



HAL
open science

Modulation of PAPP-A expression by PPARgamma in human first trimester trophoblast.

Karen Handschuh, Jean Guibourdenche, Mickaël Guesnon, Ingrid Laurendeau, Danièle Evain-Brion, Thierry Fournier

► **To cite this version:**

Karen Handschuh, Jean Guibourdenche, Mickaël Guesnon, Ingrid Laurendeau, Danièle Evain-Brion, et al.. Modulation of PAPP-A expression by PPARgamma in human first trimester trophoblast.. Placenta, 2006, 27 Suppl A, pp.S127-34. 10.1016/j.placenta.2005.10.012 . inserm-00000053

HAL Id: inserm-00000053

<https://inserm.hal.science/inserm-00000053>

Submitted on 2 Jun 2006

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Modulation of PAPP-A expression by PPAR gamma in human first trimester trophoblast

Karen Handschuh^{a,b}, Jean Guibourdenche^{a,b,c}, Mickaël Guesnon^{a,b}, Ingrid Laurendeau^d, Danièle Evain-Brion^{a,b} and Thierry Fournier^{a,b}

^a INSERM, U427, 4 avenue de l'Observatoire, 75006 Paris, France;

^b Université Paris Descartes, Faculté des Sciences Pharmaceutiques et Biologiques, 4 avenue de l'Observatoire, 75006 Paris, France;

^c APHP, Hôpital Debré, Biochimie fœto placentaire, 48 boulevard Sérurier, 75935 Paris 19, France;

^d Université Paris Descartes, Faculté des Sciences Pharmaceutiques et Biologiques, UPRES EA 3618, Laboratoire de génétique moléculaire, 75006 Paris, France

Corresponding author to whom all correspondence should be addressed:

Thierry Fournier, INSERM U427, Faculté de Pharmacie, 75006 Paris, France

TEL: 33(1) 53 73 96 03; FAX 33(1) 44 07 39 92; E-MAIL Thierry.Fournier@univ-paris5.fr

Short title: PPAR γ regulates PAPP-A in human trophoblast

Key words: rosiglitazone, syncytiotrophoblast, cytotrophoblast, differentiation,

This work was supported by “la Caisse d’Assurance Maladie des Professions Libérales-Provence”, CAMPLP, Paris la Défense, 92042, France.

ABSTRACT

Pregnancy-associated plasma protein-A (PAPP-A) is a metzincin metalloproteinase that cleaves the insulin-like growth factor (IGF)-dependent binding protein-4 and increases in maternal serum during pregnancy. In human placenta PAPP-A is expressed both in villous cytotrophoblasts (VCT) that cover the chorionic villi and in extravillous cytotrophoblasts (EVCT) of the anchoring villi. Due to the key role of PPAR γ in human trophoblast differentiation such as syncytiotrophoblast formation and EVCT invasion, we studied the effect of PPAR γ activation on PAPP-A expression using our *in vitro* model of EVCT and VCT primary cultures isolated from the same first trimester chorionic villi. First, we demonstrated that invasive EVCT expressed and secreted 10 times more PAPP-A than VCT did. Then, we showed that activation of PPAR γ inhibited PAPP-A gene expression and secretion in EVCT, whereas it had no effect in VCT. Since we have previously shown that PPAR γ agonist inhibits EVCT invasion *in vitro*, we suggest that PPAR γ -mediated inhibition of PAPP-A might decrease the amount of bioactive IGFII, a factor known to promote trophoblast invasion.

INTRODUCTION

The human placenta is characterized by extensive invasion of cytotrophoblasts into the uterus wall allowing direct contact of cytotrophoblasts with the maternal blood (hemomonochorial placentation) and by the extent and specificity of its hormonal production [1]. After the initial phase of nidation, human cytotrophoblasts differentiate along either the villous cytotrophoblast pathway leading to the formation of the syncytiotrophoblast that secretes the majority of placental hormones, or the extravillous cytotrophoblast pathway implicated in the anchoring of chorionic villi in the uterus (Fig. 1A)[2]. The specificity of human placenta has led to the development of *in vitro* models, such as explants [3-6] or primary cell cultures [7-9] allowing the study of human cytotrophoblast differentiation. We recently described a sequential enzymatic method permitting isolation, from the same first trimester chorionic villi, of extravillous and villous cytotrophoblasts that differentiate *in vitro* into invasive cytotrophoblasts or multinucleated syncytiotrophoblast by cell-cell fusion, respectively (10).

Pregnancy-associated plasma protein-A (PAPP-A) was first isolated in the 1970s from the serum of pregnant women [11, 12]. PAPP-A levels increase in the maternal circulation until the end of pregnancy [13, 14] and decline after delivery, suggesting a placental production [14, 15]. PAPP-A is secreted as a dimer of 400 kDa but circulates in maternal blood as a disulfide bound 500 kDa 2:2 complex with the proform of eosinophil major basic protein (pro-MBP). Recently, PAPP-A has been identified in different cell systems and in human pregnancy serum as a metzincin superfamily metalloproteinase, which cleaves the insulin-like growth factor (IGF)-dependent binding protein-4 [16-19]. PAPP-A is expressed in the placenta [20, 21] and more specifically in the villous and extravillous cytotrophoblasts [22, 23, 24, 25] and in the decidua [26]. *In vitro*, PAPP-A is secreted by human endometrial

cells, decidual cells and trophoblastic cells [25]. Its production is regulated by progesterone [27, 28].

Recent studies have pointed to a key regulatory role for the peroxisome proliferator-activated receptor- γ (PPAR γ) in human trophoblast differentiation and function [30-32]. PPAR γ is a member of the nuclear receptor superfamily that controls the expression of a large array of genes in a ligand-dependent manner. DNA binding of PPAR γ to its response element requires obligate heterodimerization with another nuclear receptor, the retinoid X receptor (RXR). We have shown *in situ* and *in vitro* that human villous and extravillous trophoblasts express both PPAR γ and RXR α [30, 31]. We next demonstrated that activation of PPAR γ by ligands from the thiazolidinedione family inhibits invasion of primary extravillous cytotrophoblasts isolated from first trimester placentas [30] and induces differentiation of villous cytotrophoblasts from term placentas and placental hormone gene expression as hCG, hPL and leptin [31].

Due to the key role of PPAR γ in trophoblast differentiation, the aim of this study was to investigate the role of PPAR γ in the regulation of PAPP-A gene expression and protein secretion in both villous and extravillous trophoblastic cells isolated from the same first trimester chorionic villi.

MATERIALS AND METHODS

Isolation and purification of villous (VCT) and extravillous (EVCT) cytotrophoblasts.

Placental tissues were obtained from the Department of Obstetrics and Gynecology at Broussais Hospital (Paris, France) during the first trimester (7-12 weeks of gestation) following legal voluntary interruption of pregnancy in patients who had signed informed consent forms. The tissue was washed in Ca^{2+} -, Mg^{2+} -free HBSS supplemented with 100 UI/mL penicillin and 100 $\mu\text{g}/\text{mL}$ streptomycin. Chorionic villi were dissected and rinsed for cell isolation. EVCT and VCT were isolated from the same first trimester chorionic villi by differential trypsin digestion according to Tarrade et al [10] with slight modifications.

For EVCT isolation, the tissue was incubated in HBSS (5 mL/g) containing 0.125% trypsin (Difco Laboratories, Detroit, Michigan), 4.2 mM MgSO_4 , 25 mM HEPES, 50 U/mL DNase type IV (Sigma, Saint-Quentin Fallavier, France) for 35 min at 37°C without agitation. After tissue sedimentation, the supernatant containing isolated EVCT was filtered (100 μm pores) and stored in the presence of 10% (v/v) fetal calf serum (FCS) to inhibit trypsin activity. The tissue was washed five to six times with warm HBSS and supernatant fractions pooled. Cells were centrifuged at 300 X g for 10 min, washed twice in HBSS supplemented with 10% FCS and filtered through a 50 μm pore nylon membrane. The cell suspension was carefully layered over a discontinuous Percoll gradient and centrifuged for 25 min at 1000 X g. The layer corresponding to 40-45% Percoll containing cytotrophoblasts was washed twice in HBSS supplemented with 10% FCS. Cells were diluted to a concentration of 2.5×10^5 cells/mL in DMEM supplemented with 10% FCS, 2 mM glutamine, 100 UI/mL penicillin and 100 $\mu\text{g}/\text{mL}$ streptomycin and plated on Matrigel-coated (5 mg/mL; Collaborative Biomedical Products, Le Pont de Claix, France) 35-mm Falcon culture dishes.

After 2 h of culture in 5% CO₂ at 37°C, cells were carefully washed three times to eliminate non-adherent cells. These purified primary EVCT were characterized using immunocytochemistry and real-time PCR and were shown to express *in vitro* the specific markers of human invasive EVCT *in situ* as described previously *i.e.* cytokeratin 7, human leukocyte antigen G, human placental lactogen, *c-erbB2* and $\alpha 5\beta 1$ [10].

VCT isolation was based on the methods of Kliman et al [33] and is a modified version of Tarrade et al [10]. After EVCT isolation, the same chorionic villi were incubated for 30 min at 37°C without agitation in 5 mL/g of tissue of the digestion buffer described above but containing only 0.0625% trypsin. The first 30 min trypsin digestion, containing a mix of EVCT and VCT, was discarded. The chorionic villi were then incubated in the same trypsin solution for 30 min and twice for 15 min at 37°C without agitation and finally washed with warm HBSS. Each time, the supernatant containing VCT was collected after tissue sedimentation, filtered (100 μ m pores), and incubated on ice with 10% FCS (v/v) to stop trypsin activity. After Percoll gradient fractionation, cells were diluted to a concentration of 1.25×10^6 cells/mL in DMEM supplemented with 10% FCS, 2 mM glutamine, 100 UI/mL penicillin and 100 μ g/mL streptomycin and plated on 35-mm or 60-mm TPP (Techno Plastic Products) culture dishes. VCT were incubated overnight in 5% CO₂ at 37°C and washed three times to eliminate non-adherent cells. Purified VCT cultures were characterized by the observation of cell aggregates and syncytiotrophoblast at 48 h and 72 h, respectively.

Cells were treated with 1 μ M of a PPAR γ agonist, rosiglitazone (BRL49653, Cayman Chemical, Ann Arbor, MI).

Immunocytochemistry

Cytokeratin 7 immunodetection

Cultured VCT and EVCT were fixed for 20 min in 4% paraformaldehyde. Saturation of unspecific sites was performed with 7% donkey serum diluted in PBS for 1 h at room temperature. Primary antibody (monoclonal mouse anti-CK7, 1/200, DAKO, France) diluted in PBS containing 1% BSA was added for 2 h at room temperature and amplified with the secondary antibody (FITC-conjugated donkey anti-mouse IgG1, 1/300, Jackson Immunoresearch Laboratories, West Grove, PA) diluted in PBS for 1 hour in dark at room temperature. Cells were mounted in a fluorescent Dapi mounting medium, examined and photographed on an Olympus BX60 epifluorescence microscope.

PAPP-A immunodetection

Cultured VCT were fixed for 8 min in methanol at -20°C . Saturation of unspecific sites was performed as described above. Primary antibody (APS13: monoclonal mouse anti-PAPP-A, 1/100, Cisbio international France) diluted in PBS containing 1% BSA was added for 2 h at room temperature and amplified as described above. Negative control was obtained by substituting the primary antibody by a mouse isotypic IgG1 (Jackson Immunoresearch Laboratories, West Grove, PA). Cells were mounted and examined as described above.

For cultured EVCT, human IgG (1:200, Jackson Immunoresearch Laboratories, West Grove, PA) were added to the donkey serum solution and saturation was performed overnight at 4°C . Primary and secondary antibodies were diluted in this saturation solution and immunostaining was performed as described above.

DNA quantification

Cell pellets were lysed in 5 M GuSCN, 0.1 M EDTA, pH 7 and stored at -80°C until use. DNA was quantified by fluorometry using the fluorochrome H \ddot{o} chst 33258 as described by Labarca and Paigen [34].

Quantification of specific transcripts by real-time RT-PCR

Total RNA was extracted from 48 h-cultured primary EVCT and 24, 48 and 72 h-cultured VCT, using TRIzol® Reagent. cDNA synthesis and PCR amplification were performed as described previously [35]. PAPP-A transcripts were amplified using an ABI Prism 7700 Sequence Detection System (Perkin-Elmer Applied Biosystems, Foster City, CA) and the Syber Green PCR Core Reagents kit (Perkin-Elmer Applied Biosystems). The PPIA gene coding the peptidyl prolyl isomerase A was used as the endogenous RNA control and each sample was normalized on the basis of its PPIA content. (PAPP-A primers: (+) CAGAATGCACTGTTACCTGGA, (-) GCTGATCCCAATTCTCTTTCA; PPIA primers: (+) GTCAACCCCACCGTGTTCTT, (-) CTGCTGTCTTTGGGACCTTGT)

PAPP-A assay

Each 24 hours, culture media were removed and stored, and fresh medium was added. PAPP-A concentrations in culture media of 24h-, 48h- and 72h-cultured VCT and EVCT were determined using TRACE (time-resolved amplified cryptate emission) technology on the KRYPTOR automated immunofluorescent analyzer (Brahms, Berlin, Germany) as described elsewhere [25]. The PAPP-A assay was performed with mouse monoclonal antibodies (APS 20 and APS 30) against the circulating complex PAPP-A/ProMBP [36]. This assay was standardized against World Health Organization (WHO) IRP 78/610 reference

material. The detection limit is 4 mU/L, and the within- and between-run coefficients of variation are 0.6% and 1.4%, respectively.

Statistical analysis

Values represent the mean \pm SEM of 3 to 7 separate cultures obtained from different placentas. The Mann and Whitney U test was used for comparison between groups and results were considered significant for $p < 0.05$.

RESULTS

Immunodetection of PAPP-A in villous and extravillous cytotrophoblast *in vitro*.

We investigated the *in vitro* expression of PAPP-A in culture of villous and extravillous cytotrophoblasts isolated from first trimester chorionic villi. Figure 2A shows immunodetection of PAPP-A in cultured-VCT. Mononucleated VCT isolated from early placentas adhere to plastic dish, aggregate and fuse *in vitro* within 72h to form a syncytiotrophoblast (Fig. 1B, upper panel), as described previously [33]. We detected PAPP-A *in vitro* in the cytoplasm of isolated, aggregated villous cytotrophoblasts (data not shown) and syncytiotrophoblast (Fig. 2A, left panel). Extravillous cytotrophoblasts need extracellular matrix-coated dishes for adhesion and culture. They display an invasive phenotype and emit pseudopodia after 48 h of culture on Matrigel (Fig. 1B, lower panel). They express specific markers and are able to invade Matrigel in Boyden chambers [10, 30]. PAPP-A was also detected in the cytoplasm of these cells (Fig. 2B, left panel).

Production of PAPP-A in human cytotrophoblasts *in vitro*

We next analysed the expression and the secretion of PAPP-A in VCT and EVCT cytotrophoblasts. As shown in figure 2A, PAPP-A levels in VCT culture medium increased significantly during the 72 h-culture period ($P < 0.05$ at 48 h vs 24 h and $P < 0.01$ at 72 h vs 24 h), even though the increase in mRNA levels was not significant. PAPP-A transcripts were largely expressed in 48 h-cultured EVCT while PAPP-A secretion increased in the same time as the cells acquire their invasive phenotype, with a 12-fold increase between 24 and 72 hours of culture ($P < 0.01$, Fig. 2B).

We then compared PAPP-A expression in VCT and EVCT. PAPP-A mRNA content was significantly higher in EVCT than VCT: 11 fold increase at 48 h (Fig. 2B, middle panel; $P<0.01$), and 13 and 5 fold increase at 24 h ($P<0.01$) and 72 h ($P<0.05$) respectively (data not shown). Concomitantly, PAPP-A levels in cell culture medium were higher in EVCT than in VCT (Fig.2B, right panel). At 48 h and 72 h of culture EVCT secreted 8 ($P<0.001$) and 9 ($P<0.01$) fold more PAPP-A than aggregated VCT and syncytiotrophoblast, respectively.

PPAR γ modulates PAPP-A expression and secretion in human cytotrophoblasts *in vitro*.

We previously showed that PPAR γ regulates VCT and EVCT functions, i.e. endocrine function and cell invasion, respectively. In the present work, we investigated the role of PPAR γ in the regulation of PAPP-A gene expression. VCT primary cells were incubated for 24 h, 48 h and 72h with 1 μ M of the PPAR γ activator rosiglitazone and EVCT only for 48 h, and PAPP-A transcript levels and protein secretions were analyzed (Fig. 3). In VCT, neither PAPP-A transcript levels nor PAPP-A secretion were significantly affected by PPAR γ agonist treatment irrespective of the time of culture (Fig. 3A). By contrast, after 48 h of culture, PAPP-A transcripts were significantly decreased by 70% in EVCT after PPAR γ activation (Figure 3B, left panel; $P<0.01$). Concomitantly, the PPAR γ agonist significantly decreased PAPP-A secretion by 35% in EVCT culture supernatant (Figure 3A, right panel; $P<0.05$).

DISCUSSION

In this study, using specific tools and *in vitro* models, we showed that PAPP-A was expressed and secreted by well-characterized trophoblastic cells populations [10, 37, 38] i.e. both villous and extravillous cytotrophoblasts isolated from first trimester human chorionic villi. Indeed, human placenta, but not mouse placenta [39], contains high levels of PAPP-A mRNA [21] as compared with other tissues. However, the precise sources of PAPP-A during pregnancy are controversial. Published data vary according to the sensitivity of the assay (e.g. *in situ* hybridization versus RT-PCR). The specificity of the antibodies used is also of importance because the antibodies may recognize either PAPP-A or ProMBP and, in some cases, other ligands of ProMBP such as C3 and haptoglobin [40, 21]. Monoclonal antibodies targeting either PAPP-A or proMBP have recently been developed [36, 41], but little *in vitro* data have been published to date. Using these specific tools, we recently showed that PAPP-A expression increased with *in vitro* differentiation of term villous cytotrophoblasts [25]. In this study, using the same tools, we showed that expression and secretion of PAPP-A were about ten times higher in extravillous cytotrophoblasts than in villous cytotrophoblasts isolated from the same first trimester placentas.

Considering the different functions of villous and extravillous cytotrophoblasts during pregnancy, our results suggest that placental PAPP-A from villous or extravillous origin might play different roles. Indeed, PAPP-A physiological functions are still under investigation. PAPP-A is a protease able to cleave the insulin-like growth factor (IGF)-dependent binding protein-4. PAPP-A cleavage of IGFBP-4 occurs in the presence of IGFs whereas the cleavage of IGFBP-5 is independent of IGFs [42]. This PAPP-A enzymatic activity is inhibited by proMBP [18]. Produced by the syncytiotrophoblast, PAPP-A is therefore secreted directly into the maternal blood due to the position (bathing in maternal

blood after 12 weeks of pregnancy) and the major endocrine functions of the syncytiotrophoblast. Thus, PAPP-A might be involved in the decrease of IGFBP-4 levels in maternal serum during human pregnancy [43, 44]. The high levels of PAPP-A secretion and expression by extravillous cytotrophoblasts suggest a possible important role for this protease at the implantation site by controlling the trophoblast invasion process through a paracrine or autocrine pathway. Indeed as suggested, PAPP-A might be an important regulator of local IGF bioavailability and cell growth [29]. IGFBP-4, highly expressed in placental bed [45], is a modulator of IGF activity [46] as proteolysis of IGFBP-4 enhances IGF bioactivity [19]. IGFBP-4 mRNA were reported to be expressed by the extravillous trophoblasts *in situ* [45] and was shown to stimulate trophoblast invasion *in vitro* [47].

This study pointed out for the first time the role of PPAR γ in the modulation of PAPP-A expression. Modulation of the PAPP-A gene expression is not well characterized. PAPP-A expression is stimulated in ovaries by hCG [48] and its placental production is regulated by progesterone [27-29]. In human cytotrophoblasts, we observed a different effect of PPAR γ stimulation depending upon villous or extravillous origin. In villous trophoblastic cells, PPAR γ stimulation had no effect on PAPP-A mRNA levels and protein secretion. By contrast, the PPAR γ agonist significantly inhibits PAPP-A gene expression and secretion by extravillous trophoblastic cells. Inhibition of PAPP-A expression and secretion by PPAR γ ligands might play a role in IGF bioactivity, particularly by decreasing the amount of bioactive IGFBP-4, which has been described to promote trophoblast invasion. We previously showed that PPAR γ /RXR α heterodimers control the invasive properties of human EVCT. We suggest that PPAR γ -mediated inhibition of trophoblast invasion involves the PAPP-A/IGF II cascade.

In conclusion this study shows that, depending upon the cell type, i.e., villous or extravillous cytotrophoblasts, activation of PPAR γ might have different effects on the

expression of the same gene (PAPP-A), and points to the complex mechanisms involved in human trophoblast differentiation and functions. Our results underscore a new mechanism by which PPAR γ might control human trophoblastic invasion.

Acknowledgement

We thank R. Lecomte (Brahms, France) and Gersende Flament (Robert Debré hospital biochemistry department) for their technical assistance. We are also grateful to D. Pons (Cis Bio international, France) for providing us the monoclonal antibodies against PAPP-A.

REFERENCES

- [1] **Malassine A, Frendo JL, Evain-Brion D** A comparison of placental development and endocrine functions between the human and mouse model. *Hum Reprod Update* 2003 ; 9:531-9.
- [2] **LokeYW and King A** Human Implantation. *Cell Biology and Immunology*, Cambridge ed. 1995
- [3] **Caniggia I, Taylor CV, Ritchie JW, Lye SJ, Letarte M** Endoglin regulates trophoblast differentiation along the invasive pathway in human placental villous explants. *Endocrinology* 1997 ; 138:4977-88.
- [4] **Haigh T, Chen C, Jones CJ, Aplin JD** Studies of mesenchymal cells from 1st trimester human placenta: expression of cytokeratin outside the trophoblast lineage. *Placenta* ; 20:615-25.
- [5] **Lala PK, Khoo NKS, Guimond MJ and Chakraborty C** Control mechanisms in human trophoblast proliferation and invasiveness : their derangement during trophoblastic tumor progression. *Trophoblast Res.* 1999 ; 13:119-136.
- [6] **Aplin JD, Haigh T, Jones CJ, Church HJ, Vicovac L** Development of cytotrophoblast columns from explanted first-trimester human placental villi: role of fibronectin and integrin alpha5beta1. *Biol Reprod.* 1999 ; 60:828-38.
- [7] **Genbacev O, Zhou Y, Ludlow JW, Fisher SJ** Regulation of human placental development by oxygen tension. *Science* 1997 ; 277:1669-72.
- [8] **Librach CL, Werb Z, Fitzgerald ML, Chiu K, Corwin NM, Esteves RA, Grobelyny D, Galardy R, Damsky CH, Fisher SJ** 92-kD type IV collagenase mediates invasion of human cytotrophoblasts. *J Cell Biol.* 1991; 113:437-49.

- [9] **Rong-Hao L, Luo S, Zhuang LZ** Establishment and characterization of a cytotrophoblast cell line from normal placenta of human origin. *Hum Reprod* 1996 ; 11:1328-33.
- [10] **Tarrade A, Lai Kuen R, Malassine A, Tricottet V, Blain P, Vidaud M, Evain-Brion D** Characterization of human villous and extravillous trophoblasts isolated from first trimester placenta. *Lab Invest* 2001; 81:1199-211
- [11] **Lin TM, Galbert SP, Kiefer D, Spellacy WN, Gall S** Characterization of four human pregnancy-associated plasma proteins. *Am J Obstet Gynecol.* 1974 ; 118:223-36.
- [12] **Bischof P** Purification and characterization of pregnancy associated plasma protein A (PAPP-A). *Arch Gynecol.* 1979 ; 227:315-26.
- [13] **Lin TM, Halbert SP, Spellacy WN** Measurement of pregnancy-associated plasma proteins during human gestation. *J Clin Invest.* 1974 ; 54:576-82.
- [14] **Bischof P, Duberg S, Herrmann W, Sizonenko PC** Amniotic fluid and plasma concentrations of pregnancy-associated plasma protein-A (PAPP-A) throughout pregnancy: comparison with other fetoplacental products. *Br J Obstet Gynaecol.* 1982 ; 89:358-63.
- [15] **Folkersen J, Grudzinskas JG, Hindersson P, Teisner B, Westergaard JG** Pregnancy-associated plasma protein A: circulating levels during normal pregnancy. *Am J Obstet Gynecol.* 1981 ; 139:910-4.
- [16] **Lawrence JB, Oxvig C, Overgaard MT, Sottrup-Jensen L, Gleich GJ, Hays LG, Yates JR 3rd, Conover CA** The insulin-like growth factor (IGF)-dependent IGF binding protein-4 protease secreted by human fibroblasts is pregnancy-associated plasma protein-A. *Proc Natl Acad Sci U S A.* 1999 ; 96:3149-53.
- [17] **Conover CA, Oxvig C, Overgaard MT, Christiansen M, Giudice LC** Evidence that the insulin-like growth factor binding protein-4 protease in human ovarian follicular fluid is pregnancy associated plasma protein-A. *J Clin Endocrinol Metab.* 1999 ; 84:4742-5.

- [18] **Overgaard MT, Haaning J, Boldt HB, Olsen IM, Laursen LS, Christiansen M, Gleich GJ, Sottrup-Jensen L, Conover CA, Oxvig C** Expression of recombinant human pregnancy-associated plasma protein-A and identification of the proform of eosinophil major basic protein as its physiological inhibitor. *J Biol Chem.* 2000 ; 275:31128-33.
- [19] **Byun D, Mohan S, Yoo M, Sexton C, Baylink DJ, Qin X** Pregnancy-associated plasma protein-A accounts for the insulin-like growth factor (IGF)-binding protein-4 (IGFBP-4) proteolytic activity in human pregnancy serum and enhances the mitogenic activity of IGF by degrading IGFBP-4 in vitro. *J Clin Endocrinol Metab.* 2001 ; 86:847-54.
- [20] **Schindler AM, Bordignon P, Bischof P** Immunohistochemical localization of pregnancy-associated plasma protein A in decidua and trophoblast: comparison with human chorionic gonadotrophin and fibrin. *Placenta.* 1984 ;5:227-35.
- [21] **Overgaard MT, Oxvig C, Christiansen M, Lawrence JB, Conover CA, Gleich GJ, Sottrup-Jensen L, Haaning J** Messenger ribonucleic acid levels of pregnancy-associated plasma protein-A and the proform of eosinophil major basic protein: expression in human reproductive and nonreproductive tissues. *Biol Reprod.* 1999 ; 61:1083-9.
- [22] **Tornehave D, Chemnitz J, Teisner B, Folkersen J, Westergaard JG** Immunohistochemical demonstration of pregnancy-associated plasma protein A (PAPP-A) in the syncytiotrophoblast of the normal placenta at different gestational ages. *Placenta.* 1984 ; 5:427-31.
- [23] **Bonno M, Oxvig C, Kephart GM, Wagner JM, Kristensen T, Sottrup-Jensen L, Gleich GJ** Localization of pregnancy-associated plasma protein-A and colocalization of pregnancy-associated plasma protein-A messenger ribonucleic acid and eosinophil granule major basic protein messenger ribonucleic acid in placenta. *Lab Invest.* 1994 ; 71:560-6.

- [24] **Sun IY, Overgaard MT, Oxvig C, Giudice LC** Pregnancy-associated plasma protein A proteolytic activity is associated with the human placental trophoblast cell membrane. *J Clin Endocrinol Metab.* 2002 ; 87:5235-40.
- [25] **Guibourdenche J, Frendo JL, Pidoux G, Bertin G, Luton D, Muller F, Porquet D, Evain-Brion D** Expression of pregnancy-associated plasma protein-A (PAPP-A) during human villous trophoblast differentiation in vitro. *Placenta* 2003 ; 24:532-9.
- [26] **Bischof P** Three pregnancy proteins (PP12, PP14, and PAPP-A): their biological and clinical relevance. *Am J Perinatol.* 1989; 6:110-6.
- [27] **Bischof P, Sizonenko MT, Herrmann WL** Trophoblastic and decidual response to RU486: effects on human chorionic gonadotrophin, human placental lactogen, prolactin and pregnancy-associated plasma protein-A production in vitro. *Hum Reprod.* 1986 ;1:3-6.
- [28] **Barnea ER, Sanyal MK, Brami C, Bischof P** In vitro production of pregnancy-associated plasma protein-A (PAPP-A) by trophoblastic cells. *Arch Gynecol.* 1986 ; 237:187-90.
- [29] **Giudice LC, Conover CA, Bale L, Faessen GH, Ilg K, Sun I, Imani B, Suen LF, Irwin JC, Christiansen M, Overgaard MT, Oxvig C** Identification and regulation of the IGFBP-4 protease and its physiological inhibitor in human trophoblasts and endometrial stroma: evidence for paracrine regulation of IGF-II bioavailability in the placental bed during human implantation. *J Clin Endocrinol Metab.* 2002 ; 87:2359-66.
- [30] **Tarrade A, Schoonjans K, Pavan L, Auwerx J, Rochette-Egly C, Evain-Brion D, Fournier T** PPARgamma/RXRalpha heterodimers control human trophoblast invasion. *J Clin Endocrinol Metab* 2001 ; 86:5017-24
- [31] **Tarrade A, Schoonjans K, Guibourdenche J, Bidart JM, Vidaud M, Auwerx J, Rochette-Egly C, Evain-Brion D** PPAR gamma/RXR alpha heterodimers are involved in

human CG beta synthesis and human trophoblast differentiation. *Endocrinology* 2001 ; 142:4504-14.

[32] **Schaiff WT, Carlson MG, Smith SD, Levy R, Nelson DM, Sadovsky Y** Peroxisome proliferator-activated receptor-gamma modulates differentiation of human trophoblast in a ligand-specific manner. *J Clin Endocrinol Metab.* 2000 ;85: 3874-81

[33] **Kliman HJ, Nestler JE, Sermasi E, Sanger JM, Strauss JF 3rd** Purification, characterization, and in vitro differentiation of cytotrophoblasts from human term placentae. *Endocrinology* 1986 ; 118:1567-82

[34] **Labarca C, Paigen K** A simple, rapid, and sensitive DNA assay procedure. *Anal Biochem* 1980 ; 102:344-52

[35] **Pavan L, Tarrade A, Hermouet A, Delouis C, Titeux M, Vidaud M, Therond P, Evain-Brion D, Fournier T** Human invasive trophoblasts transformed with simian virus 40 provide a new tool to study the role of PPARgamma in cell invasion process. *Carcinogenesis* 2003 ; 24:1325-36

[36] **Bersinger NA, Meisser A, Bessou T, Seguin P, Birkhauser MH, Bischof P** Production and characterization of monoclonal antibodies against pregnancy-associated plasma protein A. *Mol Hum Reprod.* 1999 ; 5:675-81.

[37] **Tarrade A, Goffin F, Munaut C, Lai-Kuen R, Tricottet V, Foidart JM, Vidaud M, Frankenne F, Evain-Brion D.** Effect of matrigel on human extravillous trophoblasts differentiation: modulation of protease pattern gene expression. *Biol Reprod.* 2002 ; 67:1628-37.

[38] **Malassiné A, Handschuh K, Tsatsaris V, Gerbaud P, Cheynet V, Oriol G, Mallet F and Evain-Brion D** Expression of HERV-W Env glycoprotein (syncytin) in the extravillous trophoblast of first trimester human placenta. *Placenta*, 2005 ; 25 :556-562.

- [39] **Qin X, Sexton C, Byun D, Strong DD, Baylink DJ, Mohan S** Differential regulation of pregnancy associated plasma protein (PAPP)-A during pregnancy in human and mouse. *Growth Horm IGF Res.* 2002 ; 12:359-66.
- [40] **Schindler AM, Bischof P** Histochemical localization of pregnancy-associated plasma protein A in fetal, infant, and adult organs and comparison between antisera. *Gynecol Obstet Invest.* 1984 ; 18:88-94.
- [41] **Qin QP, Christiansen M, Oxvig C, Pettersson K, Sottrup-Jensen L, Koch C, Norgaard-Pedersen B** Double-monoclonal immunofluorometric assays for pregnancy-associated plasma protein A/proeosinophil major basic protein (PAPP-A/proMBP) complex in first-trimester maternal serum screening for Down syndrome. *Clin Chem.* 1997 ; 43:2323-32.
- [42] **Laursen LS, Overgaard MT, Soe R, Boldt HB, Sottrup-Jensen L, Giudice LC, Conover CA, Oxvig C** Pregnancy-associated plasma protein-A (PAPP-A) cleaves insulin-like growth factor binding protein (IGFBP)-5 independent of IGF: implications for the mechanism of IGFBP-4 proteolysis by PAPP-A. *FEBS Lett.* 2001 ; 504:36-40.
- [43] **Hossenlopp P, Segovia B, Lassarre C, Roghani M, Bredon M, Binoux M** Evidence of enzymatic degradation of insulin-like growth factor-binding proteins in the 150K complex during pregnancy. *J Clin Endocrinol Metab.* 1990 ; 71:797-805.
- [44] **Giudice LC, Farrell EM, Pham H, Lamson G, Rosenfeld RG** Insulin-like growth factor binding proteins in maternal serum throughout gestation and in the puerperium: effects of a pregnancy-associated serum protease activity. *J Clin Endocrinol Metab.* 1990 ; 71:806-16.
- [45] **Han VK, Bassett N, Walton J, Challis JR** The expression of insulin-like growth factor (IGF) and IGF-binding protein (IGFBP) genes in the human placenta and membranes:

evidence for IGF-IGFBP interactions at the feto-maternal interface. *J Clin Endocrinol Metab.* 1996 ;81:2680-93.

[46] **Jones JI, Clemmons DR** Insulin-like growth factors and their binding proteins: biological actions. *Endocr Rev.* 1995 ; 16:3-34.

[47] **McKinnon T, Chakraborty C, Gleeson LM, Chidiac P, Lala PK** Stimulation of human extravillous trophoblast migration by IGF-II is mediated by IGF type 2 receptor involving inhibitory G protein(s) and phosphorylation of MAPK. *J Clin Endocrinol Metab.* 2001 ; 86:3665-74.

[48] **Hourvitz A, Kuwahara A, Hennebold JD, Tavares AB, Negishi H, Lee TH, Erickson GF, Adashi EY** The regulated expression of the pregnancy-associated plasma protein-A in the rodent ovary: a proposed role in the development of dominant follicles and of corpora lutea. *Endocrinology* 2002 ; 143:1833-44.

FIGURE LEGENDS

Fig. 1: *in vitro* models of human villous and extravillous cytotrophoblasts.

A, Structure of human implantation site. Sc : stromal core; vct : villous cytotrophoblast; st : syncytiotrophoblast; fv : floating villi; is : intervillous space; av : anchoring villi; evct : extravillous cytotrophoblast; p : proliferative evct; i : invasive evct; e : endovascular evct; gc : giant cells; d : decidual cells; usa : uterine spiral artery. **B**, Immunodetection of cytokeratin 7 in human villous and extravillous cytotrophoblast primary cultures. Upper panels – Villous cytotrophoblasts. Lower panel – Extravillous cytotrophoblasts on Matrigel. Scale bar: 20 μ m.

Fig. 2: PAPP-A expression in human cultured VCT and EVCT.

A, PAPP-A expression in villous cytotrophoblasts. Immunocytochemistry at 72 h (left panel : PAPP-A detection and isotype-matched control), Scale bar: 20 μ m; transcript levels (middle panel) and secretion of PAPP-A (right panel) in VCT. **B**, Comparative PAPP-A expression in VCT and EVCT. Immunocytochemistry in 48 h-cultured EVCT (left panel : PAPP-A detection and isotype-matched control), transcript levels at 48 h (middle panel) and secretion of PAPP-A (right panel) in VCT and EVCT.

Transcripts coding for PAPP-A were quantified by RT PCR. Values were normalized to PPIA transcript levels. Values represent the mean \pm SEM of 3 to 5 independent cultures obtained from different placentas. Supernatants from 24, 48 and 72 h-cultured VCT and EVCT were collected and PAPP-A measured as described in Materials and Methods. Protein secretions were expressed as UI/L and normalized to DNA content for comparison between VCT and EVCT. Values represent the mean \pm SEM of three independent cultures obtained from three different placentas. Mann and Whitney U test was used for statistical analysis (* $P < 0.05$ and **, \$\$ $P < 0.01$).

Fig. 3: Effect of PPAR γ agonist on PAPP-A expression in VCT and EVCT.

PAPP-A transcripts and secreted proteins were measured in VCT (**A**) and in EVCT (**B**) treated or not with 1 μ M of the PPAR γ agonist rosiglitazone. Values were expressed relative to untreated cells and represent the mean \pm SEM. For transcript determination, five independent cultures from five different placentas were run in duplicate. For protein determination, six independent cultures from six different placentas were run in duplicate.

Treated vs control: * $P < 0.05$; ** $P < 0.01$.