Osteopontin Promotes Trophoblast Invasion in the Smooth Muscle Cell-Endothelial Co-Culture At Least Via Targeting Integrin \(\alpha v\beta 3\)

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Abstract
Preeclampsia is a pregnancy disorder, whereas the underlying mechanisms and etiological factors of this complication remain elusive. Studies have reported that decreased invasiveness of trophoblast cells, immunity disorder in the maternal–fetal interface, and oxidative stress may contribute to the development of preeclampsia. In the present study, we firstly co-cultured the smooth muscle cells (SMCs) and endothelial cells (ECs) to mimic the decidua and myometrium interface and examined the effects of osteopontin (OPN) on the invasive potential of trophoblasts in the SMC-EC co-culturing system. Our results showed that HTR-8/SVneo cells after hypoxia treatment showed enhanced invasive potential in the SMC-EC co-culturing system. OPN levels in the culture media from hypoxia-treated HTR-8/SVneo cells were significantly increased. More importantly, OPN treatment upregulated integrin, beta 3 and integrin, beta 5 expression in HTR-8/SVneo cells, and promoted HTR-8/SVneo cell invasion in the transwell invasion assay and SMC-EC co-culturing system. Mechanistically, treatment with integrin \(\alpha v\beta 3\) inhibitor significantly attenuated the enhanced invasive potential of HTR-8/SVneo cells treated with OPN in the SMC-EC co-culturing system. In conclusion, our study for the first time established the SMC-EC co-culturing system to examine the invasive potential of trophoblasts. Our results indicated that OPN promoted the invasive capacity of trophoblasts via at least targeting \(\alpha v\beta 3\) in the EC-SMC co-culturing system. Future studies were required to further validate the EC-SMC co-culturing system and to determine the molecular mechanisms of OPN-mediated trophoblast invasion.

Keywords
preeclampsia, trophoblast, co-culturing system, osteopontin, integrin \(\alpha v\beta 3\), invasion

Introduction
Preeclampsia is a pregnancy disorder, which exerts a serious impact on the health of the fetus and perinatal mother\(^1\). Preeclampsia is featured by the abnormal trophoblast invasion of the uterine spiral arteries and the reduced number of converted vessels\(^2\). Under normal conditions, human placenta requires the switch of proliferative trophoblasts to an invasive phenotype, and most proliferative trophoblasts within the anchoring and floating villi differentiate to form the syncytiotrophoblasts\(^3\). The highly invasive trophoblasts will invade through the decidua and myometrium into the maternal spiral arteries\(^3\). However, in the preeclampsia patients, the normal invasive capacity of trophoblasts was impaired\(^4\). As far as we know, the precise molecular mechanisms underlying the abnormal invasive behavior of trophoblasts remain elusive.

Osteopontin (OPN) is a class of extracellular matrix glycoprotein and possesses the arginine–glycine aspartate-
binding motif, which can bind to integrin subunits including \( \alpha v, \beta 1, \beta 3, \) and \( \beta 5-7 \). OPN has been reported to play diverse biological functions, including cell proliferation, cell invasion, cell adhesion, and so on\(^8\)\(^-\)\(^10\). In the mouse studies, researchers identified the expression of OPN in the mouse blastocyst, the glandular epithelium, and uterine luminal fluid, and OPN plays an important role in blastocyst hatching, blastocyst adhesion, and embryo development in the mouse\(^11\). OPN was effective in promoting the invasion of human trophoblasts via upregulating matrix metalloproteinase-9\(^-\)\(^12\). Recently, studies by Frank et al. demonstrated that alpha v integrins could bind OPN to support trophoblast cell adhesion\(^13\). Previously, our research group demonstrated that OPN and \( \alpha v \beta 3 \) protein were expressed in the human placenta. In addition, OPN was detected in the placental syncytiotrophoblasts and the cytoplasm of capillary endothelial cells, and integrin \( \alpha v \beta 3 \) was mainly expressed on the surface of trophoblast cells\(^14\). However, the precise mechanisms of OPN and its receptor \( \alpha v \beta 3 \) in the pathogenesis of preeclampsia are not fully understood.

In the present study, we aimed to develop an \textit{in vitro} smooth muscle cell (SMC)-endothelial cell (EC) co-culture system to mimic decidua and myometrium boundary, and further mechanistic studies were performed to examine the effects of OPN on the invasive potential of the trophoblasts in the \textit{in vitro} SMC-EC co-culture system.

**Materials and Methods**

**Cell Lines and Cell Culture**

HTR-8/SVneo cells were a generous gift from Professor Fuyuan Qiao (Tongji University, Shanghai, China); human hepatic venous endothelial cells (ED25) were purchased from Cell Bank of Sun Yat-sen University (Guangzhou, China); mouse vascular SMCs (MOVAS-1) were purchased from Type Culture Collection Center of Wuhan University (Wuhan, China). All the three cell lines were cultured in Dulbecco’s modified Eagle medium (DMEM; Gibico, Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS; Gibico) in a humidified incubator at 37°C with 5% CO\(_2\). For generating the green fluorescence protein (GFP)-expressing HTR-8/SVneo cells, a lentiviral vector of pLVX-AcGFP1-C1 (#632155; Takara, Dalian, China) was transfected into 293 T cells with Lipofectamine 2000 reagent (Invitrogen, Carlsbad, USA) according to the manufacturer’s protocol. At 48 h post-transfection, viral supernatant was collected to infected HTR-8/SVneo cells. The transfection efficiency was evaluated under a fluorescent microscope.

**SMC-EC Co-Culture System**

The transwell inserts (Costar, Brooklyn, NY, USA) with polycarbonate filters in 8-\( \mu \)m pore size were precoated with 50 \( \mu \)l of 1 mg/ml Matrigel matrix (Becton Dickinson, Bedford, UK). MOVAS-1 cells (0.5 \( \times \) 10\(^5\), 1 \( \times \) 10\(^5\), 1.5 \( \times \) 10\(^5\), and 2 \( \times \) 10\(^5\) cells) in serum-free medium were seeded on the lower surface of the inverted transwell inserts. After 2 h culture, the inverted transwell inserts turned upside down and the lower chamber was filled with 2% FBS medium. After a further culture for 24 h, ED25 cells (1 \( \times \) 10\(^5\) and 2 \( \times \) 10\(^5\) cells) were seeded on the upper chamber and were cultured for another 8 h to establish the SMC-EC co-culture system.

**Hypoxia Induction and Drug Treatment**

For the hypoxia induction, the HTR-8/SVneo cells or the SMC-EC co-culture were incubated with 250 \( \mu \)M cobalt chloride (CoCl\(_2\); Sigma-Aldrich, St. Louis, USA) for 6 h before further \textit{in vitro} assays. For OPN (Sigma-Aldrich) treatment, HTR-8/SVneo cells were incubated with OPN (50 \( \mu \)g/ml) for 6 and 24 h, respectively, before further \textit{in vitro} assays. For the cilengitide (CGT; an integrin \( \alpha v \beta 3 \) inhibitor; Sigma-Aldrich) treatment, HTR-8/SVneo cells were incubated with CGT (20 \( \mu \)g/ml) for 24 h before further \textit{in vitro} assays.

**Transwell Invasion Assay Using SMC-EC Co-Culture System**

For the invasion assay, GFP-expressing HTR-8/SVneo cells (1 \( \times \) 10\(^5\) cells) in serum-free medium were seeded on the upper chamber of the SMC-EC co-culture system. After a further culture for 24, 48, and 72 h, respectively, the cells on the Matrigel side of the inserts were removed by cotton swab, and the invaded cells were examined under a fluorescent microscope.

**Transwell Invasion Assay**

For the transwell invasion assay, transwell inserts (Costar) with polycarbonate filters in 8-\( \mu \)m pore size were precoated with 50 \( \mu \)l of 1 mg/ml Matrigel matrix (Becton Dickinson). HTR-8/SVneo cells (1 \( \times \) 10\(^5\) cells) in serum-free medium were plated in the upper chamber, whereas medium with 10% FBS was added to the lower chamber. After incubating for 24 h, the cells on the Matrigel side of the inserts were removed by the cotton swab. The inserts were fixed in methanol and stained with 0.1% crystal violet (Sigma-Aldrich). The number of invaded cells attached to the lower side of the insert was counted under a light microscope.

**Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)**

Total RNA from HTR-8/SVneo cells was extracted using Trizol Reagent (Takara, Dalian, China). RNA was reverse transcribed into cDNA using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, USA). qRT-PCR was performed using SYBR Green PCR Master Mix (Applied Biosystems). Amplification and fluorescence detection were performed using an ABI Prism 7500 Real-Time PCR system (Applied Biosystems). Relative integrin subunit beta 3 (ITGB3) and integrin subunit beta 5
mRNA expression was calculated by the $2^{-\Delta\Delta Ct}$ method using glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as the reference control.

**ELISA Analysis of OPN Protein Concentrations**

The OPN protein concentrations from the culture medium were analyzed using a commercial OPN ELISA kit (#BMS2066; Thermo Fisher Scientific, Waltham, MA, USA) by following the manufacturer’s protocol.

**Western Blot Analysis**

Proteins from cells were extracted using radioimmunoprecipitation assay buffer containing protease inhibitors (Roche, Basel, Switzerland). A total of 50 μg protein samples was resuspended in sodium dodecyl sulfate sample buffer and boiled for 5 min. Equal amounts of protein samples were separated on 10% sodium dodecyl sulfate-polyacrylamide gels and were subsequently transferred to the nitrocellulose membranes. The nitrocellulose membranes were blocked with 5% bovine serum albumin (Sigma-Aldrich) for 1 h at room temperature followed by incubating with primary antibodies against ITGB3 (1:1000; #4702, Cell Signaling Technology, Danvers, USA) and ITGB5 (1:1000; #3629, Cell Signaling Technology), or GAPDH (1:2000; #2118, Cell Signaling Technology). After washing with Tris-buffered saline with 0.1% Tween 20 buffer, the membranes were incubated with horseradish peroxidase-conjugated secondary antibodies (1:2000; Cell Signaling Technology) at room temperature for 2 h. The blotting bands were visualized by enhanced chemiluminescence kit (#32106; Thermo Fisher Scientific). The intensity of the detected bands was quantified by using the Software Smartview 2001 (Shanghai Furi Science & Technology Co. Ltd., Shanghai, China). Relative intensities of each protein signal were obtained by dividing the intensities of each protein signal by those of GAPDH signals.

**Statistical Analysis**

All the data analysis was performed using the SPSS12.0 software package (IBM, Almond, USA). Unpaired Student’s
-test or one-way analysis of variance followed by Bonferroni’s post hoc test was performed to determine the statistical significance between/among different treatment groups, as appropriate. All the data were presented at mean ± SD. The level of statistical significance was set at $P < 0.05$.

**Results**

**Optimization of Cell Seeding Number for the SMC-EC Co-Culture on the Transwell Membrane**

In order to establish the SMC-EC co-culture on the transwell membrane, the SMCs were seeded on the lower surface of the transwell membrane at different densities, that is, $0.5 \times 10^5$, $1 \times 10^5$, $1.5 \times 10^5$, and $2 \times 10^5$ cells/well, and the cells were cultured for 24 h. As shown in Fig. 1A, SMCs at $1.5 \times 10^5$ cells/well cultured for 24 h reached nearly 100% confluence (Fig. 1A). After that, the ECs were seeded on the upper surface of the transwell membrane at the densities of $1 \times 10^5$ and $2 \times 10^5$ cells/well. After further culturing for 8 h, the ECs at $2 \times 10^5$ cells/well showed nearly 100% confluence (Fig. 1B). Based on the above observation, SMCs at $1.5 \times 10^5$ cells/well and ECs at $2 \times 10^5$ cells/well were used in the subsequent studies.

**Optimization of Incubation Duration for the Transwell Invasion Assay in SMC-EC Co-Culture System**

In order to optimize the incubation period for HTR-8/SVneo cells, HTR-8/SVneo cells were transfected with vectors expressing GFP. As shown in Fig. 2A, HTR-8/SVneo cells with GFP-expressing vector transfection showed green fluorescence with a transfection efficiency of ~90%, and the transfection of GFP into HTR-8/SVneo cells had no effect on the cell viability (data not shown). Furthermore, we used GFP-HTR-8/SVneo cells to determine the optimized incubating period for cell invasion in the SMC-EC co-culture membrane. The GFP-HTR-8/SVneo cells at $1.5 \times 10^5$ cells were seeded on the transwell chamber with SMC-EC co-culture membrane and were cultured for 24, 48, and 72 h, respectively. As shown in Fig. 2B, green fluorescence intensity was relatively low in the lower surface of the transwell when the GFP-HTR-8/SVneo cells were cultured for 24 and 48 h, respectively, while the green fluorescence intensity was markedly increased in the lower surface of the transwell membrane when the GFP-HTR-8/SVneo cells were cultured for 72 h. These results suggested that HTR-8/SVneo cells culturing in the SMC-EC co-culture membrane can be used for the transwell invasion assay.
Effects of Hypoxia on the HTR-8/SVneo Cell Invasion in the Transwell Co-Culture System

In order to determine the effects of hypoxia on the invasive potential of HTR-8/SVneo cells, we treated the HTR-8/SVneo cells or the SMC-EC co-cultured cells with CoCl₂ (250 µM) for 6 h, respectively. GFP-HTR-8/SVneo cells with hypoxia treatment for 6 h showed enhanced invasive potential when compared with the normoxia group (Fig. 3A and B). In addition, SMC-EC co-cultured cells exposed to hypoxia had no effect on the invasive potential of the GFP-HTR-8/SVneo cells when compared with the normoxia group (Fig. 3A and B). More importantly, we measured the OPN protein levels in the culture media from hypoxia-treated GFP-HTR-8/SVneo cells and SMC-EC co-culture cells, and OPN protein levels in the culture media from hypoxia-treated GFP-HTR-8/SVneo cells but not the SMC-EC co-culture cells exhibited higher protein levels of OPN than that in the normoxia group (Fig. 3C). These results suggest that OPN may involve in the invasive potential of HTR-8/SVneo cells induced by hypoxia.

Effects of OPN Treatment on the ITGB3 and ITGB5 Expression in the HTR-8 Cells

Furthermore, we treated the HTR-8/SVneo cells with OPN for 6 and 24 h, respectively, and qRT-PCR and Western blot assays were performed to determine the ITGB3 and ITGB5 mRNA and protein expression levels in HTR-8/SVneo cells. As shown in Fig. 4A, OPN treatment for 6 and 24 h both significantly upregulated ITGB3 and ITGB5 mRNA expression when compared with the control group. Consistently, OPN treatment increased the protein levels of ITGB3 and ITGB5 when compared with the control group (Fig. 4B).

CGT Attenuates OPN-Induced Increase in the Invasive Capacity of HTR-8/SVneo Cells

OPN has been shown to promote the HTR-8/SVneo cell invasion and migration, and consistently, we found that OPN treatment significantly promoted the invasion of HTR-8/SVneo cells when compared with the control group (Fig. 5), while treatment with CGT significantly attenuated
the increased invasive potential of HTR-8/SVneo cells treated with OPN (Fig. 5).

**CGT Attenuates OPN-Induced Increase in the Invasive Capacity of HTR-8/SVneo in the Co-Culturing System**

Furthermore, we tested the effects of OPN treatment on the invasive potential of GFP-HTR-8/SVneo cells in the co-culturing system. As shown in Fig. 6, OPN treatment significantly promoted the invasion of GFP-HTR-8/SVneo cells in the co-culturing system when compared with the control group (Fig. 6). Expectedly, CGT treatment attenuated the OPN-induced increase in the invasive capacity of HTR-8/SVneo cells in the co-culturing system (Fig. 6).

**Discussion**

Preeclampsia is a pregnancy disorder, whereas the underlying mechanisms and etiological factors of this complication remain elusive. Studies have reported that decreased invasiveness of trophoblast cells, immunity disorder in the
maternal–fetal interface, and oxidative stress may contribute to preeclampsia development. In the present study, we firstly co-cultured the SMCs and ECs to mimic the decidua and myometrium interface and examined the invasive potential of trophoblasts in the SMC-EC co-culturing system. Our results showed that trophoblast cells after hypoxia treatment showed enhanced invasive potential in the SMC-EC co-culturing system. In addition, hypoxia treatment significantly increased the OPN levels in the culture media from HTR-8/SVneo cells. More importantly, OPN treatment upregulated ITGB3 and ITGB5 expression in HTR-8/SVneo cells and promoted HTR-8/SVneo cell invasion in the transwell invasion system and SMC-EC co-culturing system. Mechanistically, treatment with integrin αvβ3 inhibitor significantly attenuated the enhanced invasive potential of HTR-8/SVneo cells treated with OPN in the SMC-EC co-culturing system. Collectively, these results suggested the potential role of OPN and integrin αvβ3 in the pathophysiology of preeclampsia.

The SMC-EC co-culturing systems have been recently applied in the investigation of cardiovascular diseases. Zheng et al. found that the phenotype of vascular SMCs co-cultured with endothelial cells is modulated by platelet-derived growth factor receptor beta/IQ motif containing GTPase activating protein 1 signaling in lipopolysaccharides-induced intravascular injury. In the aspect of preeclampsia studies, Dunk et al. developed an in vitro co-culture system in which first trimester villous explants are cultured at low oxygen tension in contact with 2-mm² sections of decidua parietalis from the same patient. Alison et al. cultured endothelial cells and vascular SMCs in hanging droplets to form spheroids representing an inverted vessel lumen and found that trophoblast-induced changes in C-X-C Motif chemokine 10 expression contributed to vascular SMC dedifferentiation during spiral artery remodeling. In our study, the ECs and SMCs were co-cultured in the upper and lower surface of the transwell, respectively, to mimic the decidua and myometrium interface, and the transwell invasion results showed that HTR-8/SVneo cells required at least 72 h to invade through the co-cultured layers, while in most of the transwell invasion assays, 24 h incubation period is sufficient for the HTR-8/SVneo cells invading through the Matrigel-coated membranes. The collective evidence suggested that the co-cultured layers have an impact on the invasive capacity of the HTR-8/SVneo cells. In early pregnancy, hypoxia is the key factor that affects trophoblast proliferation, invasion, and migration. Trophoblasts treated with 24 h hypoxia showed an enhanced invasion and migration. In our study, we showed that treating trophoblast with 6 h hypoxia did not affect the cell proliferation (data not shown), but induced an increase in the invasive capacity of trophoblasts; while treating SMC-EC co-culturing system had no effect on the trophoblast invasion, suggesting that inducting trophoblasts with hypoxia promotes trophoblast invasion in the SMC-EC co-culturing system.

OPN is a class of extracellular matrix glycoprotein and has been reported to play an important role in regulating the
invasiveness of trophoblast cells\textsuperscript{24,25}. In this study, we showed that OPN protein levels were increased in the culture media from the hypoxia-treated trophoblasts, but not in the hypoxia-treated SMC-EC co-culture, suggesting that increased OPN protein levels may be related to the enhanced invasive activity of trophoblasts. In the subsequent studies, we confirmed that OPN treatment could increase the invasive capacity of trophoblasts in the transwell invasion assay and the SMC-EC co-culturing system. In order to further elucidate the OPN-mediated trophoblast invasion, we performed the qRT-PCR and Western blot analysis and found that OPN treatment upregulated ITGB3 and ITGB5 expression in the trophoblasts. As ITGB3 is a key component of $\alpha\text{v}\beta3$ and $\alpha\text{v}\beta5$ is expressed on the surface of trophoblast cells\textsuperscript{26,28}, we speculated that $\alpha\text{v}\beta3$ may involve in OPN-mediated trophoblast invasion. OPN treatment for 6 h did not affect the cell proliferative potential of the trophoblasts in our study (data not shown). Furthermore, we found that pretreatment with $\alpha\text{v}\beta3$ inhibitor significantly attenuated the OPN-induced increase in the invasive capacity of trophoblasts in the transwell invasion assay and the SMC-EC co-culturing system. Collectively, these results may imply that OPN increased trophoblast invasion at least via targeting $\alpha\text{v}\beta3$.

Our findings are still at the preliminary stage and have several limitations. First, the current investigation is limited to the \textit{in vitro} culturing studies to mimic the decidua–myometrium interface, and future studies need to characterize this model as representative \textit{in vivo} situation in the human. Second, our study used the MOVAS line and human hepatic venous ECs, and future studies may consider to use the human primary ECs as well as vascular SMCs to further confirm our findings. Third, the effects of OPN on the cell apoptosis and oxidative stress of the HTR-8/SVneo are unknown, which still requires further studies to confirm the mechanistic actions of OPN on the trophoblasts by using the SMC-EC co-culturing system.

In conclusion, our study, for the first time, established the SMC-EC co-culturing system to examine the invasive potential of trophoblasts. Our results indicated that OPN promoted the invasive capacity of trophoblasts via at least targeting $\alpha\text{v}\beta3$ in the EC-SMC co-culturing system. Future studies were required to further validate the EC-SMC co-culturing system and to determine the molecular mechanisms of OPN-mediated trophoblast invasion.

**Authors’ Contributions**

JX conceived the study. RK and LZ designed the experiments and wrote the manuscript. RK and FZ analyzed the data and revised the manuscript.
Ethical Approval
This study was approved by our institutional review board.

Statement of Human and Animal Rights
This article does not contain any studies with human or animal subjects.

Statement of Informed Consent
There are no human subjects in this article and informed consent is not applicable.

Declaration of Conflicting Interests
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References
1. Mol BWJ, Roberts CT, Thangaratnam S, Magee LA, de Groot CJM, Hofmeyr GJ. Pre-eclampsia. Lancet. 2016;387(10022):999–1011.
2. Correa PJ, Palmeiro Y, Soto MJ, Ugarte C, Illanes SE. Etiopathogenesis, prediction, and prevention of preeclampsia. Hypertens Pregnancy. 2016;35(3):280–294.
3. Rana S, Lemoine E, Granger JP, Karumanchi SA. Preeclampsia: pathophysiology, challenges, and perspectives. Circ Res. 2019;124(7):1094–1112.
4. Fisher SJ. Why is placental abruption in preeclampsia? Am J Obstet Gynecol. 2015;213(4 Suppl):S115–122.
5. Icer MA, Gezmen-Karadag M. The multiple functions and mechanisms of osteopontin. Clin Biochem. 2018;59:17–24.
6. Hao C, Cui Y, Owen S, Li W, Cheng S, Jiang WG. Human osteopontin: potential clinical applications in cancer (Review). Int J Mol Med. 2017;39(6):1327–1337.
7. Castello LM, Raineri D, Salmi L, Clemente N, Vasciottto R, Quaglia M, Garzaro M, Gentili S, Navalesi P, Cantaluppi V, Dianzani U, et al. Osteopontin at the crossroads of inflammation and tumor progression. Mediators Inflamm. 2017;2017:4049098.
8. Pang X, Gong K, Zhang X, Wu S, Cui Y, Qian BZ. Osteopontin as a multifaceted driver of bone metastasis and drug resistance. Pharmacol Res. 2019;144:235–244.
9. Rittling SR, Singh R. Osteopontin in Immune-mediated diseases. J Dent Res. 2015;94(12):1638–1645.
10. Zhao H, Chen Q, Alam A, Cui J, Suen KC, Soo AP, Eguchi S, Gu J, Ma D. The role of osteopontin in the progression of solid organ tumour. Cell Death Dis. 2018;9(3):356.
11. Qi QR, Xie QZ, Liu XL, Zhou Y. Osteopontin is expressed in the mouse uterus during early pregnancy and promotes mouse blastocyst attachment and invasion in vitro. PLoS One. 2014;9(8):e104955.
12. Wu LZ, Liu XL, Xie QZ. Osteopontin facilitates invasion in human trophoblastic cells via promoting matrix metalloproteinase-9 in vitro. Int J Clin Exp Pathol. 2015;8(11):14121–14130.
13. Frank JW, Seo H, Burghardt RC, Bayless KJ, Johnson GA. ITGAV (alpha v integrins) bind SPP1 (osteopontin) to support trophoblast cell adhesion. Reproduction. 2017;153(5):695–706.
14. Xia J, Qiao F, Su F, Liu H. Implication of expression of osteopontin and its receptor integrin alphabeta3 in the placenta in the development of preeclampsia. J Huazhong Univ Sci Technol Med Sci. 2009;29(6):755–760.
15. Sibai B, Dekker G, Kupfermince M. Pre-eclampsia. Lancet. 2005;365(9461):785–799.
16. Steegers EA, van Dadelnsen P, Duvekot JJ, Pijnenborg R. Pre-eclampsia. Lancet. 2010;376(9741):631–644.
17. Williamson RD, McCarthy C, McCarthy FP, Kenny LC. Oxidative stress in pre-eclampsia; have we been looking in the wrong place? Pregnancy Hypertens. 2017;8:1–5.
18. Patsch C, Challet-Meylan L, Thoma EC, Uriech E, Heckel T, O’Sullivan JF, Grainger SJ, Kapp FG, Sun L, Christensen K, Xia Y, et al. Generation of vascular endothelial and smooth muscle cells from human pluripotent stem cells. Nat Cell Biol. 2015;17(8):994–1003.
19. Scott RA, Ramaswamy AK, Park K, Panitch A. Decorin mimic promotes endothelial cell health in endothelial monolayers and endothelial-smooth muscle co-cultures. J Tissue Eng Regen Med. 2017;11(5):1365–1376.
20. Zheng X, Hu X, Zhang W. The phenotype of vascular smooth muscle cells co-cultured with endothelial cells is modulated by PDGFR-beta/IQGAP1 signaling in LPS-induced intravascular injury. Int J Med Sci. 2019;16(8):1149–1156.
21. Dunk C, Petkovic I, Baczzyk D, Rossant J, Winterhager E, Lye S. A novel in vitro model of trophoblast-mediated decidual blood vessel remodeling. Lab Invest. 2003;83(12):1821–1828.
22. Wallace AE, Cartwright JE, Begum R, Laing K, Thilaganathan B, Whitley GS. Trophoblast-induced changes in C-x-C motif chemokine 10 expression contribute to vascular smooth muscle cell dedifferentiation during spiral artery remodelling. Arterioscler Thromb Vasc Biol. 2013;33(3):e93–e101.
23. Zhu J, Wang K, Li T, Chen J, Xie D, Chang X, Yao J, Wu J, Zhou Q, Jia Y, Duan T. Hypoxia-induced TET1 facilitates trophoblast cell migration and invasion through HIF1alpha signaling pathway. Sci Rep. 2017;7(1):8077.
24. Johnson GA, Burghardt RC, Bazer FW, Spencer TE. Osteopontin: roles in implantation and placentation. Biol Reprod. 2003;69(5):1458–1471.
25. Briese J, Oberndorfer M, Patschenik C, Schulte HM, Makri- giannakakis A, Loning T, Bamberger AM. Osteopontin is colocalized with the adhesion molecule CEACAM1 in the extravillous trophoblast of the human placenta and enhances invasion of CEACAM1-expressing placental cells. J Clin Endocrinol Metab. 2005;90(9):5407–5413.
26. Paparini D, Grasso E, Calo G, Vota D, Hauk V, Ramhorst R, Leiros CP. Trophoblast cells primed with vasoactive intestinal peptide enhance monocyte migration and apoptotic cell clearance through alphavbeta3 integrin portal formation in a model of maternal-placental interaction. Mol Hum Reprod. 2015; 21(12):930–941.

27. Rout UK, Wang J, Paria BC, Armant DR. Alpha5beta1, alphaVbeta3 and the platelet-associated integrin alphallbbeta3 coordinately regulate adhesion and migration of differentiating mouse trophoblast cells. Dev Biol. 2004;268(1):135–151.

28. Schmitz C, Yu L, Bocca S, Anderson S, Cunha-Filho JS, Rhavi BS, Oehninger S. Role for the endometrial epithelial protein MFG-E8 and its receptor integrin alphavbeta3 in human implantation: results of an in vitro trophoblast attachment study using established human cell lines. Fertil Steril. 2014;101(3): 874–882.