D. B., Popescu, N., Silverman, G. A., and Shapiro, S. D. (1995) J. Biol. Chem. 270, 14568–14575
7. Basset, P., Beloqui, J.-P., Wolf, C., Stoll, Y., Segain, J. P., Anglard, P., Stoll, Y., Segain, J. P., Anglard, P., and Bode, W. (1995) J. Biol. Chem. 270, 13527–13533
8. Lepage, T., and Gache, C. (1990) J. Biol. Chem. 265, 13527–13533
9. Kinosita, T., Fujukawa, H., Shimada, T., Sato, T., and Matsuda, Y. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 4693–4697
10. Pak, J. H., Liu, C. Y., Huangpu, J., and Graham, J. S. (1997) FEBS Lett. 404, 283–288
11. Yang, M., and Karkniss, K. (1998) J. Biol. Chem. 273, 17983–17990
12. Wada, K., Nakamura, T., Komin, H., Nakajima, K., Hayashi, K., and Seiki, M. (1998) Gene (Amst.) 21, 57–62
13. Werb, Z. (1977) Cell Biol. 91, 439–442
14. Massague, J., and Pandiella, A. (1993) Annu. Rev. Biochem. 62, 515–541
15. Couët, J., Sar, S., Jolivet, A., H. M. T. Y., Milgrom, E., and Miesfeld, R. (1996)

2 G. Velasco, unpublished results.