Expression of Progranulin in Early and Late Gestation Human Placentas

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ABSTRACT

Development of placenta is a complex process that is critical for the pregnancy and controlled by many factors including cytokines, hormones, growth factors and apoptotic molecules. Recently, it has been shown that progranulin (PGRN) functions in growth of embryo and trophectoderm as well as cell migration. To initiate understanding the role of PGRN in human placental development, we investigated the expression of PGRN mRNA and protein in early and late gestation human placentas, term cytotrophoblast cells and two choriocarcinoma cell lines, JEG-3 and Jar. Reverse transcriptase polymerase chain reaction identified mRNAs derived from the PGRN gene in all samples. Immunoblot analysis showed that PGRN proteins are present in early and late gestation human placentas with decreasing levels over gestation and that PGRN proteins are present in normal and transformed trophoblast cells. Immunohistochemical analysis using paraformaldehyde-fixed tissue sections taken from early and late stages of pregnancy showed that PGRN proteins are present in cytotrophoblast cells, syncytiotrophoblast and extravillous cytotrophoblast cells and that expression pattern of PGRN differed according to the stage of cell differentiation. The results of this study are consistent with the hypothesis that PGRN proteins have critical roles in placental development and suggest that PGRN may function in trophoblast cell growth and differentiation.

(Key words : Progranulin, Human, Placenta, Development, Trophoblast)

INTRODUCTION

The placenta is a temporary organ that is formed during pregnancy and unique to eutherian mammals. It is responsible for a metabolic exchange between the mother and the conceptus/the fetus and for hormone production. Placentation, formation of the placenta, is a complex process that requires the coordinated interactions between the maternal uterus and the conceptus. Abnormal placental development causes many pregnancy-related diseases, including preeclampsia, abortion and fetal growth restriction, and even fetal loss (Benirschke and Kaufmann, 2000). It has been demonstrated that growth factors, cytokines and hormones among many factors play important roles in placental development. Among those factors, it has been suggested that progranulin (PGRN; also called proepithelin, granulin/epithelin precursor, PC cell-derived growth factor, or acrogranin) may participate in placental development (Daniel *et al.*, 2000; Qin *et al.*, 2005).

PGRN is an 88 kDa growth factor, which functions in epithelial cell growth, cell migration, tumorigenecity, wound healing, and inhibition of neutrophil activation (He and Bateman, 1999; He *et al.*, 2003). Furthermore, it has been shown that PGRN activates growth of embryo and trophectoderm and involved in implantation (Diaz-Cueto *et al.*, 2000; Qin *et al.*, 2005). PGRN expression is observed in many epithelial cells, hematopoietic cells and fibroblasts as well as trophectoderm and uterine epithelia in mice (Daniel *et al.*, 2000; Diaz-Cueto *et al.*, 2000). Although it has been shown that PGRN expression is regulated by steroid hormones, estrogen and progestereone (Lu and Serrero, 1999; 2001), not much is known on the regulation of the PGRN gene expression. Analysis of the promoter region of the PGRN gene which contains numerous transcription factor binding sites, such as Sp1, AP2, NF-IL6, PPAR and C/EBP (Bhandari *et al.*, 1993; Bhandari *et al.*, 1996; Bateman and Bennett, 1998) suggests that various factors may affect expression of the PGRN gene.

To initiate studies on the role of PGRN during placental development, this study determined cell type-specific and temporal expression of PGRN mRNA and protein in early and late gestation placentas, term cytotrophoblast cells and two choriocarcinoma cell lines, JEG-3 and Jar.

MATERIALS AND METHODS

Tissue Samples and Cell Lines

Placentas from first trimester (n=6, ranging from 6 to

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12 weeks of gestation) were obtained from patients undergoing elective pregnancy termination, and term placentas (n=6) were obtained from patients at term who underwent cesarean section to alleviate fetal distress. Tissues were dissected manually and samples were taken randomly from villous placenta and reflected amniochorion. Samples were either frozen in liquid nitrogen and stored at −80℃ or fixed in 4% paraformaldehyde in phosphate buffered saline (PBS; pH 7.2) for 24 hr at 4℃ and embedded in paraffin.

Trophoblast-derived choriocarcinoma cell lines, JEG-3 (HTB-36) and Jar (HTB-144) cells, were purchased from the American Type Culture Collection (ATCC, Manassas, VA). The cell lines were grown in tissue culture flasks at 37℃ in RPMI 1640 (Sigma, St. Louis, MO) supplemented with 10% fetal bovine serum (Atlanta Biologicals, Norcross, GA) and antibiotics (Sigma).

Cytotrophoblast Cell Purification

Cytotrophoblast cells were isolated from term placenta (n=3) as described previously (Kliman *et al.*, 1986). Briefly, approximately 30 g of villous tissue was dissected from placenta, washed with sterile 0.9% (w/v) saline to remove excess blood, thoroughly minced, and repeatedly washed with sterile saline. Tissue was then digested three times at 37℃ in a shaking water bath in Hanks Balanced Salt Solution (Sigma) containing 0.25% trypsin (Sigma), 300 U/ml DNase I (Sigma) and 25 mM HEPES (pH 7.4; Sigma). Cells were centrifuged from digestion supernatants, washed with culture medium (Dulbecco Modified Eagle Medium, DMEM:Waymouth 1:1) and filtered through 100 μm nylon mesh (Becton Dickinson, Franklin Lakes, NJ). Cells were then separated by Percoll (Sigma) gradient centrifugation. Cytotrophoblast cells were further purified by removal of HLA-A, B, C-positive cells using the mouse monoclonal antibody to HLA class I antigens, W6/32 (HB-95, ATCC), and goat anti-mouse immunoglobulin-conjugated magnetic microbeads (Miltenyi Biotec Inc., Auburn, CA) according to the manufacturer's protocol. The purity was assessed by centrifuging the cells onto glass slides using a Shandon Cytospin (Pittsburgh, PA), and analyzing the cells by immunohistochemical staining reactions using mouse anti-cytokeratin 7 (DAKO, Carpinteria, CA) and anti-CD14 (Zymed, San Francisco, CA) which identify trophoblast cells and macrophages, respectively, as previously described (Ka and Hunt, 2003). We further qualified the purity by immunostaining using mouse anti-βhCG (Neomarker, Fremont, CA) to detect any contaminating syncytial fragments. Average purity was 99% cytotrophoblast cells following the magnetic bead purification procedure.

Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) Analysis

Expression of PGRN mRNA in placentas, cytotrophoblast cells and choriocarcinoma cell lines, JEG-3 and Jar, was examined by RT-PCR. Total cellular RNA was extracted using TRIzol reagent (Life Technologies, Gaithersburg, MD). Two μg of total RNA were treated with DNase I (Sigma) and reverse transcribed using Moloney murine leukemia virus reverse transcriptase (Life Technologies) to obtain cDNAs. The cDNA templates were then diluted 1:5 with sterile water and amplified by PCR using *Taq* DNA polymerase (Life Technologies) and specific primers based on human PGRN (GenBank accession # AY124489, forward, 5′-GCC ACT CCT GCA TCT TTA CC-3′; reverse, 5′-CTG CCC TGT TAG TCC TCT GG-3′), and human β-actin (GenBank accession # X00351, forward, 5′-CAC CCC GTG CTG CTG ACC GAG GCC-3′; reverse, 5′-CCA CAC GGA GTA CTT GCG CTC AGG-3′). PCR conditions were 30 cycles of 94℃ for 45 sec, 57℃ for 45 sec and 72℃ for 2 min for PGRN, and 25 cycles of 94℃ for 45 sec, 60℃ for 30 sec and 72℃ for 2 min for β-actin. Expected sizes of PCR products for PGRN and β-actin were 390 bp and 719 bp, respectively. PCR products were separated on 2% agarose gels and visualized by ethidium bromide staining. The identity of each amplified PCR product was verified by sequence analysis after cloning into the pCRII vector (In Vitrogen, San Diego, CA).

Protein Isolation and Immunoblot Analysis

PGRN proteins in placentas, cytotrophoblast cells and choriocarcinoma cell lines, JEG-3 and Jar, were analyzed by immunoblotting. To obtain cellular protein, cytotrophoblast cells, JEG-3 and Jar cells were lysed by incubating in lysis buffer (1% Triton X-100, 0.5% Nonidet P-40, 150 mM NaCl, 10 mM Tris, 1 mM EDTA, 1 mM EGTA, 0.2 mM Na_3VO_4, 0.2 mM PMSF, 50 mM NaF, 30 mM $Na_4P_2O_7$, 1 μg/ml leupeptin, and 1 μg/ml pepstatin) for 30 min at 4℃. Cell lysates were passed through a 26-gauge needle and then clarified by centrifugation (16,000 ×g, 15 min, 4℃). Samples of villous placenta were thawed and immediately homogenized in lysis buffer at a ratio of 1 g tissue per 5 ml buffer, and cellular debris was removed by centrifugation (16,000 ×g, 15 min, 4℃). The concentrations of protein in cell lysates and placental tissue extracts were determined using a Bradford protein assay (Bio-Rad Laboratories, Richmond, CA) with bovine serum albumin used as the standard. Proteins in cell lysates (30 μg/lane) or placental tissue extracts (40 μg/lane) were denatured in SDS-PAGE buffer, separated on 12% SDS-PAGE gels, and transferred to nitrocellulose membrane. Blot was blocked for 1 hr at room temperature with 5% (w/v) nonfat milk-TBST (Tris-buffered saline with 0.1% Tween-20). Duplicate blots were prepared.

One blot was incubated overnight at 4℃ with goat polyclonal anti-PGRN antibody (0.5 μg/ml, Santa Cruz Biotechnology, Santa Cruz, CA) diluted in 2% milk-TBST, the other one was incubated with normal goat IgG (0.5 μg/ml, Santa Cruz Biotechnology). Blot was rinsed for 30 min at room temperature with TBST, incubated with the peroxidase-conjugated rabbit anti-goat (Jackson ImmunoResearch Laboratories, West Grove, PA) secondary antibody for 1 hr at room temperature, and rinsed again for 30 min at room temperature with TBST. Immunoreactive proteins were detected by chemiluminescence (SuperSignal West Pico, Pierce Chemical Co., Rockford, IL) according to the manufacturer's recommendations using Hyperfilm ECL (Amersham Biosciences, Piscataway, NJ). Blot was re-blotted with rabbit polyclonal anti-actin antibody (1: 5,000, Sigma) to assess consistent loading. The optical density of PGRN and actin bands in the immunoblot was quantified by scanning densitometry using EPSON1680 (EPSON, Long Beach, CA) and GelPro Analyzer (Media Cybernetics, Silver Spring, MD). Values are presented as the ratio of PGRN integrated optical density to actin integrated optical density.

Immunohistochemical Analysis

To determine which types of cells in human placentas express PGRN protein, immunohistochemistry was applied. Sections (5 μm) were deparaffinized and rehydrated in an ethanol gradient. Tissue sections were boiled for 10 min in 10 mM citrate buffer (pH 6.0) for antigen retrieval. Tissue sections were washed with PBST (PBS with 0.3% Tween-20) three times and the peroxidase block was performed with 0.5% H_2O_2 in methanol for 30 min. Tissue sections were then blocked with 10% normal goat serum for 30 min at room temperature. Goat anti-PGRN (2 μg/ml, Neomarkers, Inc., Fremont, CA) was added and incubated overnight at 4℃ in a humidified chamber. For each tissue tested, purified normal goat IgG was substituted for primary antibody and served as a negative control. Tissue sections were washed with PBST three times and the peroxidase block was performed with 0.5% H_2O_2 in methanol for 30 min. The biotinylated rabbit anti-goat secondary antibody (Vector Laboratories, Burlingame, CA) was added and incubated for 1 hr at room temperature. Following washes with PBST, the streptavidin peroxidase conjugate (Zymed, San Francisco, CA) was added and the tissue sections were incubated for 10 min at room temperature. The sections were washed with PBST and the 3-amino-9-ethylcarbozole in N, N-dimethylformamide (AEC) color development substrate (Zymed) was added to the tissue sections, which were then incubated for 10 min at room temperature. The tissue sections were washed in water, counterstained with Mayer's hematoxylin, and coverslipped.

Statistical Analysis

Densitometry data from immunoblotting were subjected to Student's *t*-test to determine the difference of PGRN protein levels between first trimester and term placentas. Results were considered to be significant where $P<0.05$. Data are presented as means with standard error.

RESULTS

Identification of Messages Encoding PGRN in Human Placentas by RT-PCR

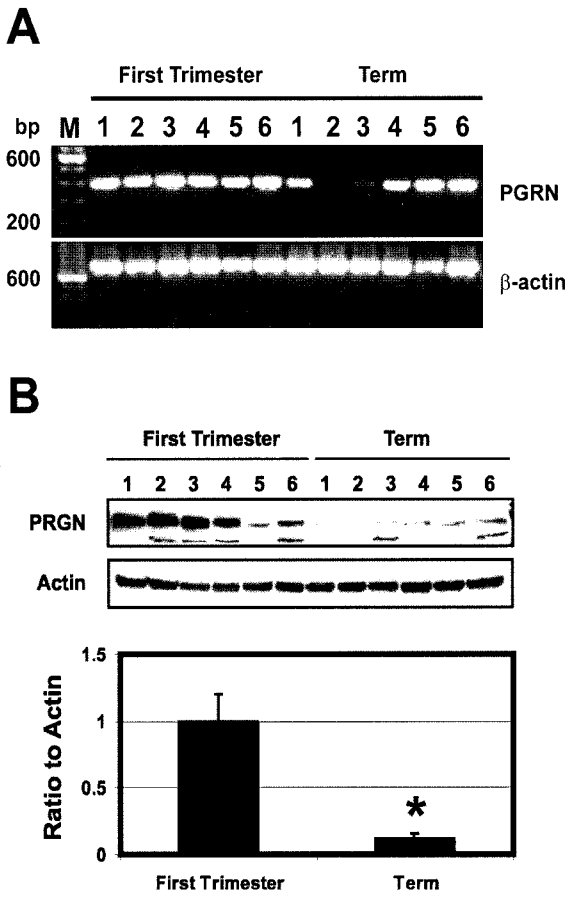

Fig. 1. RT-PCR (A) and immunoblot (B) analyses of PGRN expression in human placentas. (A) Placental tissue samples from first trimester (TM) (n=6) and term placentas (n=6) were tested. Beta-actin was used as a loading control. Molecular weight markers (M) are noted to indicate base pairs (bp) of PCR products. Sequencing experiments demonstrated authenticity of PCR products. (B) Placental tissue samples from first trimester (TM) (n=6) and term placentas (n=6) were tested. Molecular weight (kDa) of each protein is indicated on the left. Actin was used as a loading control. The ratio of each PGRN protein density to actin density was obtained by scanning densitometry. *Significantly low ($P<0.05$).

The first series of experiments determined whether PGRN mRNAs were detectable in human placentas and established expression in early and late gestation. As shown in Fig. 1A, analysis by RT-PCR demonstrated that the message for PGRN was detectable in early and late gestation of human placentas. Controls in which reverse transcriptase was omitted were negative for all sets of primers (data not shown), and each amplified PCR product was sequenced and identified as authentic.

Identification of PGRN Protein in Human Placentas by Immunoblot Analysis

Having determined that the specific message for PGRN was present in human placentas at both early and late stages of pregnancy, next sets of experiments were done to establish translation using a commercially available antibody that identify proteins in immunoblots. To assess consistency of loading, the blot was reblotted with an antibody against actin. Isotype-matched nonspecific IgG control blot was analyzed, and there was no detectable signal (data not shown). As shown in Fig. 1B, PGRN protein was detectable in all placental samples. In the densitometry analysis, signal intensities for PGRN protein were significantly ($P<0.05$) higher in first trimester than in term placentas.

Localization of PGRN Protein in Human Placentas by Immunohistochemistry

To determine which types of cells in human placentas express PGRN protein, immunohistochemical studies were done on early and term placentas to localize PGRN protein. Immunoreactivity was not detectable in control tissue sections where primary antibody was substituted with nonspecific goat IgG.

As shown in Fig. 2, immunoreactive PGRN protein was present in the cytoplasm of villous cytotrophoblast cells, syncytiotrophoblast and villous mesenchymal cells in both first trimester and term placentas. Immunostaining signals of PGRN protein in first trimester placentas were more intense in syncytiotrophoblast layer than in villous cytotrophoblast cells. In term placentas, immunostaining pattern was patchy in syncytiotrophoblast layer.

Localization of PGRN Protein in Extravillous Cytotrophoblast Cells in First Trimester Placentas and in the Term Amniochorion Membrane by Immunohistochemistry

Fig. 2 demonstrated that PGRN protein could be found in villous cytotrophoblast cells. Further analysis was done to determine whether these proteins showed any new patterns as the cells formed the columns that generate the invasive phenotype of early gestation or

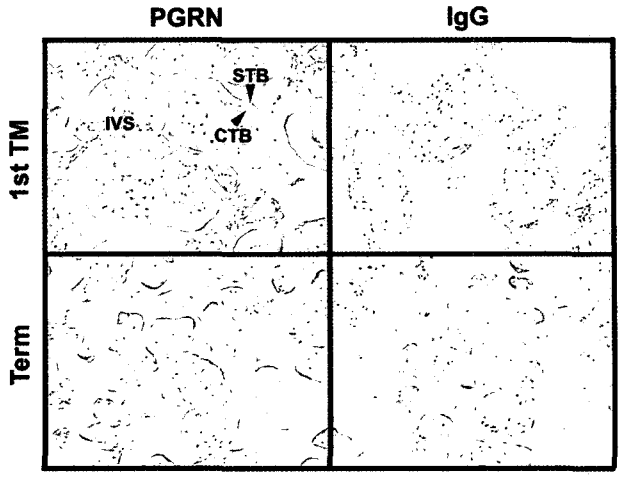

Fig. 2. Photomicrographs illustrating representative localization of PGRN protein by immunohistochemistry in human placentas. Specific immunostaining signals were not detected when the primary antibody were substituted with normal goat IgG. vCTB, villous cytotrophoblast cells; STB, syncytiotrophoblast layer; M, villous mesenchyme; IVS, intervillous space. Original magnification, ×400.

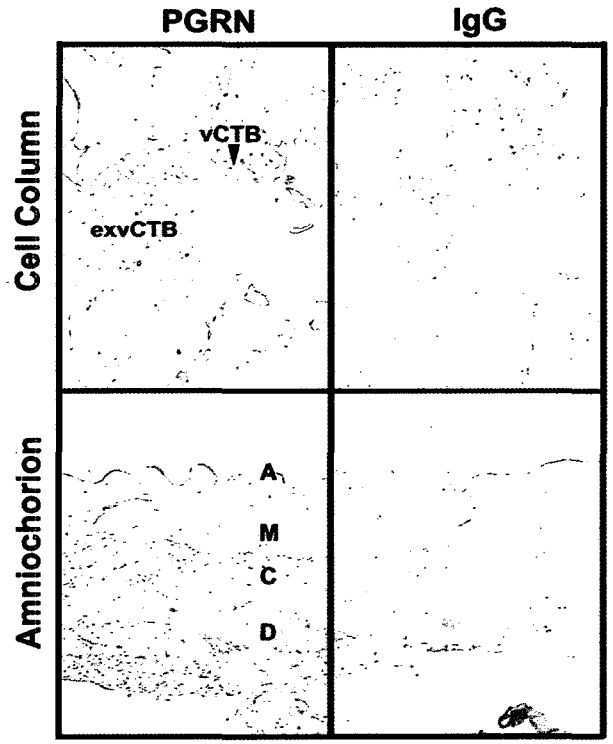

Fig. 3. Photomicrographs illustrating representative localization of PGRN protein by immunohistochemistry in extravillous cytotrophoblast cells present in cell columns of first trimester placentas and amniochorion membranes of term placentas. Specific immunostaining signals were not detected when the primary antibody were substituted with normal goat IgG. vCTB, villous cytotrophoblast cells; exvCTB, extravillous cytotrophoblast cells; A, amnion epithelia; M, mesenchyme; C, chorionic membrane; D, decidua capsularis. Original magnifications, ×400 for cell columns, ×200 for amniochorion.

formed the chorion membrane, the quiescent cell layer of extravillous cytotrophoblast cells in term membranes.

Fig. 3 illustrates that extravillous cytotrophoblast cells in cell columns observed in four different first trimester placentas expressed PGRN protein. Fig. 3 also demonstrates representative immunostaning pattern of PGRN obtained from six different term amniochorion. Amnion epithelia did not contain PGRN protein. Extravillous cytotrophoblast cells in chorion contained PGRN, as with extravillous cytotrophoblast cells in first trimester placentas. In the decidua, PGRN protein was readily detectable.

Expression of PGRN in Villous Cytotrophoblast Cells Isolated from Term Placentas and Choriocarcinoma Cell Lines

The results described above indicated that villous and extravillous cytotrophoblast cells contain PGRN protein. Because villous cytotrophoblast cells are scarce and difficult to identify in term placentas, PGRN expression was unclear. To obtain more information on this subpopulation of cells, RT-PCR and immunoblotting were conducted using highly purified preparations of cytotrophoblast cell from term placentas. As shown in Fig. 4A and 4B, term villous cytotrophoblast cells contained message and protein for PGRN.

To determine whether trophoblast-derived tumor cells might express PGRN protein as well as their normal counterpart, RT-PCR and immunoblotting were conducted on choriocarcinoma cell lines, JEG-3 and Jar. As shown in Fig. 4A and 4B, JEG-3 and Jar cells also contained messages and proteins for PGRN as with normal cytotrophoblast cells.

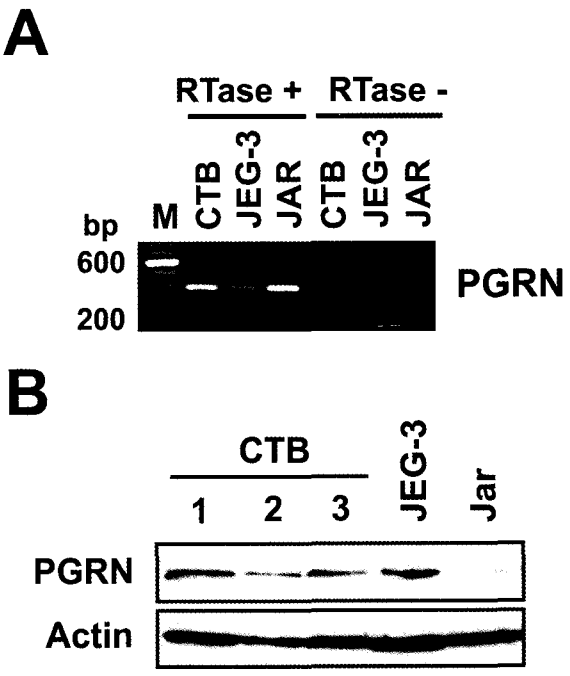

Fig. 4. RT-PCR (A) and immunoblot (B) analyses of PGRN expression in cytotrophoblast cells (CTB) and choriocarcinoma cell lines, JEG-3 and Jar. Cytotrophoblast cells for immunoblot analysis were from three different term placentas. Molecular weight markers (M) are noted to indicate base pairs (bp) of PCR products, and the molecular weight (kDa) of each protein is indicated. RTase + / −, with (+) or without (−) reverse transcriptase.

DISCUSSION

The results of this study show for the first time that 1) PGRN messages and proteins are present in human placentas at both early and late stages of gestation; 2) the level of PGRN protein expression is higher in first trimester than in term placentas; 3) the proteins are localized to specific types of cells; and 4) specific messages and proteins derived from the PGRN genes are detectable in both cytotrophoblast cells isolated from term placentas and trophoblast-derived choriocarcinoma cells.

The observations in this study show the presence of PGRN proteins in early and late gestation human placentas, and are of great potential importance to understanding the regulation of placental development. Placentas are rich sources of growth factors and cytokines such as epidermal growth factors, fibroblast growth factors, insulin-like growth factors, transforming growth factors, and tumor necrosis factor-a as well as their receptors (Maruo *et al.*, 1995; Uehara and Kitamura, 1996; Cross *et al.*, 2003; Fowden, 2003). These growth factors play critical roles in placental growth and differentiation during gestation (Benirschke and Kaufmann, 2000).

Among many growth factors, PGRN is a newly identified autocrine growth factor (He and Bateman, 2003). Since PGRN is known to induce proliferation and migration of cells, expression of PGRN in human placentas suggests that PGRN may affect placental cell functions such as proliferation of cytotrophoblast cells and migration of extravillous cytotrophoblast cells during placental growth and development.

Expression of PGRN in the human placenta was cell type-specific. PGRN in mouse embryo is expressed in trophectoderm cells at the blastocyst stage, and functions in blastocyst hatching and outgrowth (Diaz-Cueto *et al.*, 2000). In the present study using human placentas, essentially all subpopulations of trophoblast cells contained the PGRN proteins, but expression appeared to vary among the subpopulations. Especially, PGRN expression was readily detectable in the syncytiotrophoblast layer, which is the outermost cell layer in the placental villi contacting the maternal blood in human placentas (Benirschke and Kaufmann, 2000). The syncy-

tiotrophoblast layer shows various differentiated functions such as production of hormones, growth factors and cytokines, but does not undergo cell division (Benirschke and Kaufmann, 2000). Thus, PGRN produced by the syncytiotrophoblast layer may lead to differentiation rather than proliferation of syncytiotrophoblast. PGRN may also affect syncytialization process and differentiated functions of syncytiotrophoblast cell.

It was also observed that expression of PGRN in the human placenta was stage-specific. The level of PGRN expression was higher in first trimester than in term placentas. This may reflect higher growth activity of the placental villi in first trimester than in term placentas. Although speculative, it is also possible that PGRN produced by the syncytiotrophoblast acts on the villous cytotrophoblast cells for proliferation in a paracrine manner, because proliferation of the villous cytotrophoblast cells occurs more in first trimester than in term placentas and the villous cytotrophoblast cells and the syncytiotrophoblast layer contact each other. However, except for the existence of two classes of PGRN binding sites in an epithelial cell line and the size of major PGRN receptor of 120 kDa (Xia and Serrero, 1998), not much is known about the PGRN receptor. Thus, to elucidate the roles of PGRN in the placental villi, further studies need to be done on localization of the PGRN receptor, which is not cloned yet, as well as functional analysis of PGRN in syncytiotropoblast differentiation and cytotrophoblast cell proliferation.

The amniochorion membrane, which consists of the amnion, the chorion, and the maternal deciduas (Benirschke and Kaufmann, 2000), also expresses PGRN, mainly in the chorioninc extravillous cytotrophoblast cells and ducidual cells. Differentiated functions of extravillous cytotrophoblast cells include cell migration during invasion and production of hormones, growth factors and cytokines. Decidual cells also produce numerous hormones. Thus, our identification of PGRN in these membranes suggests that PGRN in the amniochorion may also affect differentiation of extravillous cytotrophoblast and decidual cells during pregnancy.

In conclusion, PGRN is expressed in a cell- and gestational stage-specific manner in the human placenta. PGRN may play an important role in placental development. These findings provide insights to our understanding of the mechanisms by which placental development is regulated in various types of cells.

REFERENCES

1. Benirschke K, Kaufmann P (2000): Pathology of the Human Placenta, 4th Ed., Springer, New York, USA, pp 50-115.
2. Bateman A, Bennett HP (1998): Granulins: the structure and function of an emerging family of growth factors. J Endocrinol 158:145-151.
3. Bhandari V, Giaid A, Bateman A (1993): The complementary deoxyribonucleic acid sequence, tissue distribution, and cellular localization of the rat granulin precursor. Endocrinology 133:2682-2689.
4. Bhandari V, Daniel R, Lim PS, Bateman A (1996): Structural and functional analysis of a promoter of the human granulin/epithelin gene. Biochem J 319: 441-447.
5. Bowen JM, Chamley L, Mitchell MD, Keelan JA (2002): Cytokines of the placenta and extra-placental membranes: biosynthesis, secretion and roles in establishment of pregnancy in women. Placenta 23:239-256.
6. Cross JC, Baczyk D, Dobric N, Hemberger M, Hughes M, Simmons DG, Yamamoto H, Kingdom JC (2003): Genes, development and evolution of the placenta. Placenta 24:123-130.
7. Daniel R, Daniels E, He Z, Bateman A (2003): Progranulin (acrogranin/PC cell-derived growth factor/granulin-epithelin precursor) is expressed in the placenta, epidermis, microvasculature, and brain during murine development. Dev Dyn 227:593-599.
8. Daniel R, He Z, Carmichael KP, Halper J, Bateman A (2000): Cellular localization of gene expression for progranulin. J Histochem Cytochem 48:999-1009.
9. Diaz-Cueto L, Stein P, Jacobs A, Schultz RM, Gerton GL (2000): Modulation of mouse preimplantation embryo development by acrogranin (epithelin/granulin precursor). Dev Biol 217:406-218.
10. Fowden AL (2003): The insulin-like growth factors and feto-placental growth. Placenta 24:803-812.
11. He Z, Ismail A, Kriazhev L, Sadvakassova G, Bateman A (2002): Progranulin (PC-cell-derived growth factor/acrogranin) regulates invasion and cell survival. Cancer Res 62:5590-5596.
12. He Z, Bateman A (1999): Progranulin gene expression regulates epithelial cell growth and promotes tumor growth *in vivo*. Cancer Res 59:3222-3229.
13. He Z, Ong CH, Halper J, Bateman A (2003): Progranulin is a mediator of the wound response. Nat Med 9:225-229.
14. He Z, Bateman A (2003): Progranulin (granulin-epithelin precursor, PC-cell-derived growth factor, acrogranin) mediates tissue repair and tumorigenesis. J Mol Med 81:600-612.
15. Ka H, Hunt JS (2003): Temporal and spatial patterns of expression of inhibitors of apoptosis in human placentas. Am J Pathol 163:413-422.
16. Kliman HJ, Nestler JE, Sermasi E, Sanger JM, Strauss JF 3rd (1986): Purification, characterization, and *in vitro* differentiation of cytotrophoblasts from human term placentae. Endocrinology 118:1567-1582.
17. Lu R, Serrero G (1999): Stimulation of PC cell-de-

rived growth factor (epithelin/granulin precursor) expression by estradiol in human breast cancer cells. Biochem Biophys Res Commun 256:204-207.
18. Lu R, Serrero G (2001): Mediation of estrogen mitogenic effect in human breast cancer MCF-7 cells by PC-cell-derived growth factor (PCDGF/granulin precursor). Proc Natl Acad Sci USA. 98:142-147.
19. Maruo T, Matsuo H, Otani T, Mochizuki M (1995): Role of epidermal growth factor (EGF) and its receptor in the development of the human placenta. Reprod Fertil Dev 7:1465-1470.
20. Ong CH, Bateman A (2003): Progranulin (granulin-epithelin precursor, PC-cell derived growth factor, acrogranin) in proliferation and tumorigenesis. Histol Histopathol 18:1275-1288.
21. Qin J, Diaz-Cueto L, Schwarze JE, Takahashi Y, Imai M, Isuzugawa K, Yamamoto S, Chang KT, Gerton GL, Imakawa K (2005): Effects of progranulin on blastocyst hatching and subsequent adhesion and outgrowth in the mouse. Biol Reprod 73:434-442.
22. Uehara Y, Kitamura N (1996): Hepatocyte growth factor/scatter factor and the placenta. Placenta 17: 97-101.
23. Xia X, Serrero G (1998): Identification of cell surface binding sites for PC-cell-derived growth factor, PCDGF, (epithelin/granulin precursor) on epithelial cells and fibroblasts. Biochem Biophys Res Commun 245:539-543.

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