Identification of a Human cDNA Sequence Which Encodes a Novel Membrane-associated Protein Containing a Zinc Metalloprotease Motif

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Abstract
We report the cloning and characterization of a human cDNA predicted to encode a novel hydrophobic protein containing four transmembrane domains and a zinc metalloprotease motif, HEXXH, between the third and fourth transmembrane domains, and have named the molecule metalloprotease-related protein-1 (MPRP-1). The MPRP-1 gene was localized to chromosome 1-p32.3 by radiation hybrid mapping, and Northern blot analysis revealed expression in many organs, with strong expression in the heart, skeletal muscle, kidney and liver. Immunohistochemical analysis showed that MPRP-1 was localized in the endoplasmic reticulum (ER), and not in the Golgi compartment. Fragments of DNA encoding a segment homologous to the HEXXH motif of MPRP-1 are widely found in bacteria, yeast, plants, and animals. These results suggest that the MPRP-1 may have highly conserved functions, such as in intracellular proteolytic processing in the ER.

Key words: metalloprotease; membrane protein; endoplasmic reticulum

In a previous study, we identified and characterized the human homologue of STE24 (hsSTE24), which was found to be a membrane-associated metalloprotease localized to the endoplasmic reticulum (ER) and possibly also to the Golgi compartment.1 Jose et al. reported cloning of hsSTE24 as Face (farnesylated protein-converting enzymes)-1, and the human FACE-1 gene mapped to 1p34.2 Although the substrate(s) for hsSTE24 is currently unknown, hsSTE24 may be involved in intracellular proteolytic processing related to protein maturation, since the hsSTE24 gene complements the Saccharomyces cerevisiae mutant lacking STE24, suggesting that hsSTE24 is involved in carboxy-terminal CAAX processing.3 The human counterpart of RCE1 (hRCE1) was similarly identified as an integral membrane protease containing multiple transmembrane domains and shown to be involved in prenyl protein processing.2,4,5

In an effort to identify novel integral membrane proteases, we examined the complete genomic sequence of the yeast S. cerevisiae and found 13 open reading frames (ORFs) containing an extensively defined metalloprotease consensus sequence, [GSTALIVN]-X(2)-H-E-[LIVMFYW]-{DEHRKP}-H-X-[LIVMFYGSPQ] (accession no. PS00142 in PROSITE database). One ORF, YKR087c, potentially encodes an integral membrane protein and contains the sequence VLAHEFAHQL, which matches the extended consensus sequence exactly. We used the translated sequence of YKR087c to search an EST database and found a portion of the DNA sequence of R02623 that has significant homology to YKR087c at the amino acid level in the region surrounding the HEXXH zinc-binding site. R02623 in the EST database shares 53% homology with YKR087c and includes the metalloprotease consensus motif. To extend the 5′ and 3′ ends of R02623, we performed 5′ and 3′ RACE-PCR on HeLa cell total RNA. Specific fragments, approximately 1 Kbp, ap-
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Figure 1. Nucleotide sequence of the MPRP-1 cDNA and deduced amino acid sequence of the MPRP-1 gene product. Nucleotides and the corresponding amino acids (single-letter code, below the nucleotide sequence) are numbered at the beginning of each line. The polyadenylation signal is underlined, and the metalloprotease motif is double underlined. The sequence data are available from DDBJ/EMBL/GenBank nucleotide sequence databases under accession no. AB048348.

Peared as a result of both the 5’ and 3’ RACE-PCR and were subcloned into a cloning vector, and several clones were recovered and sequenced to determine the full length sequence of MPRP-1. The entire sequence of MPRP-1 was 1.9 Kbp in length and coincided with a specific hybridization size detected by Northern blot analysis (Fig. 3), suggesting that the sequence encodes almost the full length of the MPRP-1 cDNA. The sequence contained an ORF of 1572 nucleotides, encoding 524 amino acids with a calculated molecular mass of 60,120 Da. An in-frame terminator codon was located 12 nucleotides upstream of a putative initiator methionine, but the typical Kozak consensus sequence was not identified at the site of the putative translation initiation codon. However, a potential polyadenylation signal, AATAAA, was identified (Fig. 1). The translated MPRP-1 has an additional 170 amino acid extension in the NH₂-terminal region compared to YKR087cp (Fig. 2A). With the exception of this extended amino-terminal region, the MPRP-1 sequence exhibits significant homology with YKR087cp (27% identity and 49% similarity) (Fig. 2A). A hydropathy plot of MPRP-1 revealed extensive hy-
Figure 2. Amino acid sequence of MPRP-1, and comparison with related sequences from other organisms. (A) The deduced amino acid sequence of Homo sapiens MPRP-1 was aligned with that of S. cerevisiae YKR087c using the CLUSTAL V program with a PAM250 substitution matrix. Asterisks indicate amino acid identities, and dots indicate conserved substitutions. The metalloprotease motifs (HEXXH) are shown in black boxes. Putative transmembrane segments are underlined. (B) Hydropathy plot of MPRP-1. The algorithm of Kyte and Doolittle was used to generate the hydropathy profile for MPRP-1 with a window of 11 amino acids. The putative transmembrane domains are numbered I through IV (top). The position of the metalloprotease motif is indicated by an arrow. (C) MPRP-1 homologues from five organisms are aligned across the segments containing the HEXXH consensus metal-binding motif in Mus musculus, Arabidopsis thaliana, S. cerevisiae, Shizosaccharomyces pombe, and Escherichia coli (Fig. 2C).

The human MPRP-1 gene was mapped to 1p32.3 by RH mapping with LODs of 11.12 to SHGC-110950 and 11.12 to SHGC-36871 and to a microsatellite marker D1S405 on chromosome 1p32. This mapping data was supported by searching DNA databases with the human MPRP-1 cDNA. The human MPRP-1 sequence was matched with clone RPC11-342F23 (50 kb; accession no. AL365187) on chromosome 1p32.

Northern blot analysis was used to investigate the tissue specificity of MPRP-1 expression. Specific hybridization was obtained at about 2 Kb in the blot, and MPRP-1 expression was widely distributed among the organs tested, with strong expression detected in the heart, skeletal muscle, kidney, and liver (Fig. 3).

The intracellular localization of MPRP-1 was investigated with a hemagglutinin (HA)-tagged MPRP-1 that was exogenously expressed in HeLa cells. When the HA-tagged MPRP-1 was detected by immunostaining with an anti-HA antibody (Ab), the HA-tagged MPRP-1 was found to exhibit a reticular staining pattern in the cytoplasm, suggesting that MPRP-1 localized in the ER and possibly in Golgi compartment (Fig. 4). To com-
In this report we describe the identification and characterization of a novel membrane-associated protein containing the neutral metalloprotease consensus sequence HEXXH. A large number of metalloproteases have been identified and have been classified into 30 families. Approximately half of the families are distinguished by the HEXXH consensus metal-binding site, which has been defined more extensively as $a\text{HE}b\text{bbH}_{c}c$, where $a$ is usually valine or threonine; $b$ is an uncharged residue; $c$ is hydrophobic; and $x$ can be any amino acid except proline. The corresponding sequence in MPRP-1 is LLGHEIAHAV, which matches the extended consensus sequence precisely except for the $a$ residue which is leucine instead of valine or threonine.

The PROSITE database contains 13 ORFs in the complete $S.\text{cerevisiae}$ genome containing the extended consensus sequence for neutral zinc metalloprotease (accession no. PS00142), and 6 out of the 13 ORFs have been identified as ion-dependent peptidases or proteases. One of the six ORFs corresponds to a zinc-containing alcohol dehydrogenase; but the functions of the other five have not yet been determined. Based on the sequence comparisons shown in Fig. 2, we think that MPRP-1 defines a conserved family of hydrophobic membrane proteins found in bacteria, yeast, plants, and animals. These sequences were deposited in databases as a result of random DNA sequencing, and none has previously been identified as a protease gene. The extensive cross-species conservation of MPRP-1 is consistent with its proposed role as a metalloprotease.

Zumiga et al. have reported that a YKR087c deletion mutant is capable of growing normally in any conditioned

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**Figure 3.** Northern blot analysis of MPRP-1 gene expression in various human tissues. Human multiple tissue blot (BD Biosciences Clontech, Palo Alto, CA) was hybridized with a probe labeled with [$\alpha$-32P]dCTP by Prime-It II Random Primer Labeling Kit (Stratagene, La Jolla, CA) using a 0.8-kbp COOH-terminal fragment of MPRP-1 cDNA as a template. Hybridization was carried out at 68°C for 1 hr using Expresshyb hybridization solution (BD Bioscience Clontech) according to the manufacturer's instructions. Finally, the blot was washed in 0.1 x SSC, 0.1% SDS at 42°C, and X-ray film was then exposed to it.
medium, and expression of YKR087c mRNA has been found to be below detectable levels in wild-type cells. In contrast to yeast YKR087c, however, the MPRP-1 gene appears to be widely expressed in human tissues, and strongly in some of them, indicating that MPRP-1 plays a physiological role in humans (Fig. 3). The MPRP-1 gene has been mapped to 1p32.3 close to the hsSTE24 gene, suggesting that MPRP-1 and hsSTE24 may have diverged from the same source. This raises the possibility that MPRP-1 may have a similar function to hsSTE24 in catalyzing CAAX proteolytic activity. The ER contains a variety of proteolytic systems involved in protein maturation, regulated protein degradation, and selective degradation of misfolded secretory proteins, and the subcellular localization of the MPRP-1 to the ER membrane (Fig. 4) suggests a role for MPRP-1 in one of these proteolytic pathways, although confirmation of its physiological function awaits further study.

References


