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Membrane-Bound Peptidases Regulate Human Extravillous Trophoblast Invasion

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Abstract

During human placentation, the extravillous trophoblast (EVT) invades maternal decidua and spiral arteries. However, the precise regulatory mechanisms by which EVT invasion is induced toward maternal arteries or limited within the uterus have not been well characterized. Recently, we found that dipeptidyl peptidase IV, a membrane-bound cell surface peptidase that can degrade chemokines, including RANTES, was expressed on EVT that had already ceased invasion. Another cell surface peptidase, carboxypeptidase-M, was also detected on EVT including the endovascular trophoblast in the maternal arteries. The inhibition of these peptidases increased cell invasion of choriocarcinoma-derived JEG-3 cells. On the other hand, CCR-1, a chemokine receptor for RANTES, was specifically expressed on EVT that migrated toward maternal arteries, while RANTES enhanced invasion of EVT that were isolated from primary villous explant culture. Platelets, which secrete RANTES and other chemokines, were detected among the endovascular trophoblast, and platelets were shown to enhance invasion of cultured EVT. Furthermore, a novel membrane-bound cell surface peptidase, laeverin, was found to be specifically expressed on EVT at deep sites in the maternal decidua. These findings suggest that membrane-bound peptidases regulate EVT invasion in cooperation with a chemokine system during early human placentation.

Keywords: Extravillous trophoblast; Dipeptidyl peptidase IV; Carboxypeptidase-M; Chemokine; Laeverin; Platelet

1. Introduction

During early placentation, the human extravillous trophoblast (EVT) invades the maternal decidual tissues in the placental site and migrates to the spiral arteries. The EVT invasion is an essential process for embryo implantation and placental formation. This infiltration by EVT leads to the loss of arterial contractility, which then maintains adequate maternal blood flow into the intervillous spaces to support placental function. If this invasion is inhibited, placental dysfunction results which may lead to obstetrical disorders such as preeclampsia [1]. EVT was reported to secrete proteases such as matrix metalloproteinases and serine proteinases, which degrade the extracellular matrix, and these proteases are considered to regulate EVT invasion in a balance with their inhibitors [2,3]. However, in contrast to malignant cells, EVT invasion is confined spatially to the uterus and temporally to early pregnancy. Although various mechanisms for EVT invasion have been proposed including growth factors [4,5], the precise mechanisms by which EVT invasion is induced toward maternal arteries or limited within the uterus are largely unknown.

Recent attention has been directed at cell surface-bound peptidases as local regulators for soluble factors including growth factors. To date several human cell surface-bound peptidases have been reported (Fig. 1). These enzymes can metabolize various peptides on their cell surface by hydrolyzing peptide bonds, as well as regulation of the local concentration of the biologically active peptides before they bind to their specific receptors on the cell surface (Fig. 2). Based on the cleavage sites of substrate peptides, membrane-bound peptidases are usually classified into three groups: aminopeptidase,

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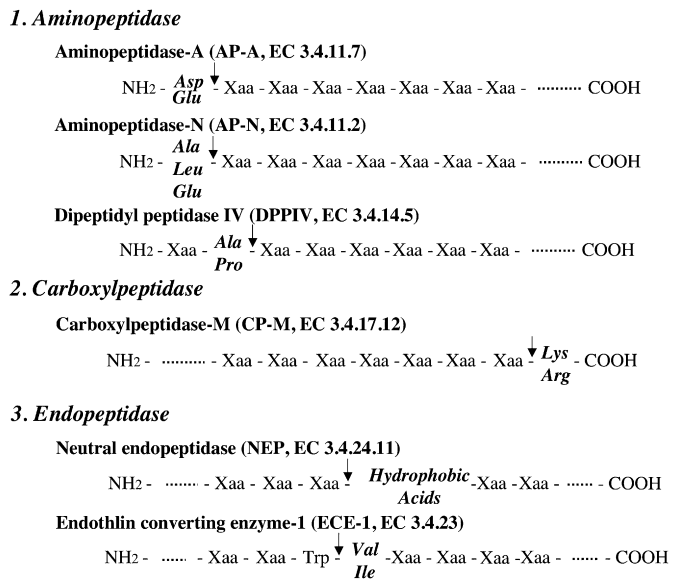

Fig. 1. Representative human membrane-bound cell surface peptidases. Aminopeptidases (aminopeptidase-N, aminopeptidase-A, and dipeptidyl peptidases IV) cleave one to three amino acid residue(s) at the N-terminal from the peptides. In contrast, carboxypeptidase-M removes amino acid residue(s) from the C-terminus. Endopeptidases (neutral endopeptidase and endothelin-converting enzyme-1) cut substrate peptides at a median site.

carboxypeptidase, and endopeptidase. Aminopeptidase is an enzyme that cleaves one to three amino acid(s) at the N-terminal of a peptide. Aminopeptidase-N (AP-N, EC 3.4.11.2), insulin-regulated aminopeptidase/placental leucine aminopeptidase (EC 3.4.11.3), aminopeptidase-A (AP-A, EC 3.4.11.7), and dipeptidyl peptidases IV (DPPIV, EC.3.4.14.5) belong to this group (Fig. 1). In contrast, carboxypeptidase removes one or two amino acid(s) from the C-terminus and carboxypeptidase-M (CP-M, EC 3.4.17.12) is classified in this group (Fig. 1). Neutral endopeptidase (NEP, EC 3.4.24.11) and endothelin-converting enzyme-1 (ECE-1, EC 3.4.23) are categorized as endopeptidases, which cut substrate peptides at a median site

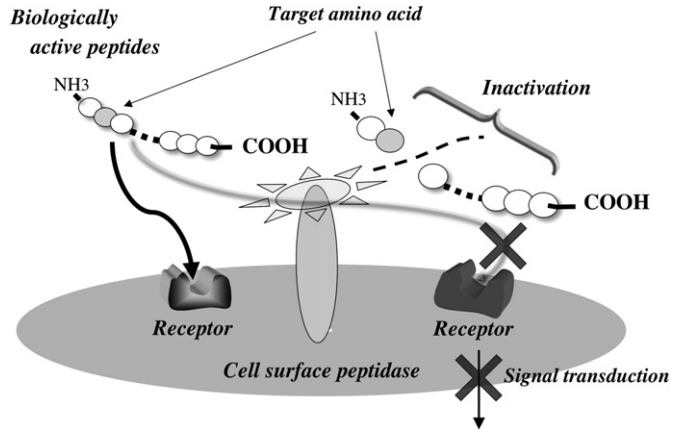

Fig. 2. Extracellular cleavage of biologically active peptides on the cell surface by peptidases. The concentration of biologically active peptides is regulated by a cell surface peptidase before the peptides access their specific receptors, showing that cell surface peptidases are important local regulators for cell function.

(Fig. 1). In general, removal of one or two amino acids from polypeptides by peptidases reduces the stability of the substrate leading to further degradation by other enzymes, or alternatively, changes the conformational structure of the substrate to decrease association with receptors. Accordingly, membrane-bound cell surface peptidases are important local regulators for cell function and differentiation [6].

We initially reported that aminopeptidase-N was a specific differentiation-related cell marker for theca interna cells in human ovarian follicles [7]. Later, its peptidase activity was shown to regulate folliculogenesis, bestatin, an inhibitor for aminopeptidase-N, is a possible pharmacologic candidate to induce follicular growth in poor responders [8–10]. In addition, other peptidases such as DPPIV, CP-M, and endothelin-converting enzyme-1 were found to be expressed on human luteinizing granulosa cells that produce progesterone after ovulation [11–14], providing evidence that cell surface-bound peptidases may be new regulators of ovarian cell function and differentiation [15].

In this context, we proposed that cell surface-bound peptidases contribute to EVT function by regulating soluble factors that are involved in EVT invasion and differentiation. Of the cell surface-bound peptidases, we previously reported that DPPIV was expressed in human EVT in the chorion laeve that has already ceased invasion [16]. Therefore, we initially examined the profiles of DPPIV expression on human EVT.

2. Possible roles of dipeptidyl peptidase IV in EVT function

Dipeptidyl peptidase IV (DPPIV) is known as T-cell activation antigen CD26 [17]. DPPIV removes a Xaa (one unspecified amino acid)-Pro or Xaa-Ala dipeptide from the N-termini of polypeptides or proteins (Fig. 2) [18]. The reported physiological substrates for DPPIV are substance P, β-casomorphin, endomorphin, neuropeptide Y, peptide YY, glucagon-like peptide, gastric inhibitory peptide, growth hormone-releasing factor, vasostatin, fibrin α-chain, and some chemokines [19].

Immunohistochemical study using monoclonal antibodies against human DPPIV showed that DPPIV was clearly expressed in the cytotrophoblast in the floating villi in the first trimester of pregnancy, and this high expression is maintained on EVT in the proximal part of the cell column of the anchoring villi. Expression rapidly decreased in the distal part of the cell column and was not detected on migrating EVT in the maternal decidual tissues. DPPIV expression, however, appeared again on EVT that migrated more deeply as pregnancy proceeded [20]. In contrast to EVT in the maternal decidual tissues, the endovascular trophoblast that invaded toward the spiral arteries lacked DPPIV expression at least up to 20 weeks' gestation.

In a primary villous explant culture system using human villous tissues derived from women in early pregnancy from 6 to 9 weeks gestation, EVT was found to proliferate and migrate toward distal sites from the villous tips, which correspond to the cell column [20]. In this culture, migrating