Upregulated unique long 16 binding protein 1 detected in preeclamptic placenta affects human extravillous trophoblast cell line (HTR-8/SVneo) invasion by modulating the function of uterine natural killer cells

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Abstract. Well-controlled trophoblast invasion at the maternal-fetal interface is crucial for normal placentation and successful pregnancy, otherwise pathological conditions of pregnancy occur, such as preeclampsia. In previous studies, it has been demonstrated that unique long 16 binding protein (ULBP)1, a ligand for the natural-killer group (NKG)2D receptor on uterine natural killer (uNK) cells, is upregulated in the placenta in patients with preeclampsia. As they are present on the majority of the decidua, uNK have an important role in pregnancy. The aim of the present study was to determine the role of ULBP1 in trophoblast cell invasion, which is closely associated with the occurrence of preeclampsia. In the present study, ULBP1 expression levels in placentas collected after cesarean section from women with preeclampsia and normal pregnant women were determined by immunohistochemistry, reverse transcription-quantitative polymerase chain reaction and western blotting. The effects of ULBP1 on extravillous trophoblast cell line (HTR-8/SVneo) invasion mediated via uNK cells and the underlying mechanisms were investigated. mRNA and protein expression levels of ULBP1 were significantly upregulated (P<0.05) in preeclamptic placentas compared with normal controls. ULBP1 inhibited HTR-8/SVneo cells via the regulation of biological functions of uNK cells, including the downregulation of NKG2D expression on uNK cells and the stimulation of production of cytokines and chemokines that affect extravillous cytotrophoblast invasion by uNK cells. ULBP1 may have an important role in the pathophysiology of preeclampsia through the modification of biological functions of uNK cells, which may affect trophoblast invasion.

Introduction

Preeclampsia is a heterogeneous pregnancy disorder that may develop in late pregnancy, and is characterized by hypertension and proteinuria which may occur after 20 weeks of gestation. It affects 2-8% of pregnancies worldwide (1,2). Preeclampsia has preclinical (symptomless) and clinical (symptomatic) stages. Until recently, only the symptomatic final stage preeclampsia could be detected by clinical screening. Although extensive research on the pathophysiology of the disease has been conducted, the etiology of preeclampsia remains poorly understood. A two-stage disorder theory is accepted for preeclampsia: Stage 1, poor placentation at the early stage of pregnancy; and stage 2, placental oxidative stress at the late stage of pregnancy. Stage 1 is preclinical and characterized by faulty trophoblast invasion and spiral artery (SA) remodeling, resulting in failure to remodel the SAs supplying the uteroplacental circulation, and placental hypoxia (3). Trophoblast differentiation, invasion and SA remodeling are regulated by several trophoblast- and/or decidua-derived factors, including cytokines, growth factors, hormones and oxygen, in the first trimester (4-6). Recent studies have reported that impaired invasion of trophoblasts, and the subsequent incomplete SA remodeling, may result in preeclampsia (6,7). Consequently, elucidating the trophoblast invasion mechanisms is crucial in understanding the pathophysiological mechanisms of preeclampsia. The decidual leukocyte population, in particular uterine natural killer (uNK) cells, is proposed to have a major role in the regulation of trophoblast invasion (8,9). Reduced numbers of uNK cells are observed in patients with preeclampsia and intrauterine growth restriction (10), which are conditions associated with poor SA remodeling and reduced trophoblast invasion in the decidua (11).

Unique long 16 binding proteins (ULBPs) which are also termed as retinoic acid early transcripts (RAET), encoded by RAET1 genes, are a family of ligands for natural-killer group 2D (NKG2D) receptors in humans that are frequently
expressed by tumor cells and mediate biological functions of NK cells (12). The majority of studies of NKG2D ligands have traditionally demonstrated their expression only on infected or transformed cells (13,14); whereas other studies have demonstrated that several normal cells and tissues also express NKG2D ligands (15,16). Constitutive intracellular presence of ULBP1-4 has been recently described in normal human bronchial epithelium (17). A study by Hedlund et al (18) demonstrated that ULBP1-5 are constitutively transcribed and expressed as proteins in human early placenta (8-16 weeks), and have localized expression on the membrane of exosomes of the multivesicular late endosomes in the syncytiotrophoblast (STB). A previous study using DNA microarray analysis and validation by reverse transcription-quantitative polymerase chain reaction (RT-qPCR), demonstrated that ULBP1 was upregulated in preeclamptic placentas (19).

Considering that inadequate invasion of trophoblasts in the first trimester may lead to preeclampsia and the role of uNK cells in the regulation of trophoblast invasion, it was hypothesized that ULBP1 may inhibit the invasion of extravillous trophoblasts (EVTs) by altering cytokines secreted by uNK cells via binding to NKG2D. Although the differential expression of ULBP1 in preeclampsia in the first trimester is difficult to determine, the differential expression of genes or proteins detected in full-term placenta may provide an indication to investigate the mechanism. The present study was performed to determine the expression levels of ULBP1 in placentas collected following cesarean section from women with preeclampsia and normal pregnant women. The functions of ULBP1 in trophoblast invasion were also investigated.

Materials and methods

Ethics statement. Ethical approval was granted by the Ethics Committee of The First Affiliated Hospital of China Medical University (Shenyang, China) and methods were carried out in accordance with the committee guidelines. Informed consent was obtained from all participating patients.

Tissue collection. The present study included 30 pregnant women with preeclampsia and 30 normal pregnant women. Human placental tissues were collected at the time of cesarean section from the Department of Obstetrics between September 2014 and August 2015, The First Affiliated Hospital of China Medical University (Shenyang, China). The clinical characteristics of the patients included in the present study are summarized in Table I. Preeclampsia was diagnosed according to the reported criteria (20). Patients enrolled in the preeclampsia group had no history of pre-existing or chronic hypertension, although they exhibited ≥140 mmHg systolic or ≥90 mmHg diastolic pressure on two occasions at least 4 h apart after 20 weeks of gestation and ≥300 mg per 24-h urine collection after 20 weeks of gestation. Chorionic tissues were obtained from four different parts of the placenta, from which the amniotic membrane and maternal decidual tissues were removed. Tissues were frozen and stored at -80˚C until use. Decidual samples were obtained from women undergoing elective surgical termination of pregnancy at 12-14 weeks of gestation (as determined by ultrasound measurement of crown rump length or biparietal diameter). Following collection, decidual tissue was immediately suspended in sterile saline, transported to the laboratory and washed two to three times in sterile phosphate-buffered saline (PBS) to remove excess blood.

Immunocytochemistry. Formalin-fixed and paraffin-embedded tissue sections of 4-µm thickness were prepared. Immunostaining was performed using a streptavidin-peroxidase method. Sections were incubated with a ULBP1 primary antibody (1:100; sc-33456; Santa Cruz Biotechnology Inc., Dallas, TX, USA) at 4˚C overnight, followed by a biotinylated goat serum anti-rabbit immunoglobulin (Ig)G secondary antibody (1:100; 0017; Ultrasensitive; Fuzhou Maixin Biotech Co., Fuzhou, China) at room temperature for 10 min. Subsequent to washing with PBS three times, the sections were incubated with horseradish peroxidase-conjugated streptavidin-biotin (1:1; 0017; Ultrasensitive; Fuzhou Maixin Biotech Co.) at room temperature for 10 min, developed using 3,3-diaminobenzidine tetrahydrochloride, lightly counterstained with hematoxylin, dehydrated in alcohol and mounted. Sections were observed under an Olympus BX51 microscope (Olympus Corp., Tokyo, Japan). As a negative control, isotype-specific IgG was used instead of primary antibody to exclude nonspecific binding of the secondary antibody.

RT-qPCR. Total RNA was extracted from human placental tissues using TRIzol reagent (Ambion; Thermo Fisher Scientific, Inc., Waltham, MA, USA) and were treated with DNase I (Promega Corp., Madison, WI, USA). cDNA was generated from 1 µg total RNA using a PrimeScript Reagent kit (Takara Biotechnology Co., Ltd., Dalian, China), according to the manufacturers’ instructions. The PCR reaction (20 µl final volume) contained 10 µl SYBR Premix Ex Taq, 2 µl cDNA, 0.8 µl of each primer and 6.4 µl double distilled H2O. qPCR analysis for ULBP1 and GAPDH was performed as follows: 95˚C for 30 sec; 45 cycles of 95˚C for 5 sec; and 60˚C for 20 sec using a Roche Lightcycler 480 (Roche Diagnostics GmbH, Mannheim, Germany). Gene-specific amplifications were confirmed with melting curve analysis following RT-qPCR. Relative gene expression levels were determined using the threshold cycle method (2^ΔΔCq) method (21) with reference to the endogenous GAPDH control. The following primers were used: ULBP1 forward, 5’-CAGCAGAGATTGAGACCATT-3’ and reverse, 5’-GACAGAACGGTGGCAGAAGGTTG-3’; and GAPDH forward, 5’-CATGAGAAGATGACCAACAGCT-3’ and reverse, 5’-AGTCTTTCCACAGTACCAAAGT-3’.

Western blotting. Human placental tissues were lysed in a radioimmunoprecipitation assay lysis buffer (Nanjing KeyGen Biotech Co., Ltd., Nanjing, China), and protein concentrations were determined using an Enhanced BCA Protein Assay kit (Beyotime Institute of Biotechnology, Beijing, China). A total of 20 µg of proteins were separated by 10% SDS-PAGE and transferred onto a polyvinylidene difluoride membrane (Merck Millipore, Darmstadt, Germany). Membranes were blocked for 1 h at room temperature with TBST (50 mM Tris-HCl, 150 mM NaCl, 0.05% Tween-20, pH 7.0) containing 5% nonfat dry milk and incubated with primary antibodies against ULBP1 (1:200; sc-33456; Santa Cruz Biotechnology)
Table I. Clinical characteristics of pregnant women enrolled on the present study.

| Clinical characteristic | Normal pregnancy (n=30) | Preeclampsia (n=30) | P-value |
|-------------------------|-------------------------|---------------------|---------|
| Maternal age (years)    | 29.50±3.21              | 29.8±3.05           | 0.71    |
| Body mass index (kg/m²) | 24.82±1.89              | 25.67±1.45          | 0.06    |
| Systolic blood pressure (mmHg) | 119.67±9.24          | 155.05±10.53        | <0.05<sup>a</sup> |
| Diastolic blood pressure (mmHg) | 68.5±6.33            | 105.13±9.18         | <0.05<sup>a</sup> |
| 24 h urine protein (g)  | 0.047±0.025             | 2.54±1.40           | <0.05<sup>a</sup> |
| Gestational age at delivery (weeks) | 38.84±0.54          | 37.26±1.91          | <0.05<sup>a</sup> |
| Fetal birth weight (g)  | 3433.33±320.78          | 2580.00±600.87      | <0.05<sup>a</sup> |

Data are presented as the mean ± standard error of the mean. *P<0.05, significant difference between normal pregnancy and preeclampsia.

and β-actin (1:8,000; 66009-1; Proteintech Group, Inc., Chicago, IL, USA) overnight at 4°C. The membranes were washed with TBST three times for 10 min, followed by incubation with horseradish-peroxidase-labeled secondary antibodies (1:12,000; SB-0071; Dingguo, Beijing, China) for 1 h at room temperature. Protein expression was visualized using an enhanced chemiluminescence system (Tanon5200; Tanon Science and Technology Co., Ltd., Shanghai, China). Bands were analyzed using densitometry with ImageJ software (version 1.46r; National Institutes of Health, Bethesda, MA, USA).

**uNK cell isolation.** Total decidual cell isolates and purified CD56<sup>-</sup>CD3<sup>-</sup> uNK cell isolates were prepared by enzymatic disaggregation and immunomagnetic selection (MACS) as previously described (22,23). The tissue was trimmed into 1-mm pieces and enzymatically digested for 20 min, using vigorous shaking, with type I DNase and IV collagenase in RPMI 1640 medium (Hyclone; GE Healthcare Life Sciences, Logan, UT, USA). Supernatants were collected and incubated overnight in a tissue culture dish in a standard 37°C environment with 5% CO<sub>2</sub>. Nonadherent cells were collected and loaded onto Ficoll-Paque density gradient media (GE Healthcare Bio-Sciences, Pittsburgh, PA, USA) to purify the lymphocyte population. Cell suspensions were subjected to MACS (Miltenyi Biotech GmbH, Bergisch Gladbach, Germany) with an NK Cell Isolation kit (Miltenyi Biotech GmbH) to obtain uNK cell suspensions. uNK cells were plated on a 24-well plate at 5x10<sup>4</sup> cells/well in 600 µl RPMI-1640 medium supplemented with 10% FBS in a standard 37°C environment (5% CO<sub>2</sub>) in an air incubator.

**Invasion assay.** Cell invasion assays were performed to investigate the effect of ULBP1 on the invasion of HTR-8/SVneo cells. HTR-8/SVneo cells were placed in the upper chamber of an 8-µm Transwell plate (Costar, New York, NY, USA) in 80 µl growth-factor-reduced Matrigel (1:9; BD Biosciences, San Jose, CA, USA). Inserts were pre-coated with 80 µl Matrigel matrix (1:9; BD Biosciences). The different uNK cell conditioned media (cultured with or without the ULBP1 protein, 33% v/v) was added to the lower chambers. To investigate whether trophoblast invasion was mediated by cytokines secreted by uNK cells, two representative neutralizing antibodies for cytokines that inhibit or stimulate trophoblast cell invasion were added to the 72-h cell supernatants cultured with ULBP1. These antibodies included specific anti-interferon (IFN)-γ (mouse anti-human IFN-γ, 10 µg/ml; D044-3) for stimulating trophoblast cell invasion and a specific anti-interleukin (IL)-8 neutralizing antibody (goat anti-human IL-8, 0.5 µg/ml; MAB208; both R&D Systems Europe, Ltd.) for inhibition of trophoblast cell invasion. A total of 1x10<sup>5</sup> HTR-8/SVneo cells in 200 µl of serum-free medium were plated in the upper chamber, and a total of 600 µl media was added in the lower chamber. Following 24 h, the membranes of the Transwell chamber were fixed in methanol and stained with hematoxylin and eosin (OriGene Technologies, Beijing, China). Finally, the number of cells was counted by capturing images of the membrane with an Olympus CKX41 microscope (Olympus Corp.). Each experiment was performed in duplicate in 10 cases. The invasion index was the average number of invaded cells in test filters divided by the average number of invaded cells in control filters for any given experiment.

**Flow cytometry.** Following 72 h of incubation of uNK cells with or without recombinant ULBP1-Fc (5 µg/ml), surface antigen fluorescence-activated cell sorting analysis was performed to detect the modulation of NKG2D by recombinant ULBP1-Fc. Cells were washed twice with ice-cold PBS. The washes were followed by incubation of the cells with mouse anti-NKG2D antibody (320805; BioLegend, Inc., San Diego, CA, USA) for 30 min on ice. Subsequent to two washes with PBS, a FACScan flow cytometer (BD Pharmingen, San Diego, CA, USA) was used for analysis. Background staining supplemented with 10% FBS in a standard 37°C environment (5% CO<sub>2</sub>) in an air incubator.
was estimated after incubation with phycoerythrin-labeled isotype antibodies (400113, 400107 and 400117; 5 µl per 10⁶ cells in 100 µl staining volume, all BioLegend, Inc., San Diego, CA, USA). ELISA kits (R&D Systems Europe, Ltd.) were used to measure tumor necrosis factor (TNF)-α (DTA00C), IFN-γ (DIF50), transforming growth factor (TGF)-β1 (DB100B), IL-6 (D6050) and IL-8 (D8000C). Cell-free supernatants were analyzed using ELISA following 72 h incubation of uNK cells with or without recombinant ULBP1-Fc (5 µg/ml). Each experiment was performed in triplicate in 10 cases.

**Statistical analysis.** Statistical analyses were performed using SPSS version 17.0 (SPSS, Inc., Chicago, IL, USA). Results are presented as the mean ± standard error. Differences between two groups were analyzed using Student’s t-test. P<0.05 was considered to indicate a statistically significant result.

**Results**

**uNK cell isolation.** CD56⁺CD3⁻ cell-enriched isolates were demonstrated to be consistently >95% pure by flow cytometry (Fig. 1).

**mRNA and protein expression levels of ULBP1 are upregulated in preeclamptic placentas.** RT-qPCR was used to investigate the mRNA expression levels of ULBP1 in preeclamptic and normal placentas. As shown in Fig. 2A, the mRNA expression levels of ULBP1 in preeclamptic placentas were significantly upregulated compared with that in normal placentas (P<0.05). In agreement with the results of RT-qPCR, western blotting demonstrated that ULBP1 protein expression levels were significantly higher in preeclamptic placentas compared with that of normal placentas (P<0.05; Fig. 2B). Immunocytochemistry demonstrated that ULBP1 was predominantly located in the cytoplasm of STB and that normal and preeclamptic placentas expressed ULBP1; however, ULBP1 expression levels were greater in preeclamptic placentas, as compared with normal placentas (Fig. 2C).

![Figure 1](image1.png)

**Figure 1.** Flow cytometric analysis of the purity of sorted uterine natural killer cells following immunomagnetic selection. The CD56⁺CD3⁻ cell-enriched isolates were demonstrated to be consistently >95% pure.

![Figure 2](image2.png)

**Figure 2.** Expression levels of ULBP1 in placentas from pregnant women with PE and women with NP were determined by RT-qPCR, western blotting, and immunohistochemistry analysis. (A) RT-qPCR analysis of ULBP1 mRNA expression levels in placentas from PE and NP women (n=30 for each group). (B) Western blot analysis of ULBP1 protein expression in placentas from PE and NP women. Upper panel, a typical result of western blotting; lower panel, bar chart according to the statistical analysis based on the result of three independently repeated experiments (n=30 for each group). (C) Immunostaining of ULBP1 in placentas from PE and NP women. Data are presented as the mean ± standard error of the mean. Left panel, PE; middle panel, NP; right panel, negative control; scale bars, 200 µm. *P<0.05, PE vs. NP. ULBP1, unique long 16 binding protein 1; PE, preeclampsia; NP, normal pregnancy; RT-qPCR, reverse transcription-quantitative polymerase chain reaction.
uNK cell culture supernatants with ULBP1 inhibit the invasion of HTR-8/SVneo cells. Following incubation of uNK cells with or without ULBP1 for 72 h, it was possible to determine the effect of ULBP1 on the invasion of EVT. When EVT was cultured in the presence of uNK cell culture supernatants with ULBP1, there was a significant decrease in EVT invasion compared to the cell culture supernatants without ULBP1 (P<0.05; Fig. 3A and B). Following the addition of specific anti-IFN-γ or anti-IL-8 neutralizing antibody to uNK cell culture supernatants with ULBP1, anti-IFN-γ neutralizing antibody significantly decreased the inhibitory effect (P<0.05; Fig. 3C) and anti-IL-8 neutralizing antibody enhanced the inhibitory effect of ULBP1 (P<0.05; Fig. 3D).

ULBP1 stimulates the production of TNF-α, IFN-γ, TGF-β1, IL6 and IL-8 by downregulating NKG2D expression. ULBP1 decreased surface NKG2D expression levels, as monitored by staining with anti-NKG2D antibody, after incubation of uNK cells for 72 h (Fig. 4). Cell-free supernatants were analyzed,
using ELISA, following 72 h incubation for the presence of TNF-α, IFN-γ, TGF-β1, IL-6 and IL-8. TNF-α, IFN-γ, TGF-β1, IL-6 and IL-8 were all significantly increased in cell-free supernatants incubated with ULBP1 (P<0.05; Fig. 5) compared with the controls.

Discussion

uNK cells, CD56⁺CD16⁺, are distinct from peripheral blood (pb)NK cells and constitute up to 70% of the decidual leukocyte population in the first half of pregnancy (24). uNK cells are considered to have a cytokine-secreting role rather than a cytotoxic defensive role, which is the predominant role of pbNK cells (25). As previously demonstrated, certain cytokines have the potential to inhibit trophoblast invasion in *in vitro* invasion assays. These cytokines include TNF-α (26), TGF-β1 (9) and IFN-γ (27). Certain cytokines stimulate EVT invasion, including IL-8 (8,28) and IL-6 (29). A study by Hanna et al (8) demonstrated that uNK cells induced EVT invasion; however, pbNK cells were unable to do this. It is evident that uNK cells are important for the achievement and maintenance of pregnancy. Although uNK cells have reduced cytotoxic-defensive ability compared with pbNK cells, they do retain low cytotoxic activity (30,31), uNK cells express cytotoxic proteins, including perforin, granzymes A and B and granulysin; therefore, uNK cells have cytolytic capacity. This cytotoxic machinery does not result in apoptosis of the invading trophoblast, although apoptosis may occur when responding to infection (32); however, the ability of uNK cells to destroy EVT remains unclear, with some evidence demonstrating that expression of human leukocyte antigen-G by EVT protects EVT from uNK cell attack (33).

The specific gestational period span of uNK cells selected for the present study was based on the research of Lash et al (34), which demonstrated that uNK cell supernatants were able to stimulate EVT invasion at 12-14 weeks gestation; however, they were unable to stimulate EVT invasion at 8-10 weeks gestation. It was suggested that, prior to 10 weeks gestational age, uNK cells had a role in the initial stages of trophoblast-independent SA remodeling and that, after 12 weeks gestational age, uNK cells had a role in regulating EVT invasion. The focus of the present study was on EVT invasion regulated by ULBP1 via uNK cells, therefore, uNK cells at 12-14 weeks were used.
NK cell function is regulated by a delicate balance of signals initiated from various activating and inhibitory receptors. NKG2D was originally identified, in 1991, as a key activating receptor on all NK cells (35), including uNK cells (8). In humans, NKG2D was first identified as a receptor for major histocompatibility complex class I chain-related A and B and, subsequently, for ULBP 1-5 molecules (36). Binding of ULBPs to NK cells has several biological consequences, including the downregulation of NKG2D expression (37) and the increased production of cytokines and chemokines (38). Flow cytometry analysis demonstrated that ULBP1 downregulated the expression of NKG2D on uNK cells in the present study and, subsequently, all measured cytokines and chemokines secreted by uNK cells increased. The effect of ULBP1 on uNK cells was the same as pbNK cells (37).

In the present study, ULBP1 expression in the placenta was predominately located in STB, which is consistent with a previous study (18). Additionally, it was demonstrated that ULBP1 expression was upregulated in preeclamptic placentas. A study by Hedlund et al (18) demonstrated that ULBP1-5 were all expressed in the placenta; however, a different study demonstrated differential expression of ULBP1 only. This may have been due to the different binding capacity to NKG2D, which may have induced varying secretory ability (37). Further investigation is required to investigate whether other ULBPs are involved in preeclampsia.

As a ligand of NKG2D, ULBP1 is associated with tumor cells (39,40). The present study demonstrated that ULBP1 is associated with preeclampsia. NKG2D ligands may be upregulated in response to the stresses of pregnancy, such as hypoxia (41). Persistent hypoxia may result in the failure of trophoblasts to differentiate from the proliferative to invasive phenotype, resulting in shallow trophoblast invasion and inadequate transformation of the spiral arteries (42). Considerable evidence supports a role for hypoxia in creating an environment that predisposes women to implantation disorders and preeclampsia (3,42). This may explain the upregulation of ULBP1 in preeclampsia.

ULBP1 is produced and retained in endosomal multivesicular bodies of the STB on exosomes in the first trimester (18). ULBP1 may bind to uNK cells on decidua via exosomes that are able to carry proteins to target cells. Therefore, there may be an association between ULBP1 and EVT invasion mediated by uNK cells. The present study focused on the secretion from uNK cells following binding of ULBP1. The secretion of cytokines related to the invasion of EVT was detected. The human EVT cell line HTR-8/SVneo, which is widely used to study trophoblast biology (43,44), was utilized in the present study. The expression of NKG2D was downregulated when uNK cells were incubated with ULBP1. Cytokine secretion of TNF-α, TGF-β1, IFN-γ, IL-8 and IL6 by uNK cells significantly increased in the present study following ULBP1 binding. Human uNK cells produce a variety of cytokines and growth factors, including TNF-α, IL-10, granulocyte-macrophage colony-stimulating factor, IL-1, TGF-β1, colony-stimulating factor-1, leukemia inhibitory factor, IFN-γ (45-47), IL-6 (9), IL-8 and interferon-inducible protein-10 (8), and the roles that these cytokines and growth factors have in EVT invasion has been investigated in previous studies. A study by Bauer et al (26) demonstrated that TNF-α inhibits trophoblast invasion through the elevation of plasminogen activator inhibitor-1. A study by Lash et al (27) demonstrated that IFN-γ inhibits EVT cell invasion by a mechanism that involves alterations to apoptosis and protease levels. It was also demonstrated that invasion of EVT cells was inhibited in the presence of TGF-β1, 2 and 3, which was associated with a decrease in secreted matrix metalloproteinase (MMP)-9 and urokinase plasminogen activator levels (7). A study by De Oliveira et al (28) concluded that IL-8 stimulates trophoblast invasion by a mechanism that may involve increased secretion of MMP-2 by EVT. IL-6 stimulates trophoblast cell migration and invasion, which may be partly attributable to stimulation of expression of the integrin subunits (29); however, different results on the effect of IL-6 on EVT invasion have been demonstrated, with the study by Champion et al (48) demonstrating that IL-6 has no effect on EVT invasion. Such discrepancies between results may be attributable to differences in cell type and quantification methods used. The present study measured IL-6 as the cell line used was the same as in the study conducted by Jovanović and Vićovac (29). In addition, the present study aimed to indicate the dysfunction of uNK cells in preeclampsia by means of measuring cytokine levels. The levels of cytokines secreted by uNK cells, regardless of their effect on EVT invasion, became unbalanced after ULBP1 binding to uNK.

In conclusion, trophoblast invasion is regulated by crosstalk between trophoblasts and decidual cells in a parasitic and autocrine manner, which involves growth factors, cytokines, chemokines and adhesion molecules. uNK cells produce a wide range of cytokines, chemokines and growth factors that may have diverse effects on trophoblast invasion. Upregulation of ULBP1 disturbs the reproductive balance on the maternal-fetal surface. This balance is complicated and is based on various factors, rather than on one particular factor. The results of the present study demonstrate that ULBP1 is significantly higher in preeclampsia placentas compared with normal placentas. As an active ligand of NK cells, ULBP1 results in the secretion of cytokines that increase EVT invasion. As a result of imbalance, invasion of EVT was inhibited, which is a characteristic of preeclampsia. This finding suggests that inappropriate crosstalk at the fetal-maternal interface disturbs the physiological balance and induces adverse pregnancy. Further investigation is required to determine whether it is possible for ULBP1 to be used as a predictor for other pregnancy complications.

References

1. Altinbas S, Toğrul C, Orhan A, Yücel M and Danisman N: Increased MPV is not a significant predictor for preeclampsia during pregnancy. J Clin Lab Ana 26: 403-406, 2012.
2. Kaleli I, Kaleli B, Demir M, Yildirim B, Cevahir N and Demir S: Serum levels of neopterin and interleukin-2 receptor in women with severe preeclampsia. J Clin Lab Anal 19: 36-39, 2005.
3. Amash A, Huleiel M, Sheiner E, Sapir O and Holcberg G: Preeclampsia as a maternal vascular disease. Harefush 146: 707-712, 2007.
4. Kharfi A, Giguère Y, Sapin V, Massé J, Dastugue B and Forest JC: Trophoblastic remodeling in normal and preeclamptic pregnancies: Implication of cytokines. Clin Biochem 36: 323-331, 2003.
5. Lala PK and Chakraborty C: Factors regulating trophoblast migration and invasiveness: Possible derangements contributing to pre-eclampsia and fetal injury. Placenta 24: 575-587, 2003.
Carbon monoxide promotes proliferation of placenta-derived soluble functional expression and regulation of extravillous trophoblast invasion.

References:
1. Li et al: ULBPI AFFECTS HTR-8/SVneo INVASION VIA uNK CELLS.
44. Graham CH, Hawley TS, Hawley RG, MacDougall JR, Kerbel RS, Khoo N and Lala PK: Establishment and characterization of first trimester human trophoblast cells with extended lifespan. Exp Cell Res 206: 204-211, 1993.

45. Jokhi PP, King A, Sharkey AM, Smith SK and Loke YW: Screening for cytokine messenger ribonucleic acids in purified human decidual lymphocyte populations by the reverse-transcriptase polymerase chain reaction. J Immunol 153: 4427-4435, 1994.

46. Jokhi PP, King A and Loke YW: Cytokine production and cytokine receptor expression by cells of the human first trimester placental-uterine interface. Cytokine 9: 126-137, 1997.

47. Rieger L, Kammerer U, Hofmann J, Sütterlin M and Dietl J: Choriocarcinoma cells modulate the cytokine production of decidual large granular lymphocytes in coculture. Am J Reprod Immunol 46: 137-143, 2001.

48. Champion H, Innes BA, Robson SC, Lash GE and Bulmer JN: Effects of interleukin-6 on extravillous trophoblast invasion in early human pregnancy. Mol Hum Reprod 18: 391-400, 2012.