THE EXPRESSION OF PEPTIDASE ANTIGENS, CD10/NEUTRAL ENDOPEPTIDASE, CD13/AMINOPEPTIDASE N, AND CD26/DIPEPTIDYL PEPTIDASE IV IN HUMAN ENDOMETRIUM

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INTRODUCTION

Peptidase enzymes are known to be widely distributed in plasma and tissues. As such, they are considered to play important roles in the local regulation of biologically active peptides, including peptide hormones, growth factors, and cytokines. In the uterine endometrium, it has been suggested that some peptidase enzymes in the endometrial epithelium play an important role in blastocyst implantation. In rabbits, the uterine epithelium undergoes extensive morphological change during the peri-implantation phase, and dynamic changes in aminopeptidase and dipeptidyl peptidase IV activities on the surface of endometrial epithelial cells have been reported in histochemical experiments (Classen-Linke et al., 1987). Furthermore, proteinase inhibitors have been shown to block implantation in rabbits (Denker, 1977). In humans, we have reported that the cluster of differentiation (CD) antigens, CD10 and CD13, were expressed in the endometrial stromal cells, and that CD26 antigens were localized in the glandular epithelial cells (Imai et al., 1992a, 1992b). CD10, CD13, and CD26 antigens were shown to be identical to neutral endopeptidase (NEP) (Letarte et al, 1988), aminopeptidase N (APN) (Look et al.1989), and dipeptidyl peptidase IV (DPP IV) (Mattern et al., 1989; Stein et al., 1989;Ulmer et al., 1990) respectively, all of which are cell surface peptidases. Since a variety of peptide factors, including peptide hormones, growth factors, and cytokines, have been suggested to play important roles in regulating endometrial cell proliferation/differentiation, these peptidase enzymes are thought to be involved in the regulation of human endometrial function and the implantation process.

IMMUNOHISTOCHEMICAL STAINING FOR PEPTIDASE ANTIGENS

Cryostat sections were prepared from human endometria at various phases of development and from the decidua of early pregnancy. Indirect immunofluorescence staining was carried out using monoclonal antibodies for CD2, CD10, CD11b, CD13, CD14, CD20, and

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CD26. It was necessary to examine the lymphohematopoietic cell markers CD2 (T lymphocytes), CD11b (monocytes/granulocytes), CD14 (monocytes/granulocytes), and CD20 (B lymphocytes) in order to evaluate antigen expression in endometrial cells. Peptidase antigens of CD10/NEP, CD13/APN, and CD26/DPP IV have been identified as surface markers for lymphoid progenitor cells, granulocytes/monocytes, and activated T lymphocytes, respectively. In the proliferative phase, a majority of cells in the endometrial stroma demonstrated weak expression of CD10 and CD13 antigens (Fig. 1A, 1B), while endometrial glandular epithelial cells showed weak CD26 antigen expression (Fig. 1C). In the secretory phase, the expression of CD10 and CD13 antigens on endometrial stromal cells increased significantly (Fig. 2A, 2B). While endometrial glandular cells expressed CD26 antigen weakly to moderately in the early secretory phase. CD26 was expressed moderately in the mid-secretory phase, and strongly in late secretory phase (Fig. 2C). Furthermore, the endometrial surface epithelium expressed CD26 antigen at the same intensity as the glandular epithelium. No cells in the glandular epithelium expressed CD10 or CD13 antigens, and only a few cells in the stroma expressed CD26 antigen. Vascular endothelial cells in the uterine myometrium expressed CD26 antigen moderately throughout the menstrual cycle. In early pregnancy decidua, stromal cells with decidual transformation expressed CD13 antigen more strongly than secretory endometrial cells (Fig. 3B). CD10 antigen expression in decidual cells, however, was much weaker than in secretory endometrium (Fig. 3A). Moderate or strong expression of CD26 antigens was observed in the glandular epithelial cells of early pregnancy decidua (Fig. 3C). It appeared that CD26 antigen expression in the apical membrane of glandular cells and surface epithelium was stronger than in the basolateral membranes (Fig. 2C, 3C). In serial sections prepared during these experiments, CD2, CD11b, CD14 or CD20 antigen-bearing cells were sparsely distributed in the endometrium throughout the menstrual cycle and during the first

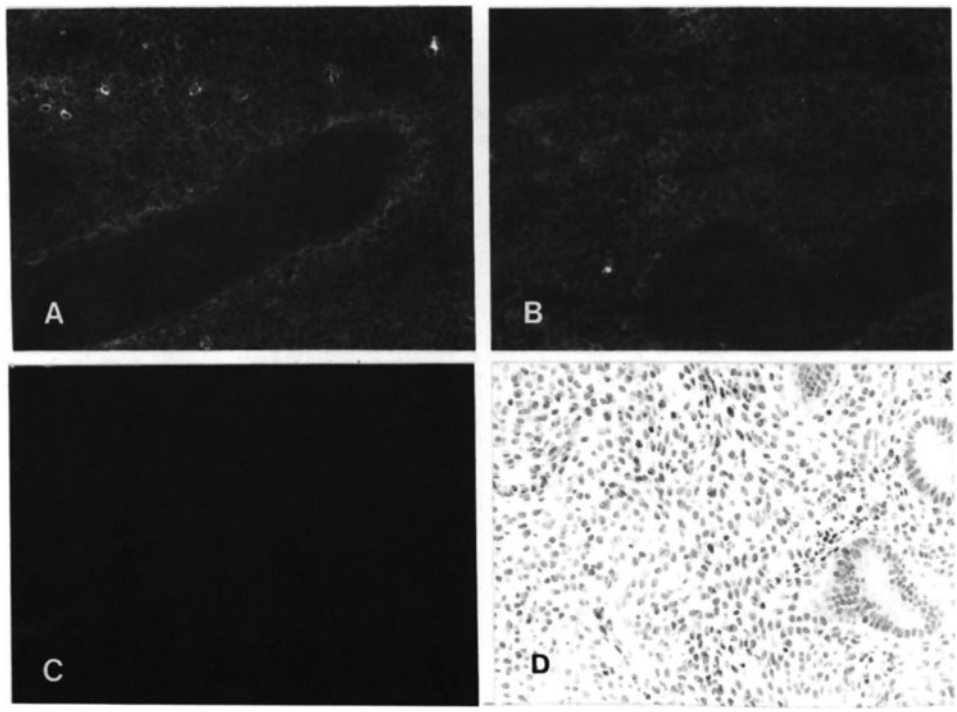

Fig. 1. Indirect immunofluorescence staining of human endometrium in proliferative phase. Cryostat sections were stained by using following monoclonal antibodies: A, CD10/Nu-N1; B, CD13/MCS2; C, CD26/Ta1; D, hematoxylin and eosin.

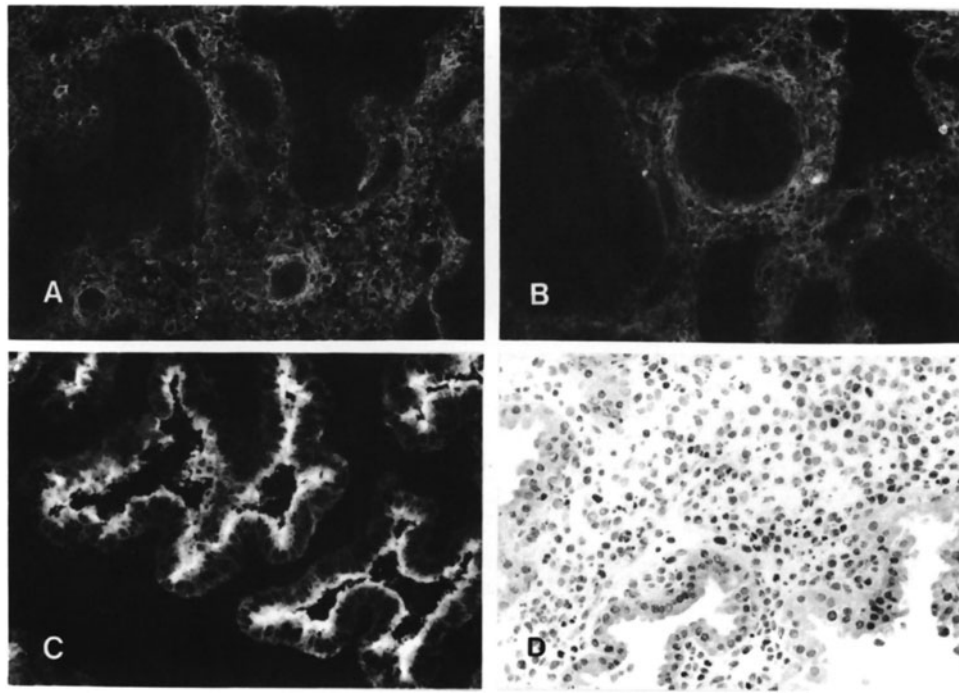

Fig. 2. Indirect immunofluorescence staining of human endometrium in secretory phase. Cryostat sections were stained by using following monoclonal antibodies: A, CD10/Nu-N1; B, CD13/MCS2; C, CD26/Ta1; D, hematoxylin and eosin.

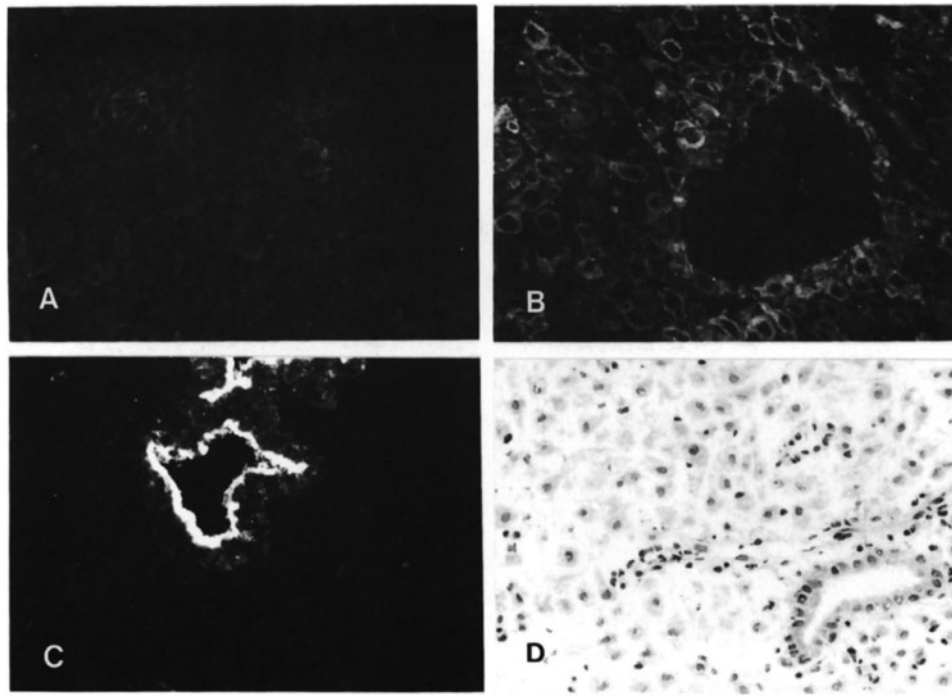

Fig. 3. Indirect immunofluorescence staining of human decidua of 7 weeks gestation. Cryostat sections were stained by using following monoclonal antibodies: A, CD10/Nu-N1; B, CD13/MCS2; C, CD26/Ta1; D, hematoxylin and eosin.

trimester of pregnancy. Therefore, it was apparent that CD10/NEP and CD13/APN antigens were expressed in stromal cells/decidual cells while CD26/DPP IV antigen was expressed in glandular cells. The intensity of CD10 and CD26 antigen expression was maximal during the secretory phase (peri-implantation periods), whereas the intensity of CD13 expression increased steadily following the establishment of pregnancy.

FLOW CYTOMETRIC ANALYSIS OF ENDOMETRIAL STROMAL CELL-ENRICHED PREPARATIONS

Human endometrial stromal cell-enriched preparations were obtained as described elsewhere (Kariya et al., 1991). The cells were stained with monoclonal antibodies against CD2, CD10, CD11b, CD13, CD14, and CD20 antigens, then examined using a flow cytometer. More than 80% of the cells examined were positive for CD10/NEP and CD13/APN antigens in every experiment, and the fluorescence intensity was relatively strong. CD2, CD11b, CD14 or CD20 antigen-bearing lymphohematopoietic cells usually accounted for less than 10% of observed fluorescence (Fig. 4). These indings confirmed the results of previous immunohistochemical experiments indicating that the stromal cells themselves express CD10/NEP and CD13/APN antigens in human endometrium. Dynamic changes in peptidase antigen expression during the menstrual cycle imply the involvement of ovarian gonadal steroids. Accordingly, the effect of estrogen, progesterone, or androgen on the expression of CD10/ NEP and CD13/APN antigens in cultured stromal cells was subsequently examined. Cells were cultured for 7 days with or without estradiol (10^{-8}M), progesterone (10^{-7}M), or testosterone (10^{-8}M), and CD10/NEP and CD13/APN antigen expressions on these cells were assessed by mean fluorescence intensity. During the culture period, CD10 antigen

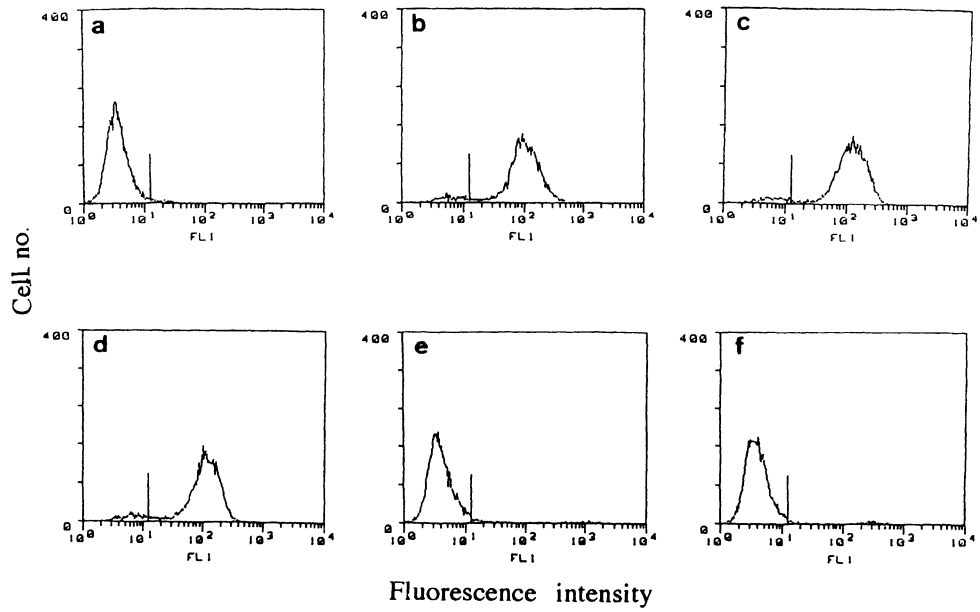

Fig. 4. Flow cytometric analysis of human ESC-enriched preparation from late-proliferative endometrium. Cells were stained as follows : a, a second antibody only (negative control); b, CD13/My7; c, CD13/MCS2; d, CD10/Nu-N1; e, CD14/LeuM3; f, CD11b/Bear-1. Percent positivity was as follows: a, 2.1; b, 92.8; c, 93.1; d, 92.2; e, 3.9; f, 3.3. Over 90% of the cells were positive for CD10 and CD13 antigens.

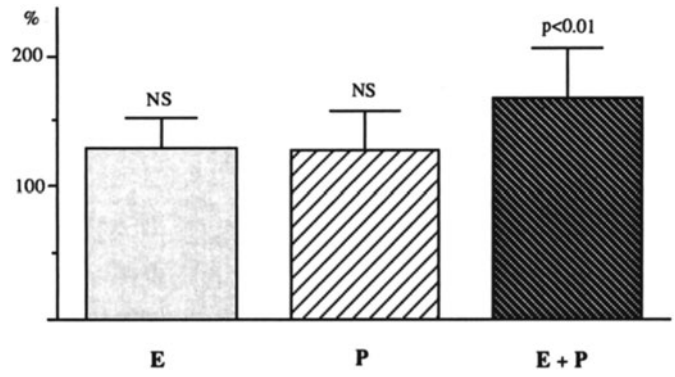

Fig. 5. CD13/APN antigen expression on endometrial stromal cells after 7 days culture with ovarian steroids. Mean fluorescence intensity was assessed by flow cytometry. Results were expressed as % of control cultures without ovarian steroids. (N=7)

expression on the stromal cells rapidly decreased, and the addition of gonadal steroids had no apparent effect on this expression. This observation suggests that some indispensable factor(s) are present *in vivo* for the maintenance of expression of NEP molecules on the cell surface. In contrast, CD13/APN antigen expression on stromal cells did not decrease even after 14 days' culture, and the expression of CD13/APN antigen was similarly unaffected by the addition of estrogen, progesterone, or androgen alone. A significant enhancement of CD13/APN antigen expression was observed, however, when both estradiol and progesterone were added to the cultures (Fig. 5). This *in vitro* experiment strongly suggests that the increase in APN expression in stromal cells during the proliferative phase and during decidualization is regulated by the effects of both estrogen and progesterone.

DETECTION OF AMINOPEPTIDASE AND DIPEPTIDYL PEPTIDASE IV ACTIVITIES

Aminopeptidase activity of the endometrial stromal cell-enriched preparation was examined using a method based on the hydrolysis of alanine-*p*-nitroanilide, to yield *p*-nitroaniline and alanine (Amoscato et al., 1989). The endometrial stromal cell-enriched preparation was suspended in PBS containing 0.4mM alanine-*p*-nitroanilide and incubated at 37°C with continuous stirring. The reaction was stopped by the addition of cold sodium acetate-acetic acid buffer. The optical density of the supernatant at 385nm was examined with a spectrophotometer, and the amount of *p*-nitroaniline formed was determined from a standard curve. Endometrial stromal cell-enriched fractions showed distinct aminopeptidase activity, and the amount of *p*-nitoaniline formed was linearly related to the number of the stromal cells (Fig. 6) and to the duration of the incubation period. This experiment demonstrated biochemically that human endometrial stromal cells have aminopeptidase activity. Interestingly, a significant increase in the *in vitro* peptidase activity was observed in the cultured endometrial stromal cells in the presence of both estrogen and progesterone, whereas estrogen, progesterone or androgen alone had no apparent effect (Fig. 7).

Histochemical detection of DPP IV activity was performed according to Lodja's method (1979). Briefly, 4mg of glycyl-prolyl-4-methoxy-β–naphthylamide was dissolved in 0.5ml N,N-dimethylformamide and mixed with 10mg Fast Blue B salt dissolved in 10ml 0.1M phosphate buffer. The mixture was filtered and overlaid on frozen sections of human endometrium and early pregnancy decidua. After 6-7 minutes of incubation, the slides were

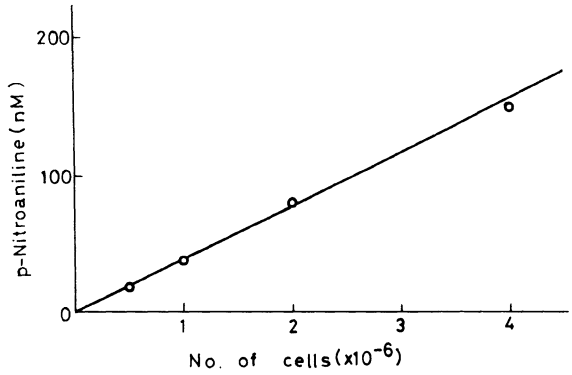

Fig. 6. Assay of peptidase activity of endometrial stromal cell-enriched preparation from the endometrium in mid-secretory phase. The amount of *p*-nitroaniline hydrolyzed from alanine-*p*-nitroanilide is linearly related to the number of cells. Incubation time: 20 min.

washed and red reaction products were observed with a light microscope. During the proliferative to early secretory phases, no DPP IV activity was observed in the endometrium (Fig. 8A). In the mid-secretory phase, most endometrial glandular cells showed weak to moderate DPP IV activity, and in the late secretory phase, glandular cells showed moderate to strong DPP IV activity (Fig. 8B). In the early pregnancy decidua, glandular cells showed moderate DPP IV activity (Fig. 8C), while vascular endothelium in the uterine myometrium had weak DPP IV activity. Lojda (1979) reported that, histochemically, DPP IV activity in the endothelium of blood vessels was heterogenous. In our study, most of the endothelium in the muscle layer was DPP IV positive, but the endothelium in the endometrial layer did not show DPP IV, either histochemically or immunohistologically.

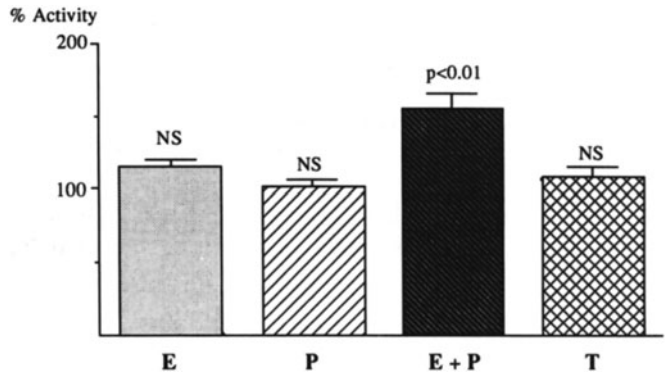

Fig. 7. Aminopeptidase activity of cultured endometrial stromal cells. Endometrial stromal cells were cultured for 7 days with ovarian steroids: E, 10^{-8}M; P, 10^{-7}M; T, 10^{-8}M. Results were expressed as % of control cultures without ovarian steroids. (N=6)

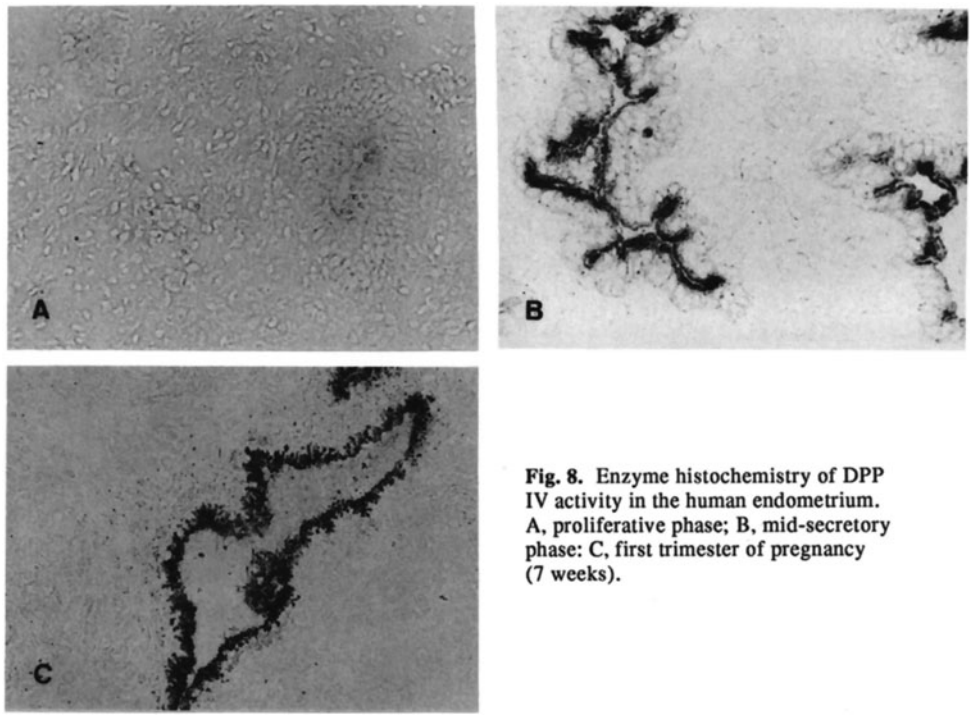

Fig. 8. Enzyme histochemistry of DPP IV activity in the human endometrium. A, proliferative phase; B, mid-secretory phase: C, first trimester of pregnancy (7 weeks).

PEPTIDASES AS POSSIBLE LOCAL REGULATORS OF PEPTIDE FACTORS

We have shown that human endometrial stromal cells express CD10/NEP and CD13/APN antigens, and that endometrial glandular cells express CD26/DPP IV antigen; the intensity of expression changes with endometrial differentiation. All three enzymes have their active domain exposed on the extracellular surface. NEP cleaves peptides at the amino side of hydrophobic residues, such as enkephalins, bradykinin, oxytocin, substance P, neurotensin, chemotactic peptides (fMet-Leu-Phe), gastrin, atrial natriuretic peptide, interleukin 1 (Erdös et al., 1989) etc. APN catalyzes the removal of N-terminal amino acids from peptides, such as met-lys-bradykinin and met-enkephalin, somatostatin (Sidorowicz et al., 1981), (Asn^1) angiotensin II (Ward et al., 1990) etc. DPP IV removes dipeptides from the N-termini of polypeptides. Its substrates include human gastrin-releasing peptide, human pancreatic polypeptide, α chain of human chorionic gonadotropin (Nausch et al., 1990), and substance P (Püschel et al., 1982). These peptidases are well known to have roles in the final steps of digestion in the intestinal canal. Recently, these enzymes have been proven to have a wide distribution on the surfaces of various cell types, and it has been suggested that they might have specific roles in the control of growth and differentiation in both hemopoietic and epithelial cell systems. In addition to having proteolytic activity, APN was reported to be a major receptor molecule for human coronavirus, and its significance in viral infection has been discussed (Delmas et al., 1992; Yeager et al., 1992). DPP IV has been shown to be involved in cellular adhesion in extracellular matrix proteins (Hanski et al., 1985, 1988; Piazza et al., 1989; Dang et al., 1990). Since DPP IV is expressed on the apical membrane of endometrial epithelium during the peri-implantation period, this antigen may have a role in the attachment of fertilized eggs to the endometrial surface.

It has become apparent that peptide factors including peptide hormones, growth factors, and cytokines play important roles during the peri-implantation period, and many of these peptides are possibly inactivated or functionally modulated by peptidase enzymes. Therefore, these enzymes may have a key role in the control of growth and differentiation of cells in human endometrium through the activation or inactivation of peptide factors and the regulation of their access to target cells.

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