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Review

Gene regulation and physiological function of placental leucine aminopeptidase/oxytocinase during pregnancy

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Abstract

Human pregnancy serum and placenta have the ability to degrade uterotonic peptide oxytocin (OT). Placental leucine aminopeptidase (P-LAP), which is also called cystine aminopeptidase, is the only membrane aminopeptidase known to functionally degrade OT as oxytocinase (OTase). P-LAP/OTase hydrolyzes several peptides other than OT including vasopressin and angiotensin III. P-LAP/OTase predicted from cDNA sequence is a type II integral membrane protein, which is converted to a soluble form existing in maternal serum by metalloproteases, possibly ADAM (a disintegrin and metalloproteinase) members. P-LAP/OTase activity increases with normal gestation, while decreases in the patients with preterm delivery and severe preeclampsia. In placenta, P-LAP/OTase is predominantly expressed in differentiated trophoblasts, syncytiotrophoblasts. Activator protein-2 (AP-2) and Ikaros transcription factors play significant roles in exerting high promoter activity of P-LAP/OTase in the trophoblastic cells. Moreover, P-LAP/OTase is transcriptionally regulated in a trophoblast-differentiation-dependent fashion via up-regulation of AP-2, putatively AP-2α. P-LAP/OTase may be involved in maintaining pregnancy homeostasis via metabolizing peptides such as OT and vasopressin.

Keywords: Aminopeptidase; Oxytocin; Placenta; Pregnancy; Transcriptional regulation; Trophoblast differentiation

1. Introduction

During pregnancy, oxytocin (OT) is the most potent uterotonic peptide hormone and plays a critical role in the regulation of labor. Human pregnancy serum and placenta are known to have the ability to degrade OT [1], which should be involved in regulating OT action, because local concentrations of OT in the feto-placental–maternal unit depend upon a balance between synthesis and degradation. OT has a cystine ring structure at its N-terminus that is essential for exerting its uterotonic activity. Of two types of peptidases that metabolize OT, post-proline endopeptidase and aminopeptidase, aminopeptidase is able to open the ring structure and destroy OT activity, but whether post-proline endopeptidase has similar potential remains unclear [2–4]. Thus, aminopeptidase should be regarded as oxytocinase (OTase) [5]. This enzyme is named cystine aminopeptidase, cystinyl aminopeptidase, CAP and placental leucine aminopeptidase (P-LAP; EC 3.4.11.3) after a chemical method to measure the enzymatic activity. For the remainder of this review, I will use the term P-LAP/OTase.

The major protein in insulin-responsive glucose transporter isotype GLUT4 vesicles in rat adipocytes, which co-translocates with GLUT4 to the cell surface in response to insulin, was found to have aminopeptidase activity [6,7]. Its cDNA cloning and characterization confirmed that the protein is a member of zinc-dependent membrane aminopeptidase family [8], hence it has been named insulin-regulated membrane aminopeptidase (IRAP). Interestingly, independent cDNA cloning of human P-LAP/OTase [9] showed that rat IRAP and human P-LAP/OTase were 87% identical at amino acid levels, indicating that IRAP is a rat

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homologue of human P-LAP/OTase. As suggested by this finding, contrary to the initial hypothesis that P-LAP is a placenta-specific enzyme, P-LAP/OTase has a widespread tissue distribution [9,10]. In addition, success in cDNA cloning of human P-LAP/OTase further led us to isolate human P-LAP genome [11] and investigate the regulatory mechanisms of human P-LAP/OTase gene.

In this review article, we have overviewed biochemical characteristics and functions of human P-LAP/OTase, especially with focusing on the possible physiological roles and gene regulation of P-LAP/OTase during pregnancy.

2. Biochemical characteristics of P-LAP/OTase

We and another group have cloned the cDNA encoding human P-LAP/OTase from human placental cDNA library [9,12]. P-LAP/OTase cDNA cloning enabled us to deduce protein structure of P-LAP/OTase and prepare recombinant proteins. The finding that P-LAP/OTase is a type II membrane protein raised the question how P-LAP/OTase is released into the serum of pregnant women.

2.1. Structural and enzymatic characteristics of P-LAP/OTase

The predicted P-LAP/OTase contains three domains; an N-terminal 108 amino acid cytoplasmic domain, a 23-amino acid single transmembrane domain and an 893-amino acid extracellular domain (Fig. 1). Intracellular trafficking of P-LAP/OTase in response to insulin and OT was demonstrated in adipocytes and vascular endothelial cells, respectively [8,13]. The N-terminal cytoplasmic domain of P-LAP/OTase is thought to interact with a retention/sorting protein that also regulates the distribution of GLUT4 [14]. In addition, Ser-80 and Ser-91 of this domain are the major phosphorylation sites by protein kinase C-ζ, which may be involved in P-LAP/OTase trafficking [15]. A large extracellular domain contains the GAMEN motif and HEXXH-(18X)-E consensus sequence of zinc-binding site, which constitute the active site of gluzincin aminopeptidase family [16].

Based on the cDNA of human P-LAP/OTase, a large-scale production system of recombinant human P-LAP/OTase was established [17], which confirmed and newly provided enzymatic characteristics of P-LAP/OTase using molecular defined proteins. P-LAP/OTase preferentially hydrolyzes small peptides which possess an N-terminal ring structure, such as OT and vasopressin [17,18]. However, P-LAP/OTase dose not hydrolyze all the hormones with this structure, such as endothelins and calcitonin [17], indicating that whether or not one peptide hormone is cleavable by P-LAP/OTase may depend on the P′1 site and/or the molecular size of the peptide. Additionally, P-LAP/OTase also releases an N-terminal neutral or basic, not acidic, amino acids of small peptides including angiotensin III, somatostatin, Lys-bradykinin, Met-enkephalin, dynorphin A and nurokinin A [17–20].

2.2. P-LAP/OTase secretase

The presence of P-LAP/OTase activity in the serum of pregnant women has been known for a half century, which is consistent with the finding that P-LAP/OTase cDNA was cloned using P-LAP/OTase purified from retroplacental serum [9]. However, predicted structure of P-LAP/OTase from cDNA is a type II integral membrane protein, suggesting that P-LAP/OTase exists as a soluble form in maternal serum and a membrane-bound form in placenta. Soluble P-LAP/OTase in maternal serum is truncated

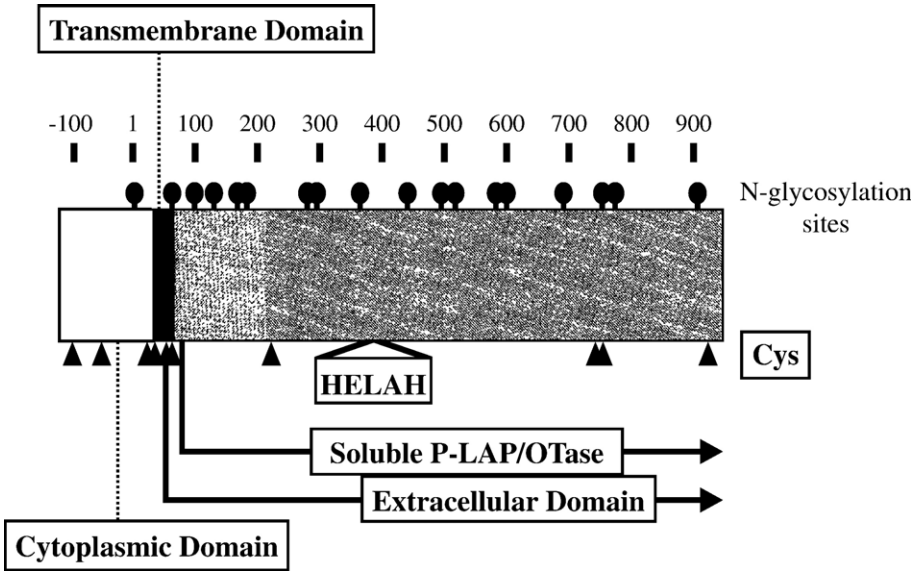

Fig. 1. Domain structure of P-LAP/OTase predicted from cDNA. Black box represents a putative transmembrane domain. Positions of the consensus sequence of the zinc-binding site and the N-terminal end of soluble P-LAP/OTase are also shown.