Pregnancy-associated plasma protein-E (PAPP-E) 1

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Abstract

A full-length cDNA encoding a novel human protein was cloned from placenta cDNA. The corresponding 1542 amino acid protein sequence was termed ‘pregnancy-associated plasma protein-E’ (PAPP-E) as it shows a 62% homology to the human pregnancy-associated plasma protein-A (PAPP-A) that is a diagnostic marker for trisomies, especially Down syndrome. The conserved domain structure contains five motifs related to the short consensus repeats of complement proteins and selectins, three motifs related to the lin-notch motifs of proteins regulating early tissue differentiation, and a putative zinc-binding motif and active site of the metzincin-superfamily of metalloproteases. The PAPP-E gene was localized to chromosome 1q23–25. Northern blot analysis showed that PAPP-E is predominantly expressed in placenta. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Novel human cDNA; Pregnancy-associated plasma protein; Chromosome 1q23–25; Placenta

Pregnancy-associated plasma protein-A (PAPP-A) is a maternal serum protein of placental origin that is disulfide-bonded to the proform of eosinophil major basic protein [1]. The complex is detectable from the fourth to sixth week of pregnancy, and reaches concentrations up to 50 mg/l in the last trimester [2]. As the serum PAPP-A concentration is reduced by about 50–77% in the sixth to 14th week of pregnancies with fetal trisomies, PAPP-A in combination with other markers is used for non-invasive, early detection of trisomies [3]. The biological role of PAPP-A is not known, but a recent study has demonstrated that PAPP-A cleaves the insulin-like growth factor binding protein-4 (IGFBP-4) and could be involved in IGF-signaling [4]. Proteolysis might result from the putative zinc-coordination site followed by a putative Met-turn that are conserved active site motifs of the metzincin-superfamily of metalloproteinases [5,6].

Using the tblastn program [9] we have screened the GenBank human EST entries with the cDNA sequence of the catalytic domain of the human metzincin MMP-8 (matrix metalloproteinase-8, neutrophil collagenase). Besides known sequences of MMPs we have found a 315 bp EST (EST79446, gi: 2020543) that codes for the conserved zinc-binding motif of metzincins and is similar to the PAPP-A cDNA. We have cloned this EST by preparing RNA from placenta using the RNeasy Total RNA System (Qiagen) and performing reverse transcription with SuperScript II Rnase H 3 reverse transcriptase (Life Technologies), the polymerase chain reaction (PCR) with Taq DNA Polymerase (Roche) and the oligonucleotides 5'tgaaggagctgaaggaggccctgc-3P and 5'cggtgtcggaca-gagggtcctccg-3P, and TOPO TA Cloning (Invitrogen). Subsequent 5'- and 3'-RACE (rapid amplification of cDNA ends) with Marathon-Ready Placenta cDNA and Advantage 2 Polymerase Mix (Clontech) and TOPO XL PCR Cloning kit (Invitrogen) resulted in three cDNA fragments comprising a 4872 bp open reading frame with 5'- and 3'-flanking sequences (Fig. 1). Re-screening of the GenBank entries shows that the genomic sequences AL031734.9 (HS652L8) and AL031290.1 (HS774I24) partially cover the 3' and 5' PAPP-E cDNA. The deduced PAPP-E amino acid sequence shows global similarity only with PAPP-

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1 The cDNA and protein data reported here have been deposited in the EMBL Nucleotide Sequence Database under accession number AJ278348, HSA278348.
Fig. 1. cDNA and deduced protein sequence of PAPP-E. The putative prepropeptide is in italics and according to the PAPP-A sequence [5] negatively numbered; start and stop codons are boldface.

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A. Both proteins have about 44% identical and 62% similar amino acids [10], a corresponding size of 1547 and 1542 (PAPP-E) amino acids, a conserved pattern of cysteine residues, and a homologous domain structure (Fig. 2). Therefore, we named the novel sequence 'pregnancy-associated plasma protein-E' (PAPP-E). (In the 1970s, the terms PAPP-B, -C, and -D were assigned to a 1300 kDa octadecamer protein, the pregnancy-specific L1-glycoprotein and the placental lactogen, which are not related with PAPP-A and PAPP-E [11^13].)

According to the PAPP-A motifs [5], the PAPP-E sequence comprises a putative prepropeptide, a putative zinc-binding site and Met-turn, five motifs that are related to short consensus repeats of complement proteins and selectins, and three motifs that are related to the lin-notch motifs of proteins regulating early tissue differentiation (Fig. 3). For example, proteins showing significant partial homologies with PAPP-E are P-, E-, and L-selectins (CD62), complement decay-accelerating factor (CD55), complement receptor types 1 and 2, complement factor H, neurogenic locus notch (homolog) proteins, Caenorhabditis elegans lin-12 protein, and the catalytic domains of 356

Table 1

<table>
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<th>Parameter</th>
<th>PAPP-A</th>
<th>PAPP-E</th>
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<tbody>
<tr>
<td>Amino acids</td>
<td>1547/80</td>
<td>1542/82</td>
</tr>
<tr>
<td>Cysteines</td>
<td>82/2</td>
<td>86/0</td>
</tr>
<tr>
<td>M_r (kDa) without glycosylation</td>
<td>172.3/8.7</td>
<td>171.1/9.4</td>
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<tr>
<td>Putative glycosylation sites</td>
<td>14×Asn; 7×Ser</td>
<td>15×Asn; 12×Ser</td>
</tr>
<tr>
<td>Theoretical pf</td>
<td>5.4/12.3</td>
<td>5.0/10.7</td>
</tr>
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<td>Predominant expression</td>
<td>Placenta</td>
<td>Placenta</td>
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<tr>
<td>Chromosomal localization</td>
<td>9k33.1</td>
<td>1q23-25</td>
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Values are mature protein/prepropeptide. Asn, putative N-glycosylation site; Ser, putative sites for the attachment of glycosaminoglycans. PAPP-A data are according to [5].
Fig. 2. Alignment of PAPP-E and PAPP-A sequences and putative motifs. LNR, lin-notch repeat; SCR, short consensus repeat; Zn²⁺, zinc-binding site; F, N-glycosylation site Asn^Xaa^Ser/Thr; 8, site for attachment of glycosaminoglycans.
metzincins [9]. Primary structure analysis with the ExPASy proteomics tools shows that corresponding to PAPP-A, PAPP-E has a molecular mass (without glycosylation) of about 170 kDa, several putative sites for N-glycosylation and the attachment of glycosaminoglycan groups (Fig. 2 and Table 1), and an acidic theoretical isoelectric point (pI) of mature protein and a basic pI of prepropeptide (Table 1).

For Northern blot analysis, the 315 bp PAPP-E cDNA fragment coding for the putative active site was biotinylated with the North2South Biotin Random Prime kit (Pierce) and used for hybridization with the MTE Array 2 (Clontech), which represents expression of 75 human tissues. Chemiluminescent detection was performed with a streptavidin-horseradish peroxidase conjugate and luminol peroxidase reaction using North2South Chemiluminescent Hybridization and Detection (Pierce) and CL-Xposure X-ray film (Pierce) with Eukobrom paper developer and Superfix fixing solution (Tetenal). The only significant signal was produced by placenta mRNA (Fig. 4), so that placenta seems to be the main source of PAPP-E expression. This correlates with the predominant expression of PAPP-E by the trophoblastic tissue of the placenta [14] and defines both proteins as pregnancy-associated proteins.

To clarify the chromosomal localization of the PAPP-E gene, radiation hybrid mapping [15] with the human/hamster Genebridge 4 RH Panel (Research Genetics) and the oligonucleotides 5'-agcccagcatattatgggatgcctggcc-3' and 5'-ggtgtcggcacagaggtctcccgtttcc-3' for PCR with Taq DNA polymerase (Qiagen) was performed. Analysis of the resulting data vector shows that the PAPP-E gene is localized between the markers D1S242 and AFM210WC11 which are in the q23^25 region of chromosome 1 (Fig. 5).

In conclusion, we have cloned a novel human cDNA encoding a PAPP-A homologous protein that has been named pregnancy-associated plasma protein-E (PAPP-E). Both proteins share the same domain structure and probably form a new subfamily of metzincins. The PAPP-E gene has been assigned to chromosome 1 and is predominantly expressed in placenta. The biological role of PAPP-E regarding its putative proteolytic and regulatory functions and involvement in pregnancy and trisomy re...
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<th>heart</th>
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<th>kidney</th>
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<th>liver</th>
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<td>pituitary gland</td>
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<td>thymus</td>
<td>uterus</td>
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<td>ileum</td>
<td>peripheral blood leukocyte</td>
<td>prostate</td>
<td>salivary gland</td>
<td>Burkitt’s lymphoma, Raji</td>
<td>fetal spleen</td>
<td>poly t(A)</td>
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<tr>
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<td>ileocecum</td>
<td>lymph node</td>
<td>testis</td>
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<td>fetal thymus</td>
<td>human Ct-1 RNA</td>
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<td>appendix</td>
<td>bone marrow</td>
<td>ovary</td>
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Fig. 4. Northern blot analysis of PAPP-E mRNA. Chemiluminescent detection of a 75-tissue expression array (Clontech) hybridized with a biotinylated PAPP-E cDNA fragment.
mains to be analyzed. The recombinant expression for biochemical characterization of the new human protein is in progress.

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References