Hypertension Research (2021) 44:318–324
https://doi.org/10.1038/s41440-020-00561-6

ARTICLE

Abnormal cGMP-dependent protein kinase I-mediated decidualization in preeclampsia

Jingsi Chen[1,2,3,4,5] · Wenlu Ren[1,3,4,5] · Lin Lin[1,3,4,5] · Shanshan Zeng[1,3,4,5] · Lijun Huang[1,3,4,5] · Jingman Tang[1,3,4,5] · Shilei Bi[1,3,4,5] · Jiahong Pan[1,3,4,5] · Dunjin Chen[1,3,4,5] · Lili Du[1,3,4,5]

Received: 29 June 2020 / Revised: 27 August 2020 / Accepted: 5 September 2020 / Published online: 23 October 2020

Abstract

Defective decidual function contributes to the pathogenesis of preeclampsia. However, the precise mechanism of defective decidua during preeclampsia has not been characterized. During decidualization, endometrial stromal cells undergo phenotypic changes that are consistent with mesenchymal-epithelial transition (MET). cGMP-dependent kinase protein I (PKGI)/VASP signaling is important in cell motility proliferation, differentiation and cell adhesion. To investigate this aim, we analyzed PKGI levels, phosphorylated VASP protein levels, and eNOS and sGC protein expression levels during pregnancy complicated by preeclampsia, which indicated that PKGI/VASP signaling function is decreased by the condition. Moreover, we evaluated the differential expression of genes that regulate MET in the decidua resulting from preeclampsia and healthy pregnancies. We discovered that vimentin mRNA levels are decreased in the decidua of preeclampsia, which indicates that excessive MET occurs in the decidua of preeclampsia pregnancies. A fundamental developmental MET program occurred in response to signaling pathways. These results suggest the important role of decreased PKGI/VASP signaling during excessive MET in the pathogenesis of preeclampsia.

Keyword PKGI · VASP · Mesenchymal-epithelial transition · Decidualization · Preeclampsia

Introduction

Preeclampsia is one of the leading causes of maternal and perinatal mortality [1, 2]. Abnormal placentation plays an important role in preeclampsia [3]. Although past work indicated that deficient cytotrophoblast invasion of uterine spiral arterioles is an important characteristic of pre-eclampsia placenta, recent studies observed that defective decidualization also contributes to abnormal placentation in preeclampsia, which also decreased invasion of trophoblast cells [4, 5]. During decidualization, endometrial stromal cells undergo phenotypic changes in a manner that is consistent with mesenchymal-epithelial transition (MET) [6], which appears to be critical for decidualization during normal pregnancies [7]. However, little information exists about how MET in decidual stromal cells might be disrupted during placentation in patients with preeclampsia.

Nitric oxide (NO)/guanosine 3',5'-cyclic monophosphate (cGMP) signaling also plays an important role in human placentation. The NO/cGMP signaling pathway regulates trophoblast invasion and placentation in preeclampsia [8, 9]. NO stimulation of soluble guanylyl cyclase (sGC) activity has been found to induce extravillous trophoblast differentiation

These authors contributed equally: Jingsi Chen, Wenlu Ren

✉ Dunjin Chen
gzdrchen@gzhmu.edu.cn

✉ Lili Du
lilidugysy@gzhmu.edu.cn

[1] Department of Obstetrics and Gynecology, The Third Affiliated Hospital of Guangzhou Medical University, Guangzhou 510150, China

[2] Department of Fetal Medicine and Prenatal Diagnosis, The Third Affiliated Hospital of Guangzhou Medical University, Guangzhou 510150, China

[3] Obstetrics & Gynecology Institute of Guangzhou, Guangzhou 510150, China

[4] Key Laboratory of Reproduction and Genetics of Guangdong Higher Education Institutes, Guangzhou 510150, China

[5] Key Laboratories for Major Obstetric Diseases of Guangdong Province, Guangzhou 510150, China

specifically through downstream cGMP signaling involving cGMP-dependent protein kinase I (PKGI) [10]. PKGI acts as a key mediator of the NO/cGMP signaling pathway and has two isoforms, PKGIα and PKGIβ. *PRKG1* is a protein-coding gene of PKGI. Vasodilator stimulating phosphoprotein (VASP) is a PKGI target. A previous study indicated that PKGIα and VASP were involved in placenta artery relaxation function [11]. VASP has been reported to be phosphorylated by PKGI, which participates in cell adhesion and motility [12]. Moreover, VASP also plays an important role in human placental development [13].

In other cell types, previous studies indicated that NO/cGMP/PKGI signaling pathways have an important role in modulating mesenchymal-epithelial transition [14, 15]. Although NO and cGMP are reported to be produced at the blastocyst implantation site, which prepares the endometrium for receptivity [16], whether NO/cGMP signaling regulates mesenchymal-epithelial transition in decidual cells of preeclampsia through PKGI has not been elucidated. In our studies, VASP was used as a functional marker of PKGI disruption. We tested the hypothesis that PKGI/VASP signaling is abnormal in MET-induced aberrant decidualization of preeclampsia.

Methods

Patients and decidual sample collection

The study was carried out in the Third Affiliated Hospital of Guangzhou Medical University with explicit approval by the institutional ethics committee. All patients provided informed signed consent for the study. Twenty-eight preeclampsia patients who terminated by cesarean section were enrolled in the preeclampsia group (PE group). Preeclampsia was diagnosed using the guidelines of the American College of Obstetricians and Gynecologists [17]. The normal group consisted of twenty-eight healthy pregnant women who delivered by cesarean section due to obstetric indications (e.g., breech presentation, maternal request or previous cesarean section). Patients with chronic hypertension, maternal diabetes, multiple pregnancies, and fetuses with abnormalities were excluded from the study.

Decidua basalis tissues were collected from the placenta bed immediately at the time of cesarean section delivery, as described previously [5]. After being washed with sterilized saline, the tissues were frozen in liquid nitrogen immediately and stored at −80 °C until analysis.

RNA microarray

RNA was obtained from decidua and analyzed using Agilent 8×60K RNA microarray. Briefly, total RNA was extracted from human decidual tissue using the Ribo Pure Kit (Thermo Fisher, Scientific Inc.) and purified using the QIAGEN RNeasy® Mini Kit (QIAGEN). The quality and quantity of the RNA samples were measured using the Nanodrop 2000C and Agilent BioAnalyzer 2100 instruments, respectively. An Agilent amplification and labeling method was adopted. Appropriate numbers of probes were taken for hybridization. The hybridization method was at 65 °C for 17 h of rolling hybridization, and the film was washed at room temperature. The results were scanned by an Agilent C high-resolution scanner. Agilent Feature Extraction software read the data (scan resolution 5 μm, PMT 100%), and finally, feature extraction was used for normalization. Genes with P values less than 0.05 were selected.

Cell culture

Human endometrial stromal cells (HESCs) were purchased from the American Type Culture Collection (ATCC^{R} CRL-4003^{TM}) and cultured according to the manufacturer's instructions. Briefly, HESCs were cultured in DMEM/F12 (Sigma) supplemented with 10% charcoal-stripped fetal bovine serum (CS-FBS, Biological Industries) at 37 °C in a humidified chamber with 5% CO2. After seeding in 6-well plates, cells were serum-restricted overnight using medium containing 2% CS-FBS. Cells were approximately 30% confluent in each well. Then, they were divided randomly into different treatment groups. For the PKGI activator 8-Br-cGMP groups, cells were treated using medium containing 100 μM 8-Br-cGMP for 24 h. Next, to induce decidualization, HESCs were treated with medium containing 1 μM medroxyprogesterone 17-acetate (MPA, Sigma) and 0.5 mM dibutyryl cAMP (dbcAMP, Sigma) as described previously [18] for 5 days. Cells in the PKGI inhibitor Rp-8-pCPT-cGMP groups were treated with medium containing 10 μM Rp-8-pCPT-cGMP for 6 h. Subsequently, decidualization was induced by the methods as stated above. During decidualization treatment, the medium was changed every 48 h. After 5 days of decidualization treatment, the confluence of cells was over 90%, and cells were collected for analysis.

Immunohistochemistry

Decidua tissues were fixed in 10% buffered formalin for 24 h before paraffin embedding, sectioning and rehydration, as described above. Subsequently, the sections underwent antigen retrieval using EDTA, inhibition of endogenous peroxidase, and treatment with 5% goat serum. The tissue sections were then incubated overnight at 4 °C with primary antibody for PKGI (1:100; Proteintech), which recognized PKGIα and PKGIβ, or vimentin (1:100; Proteintech). After

extensive washing with PBS, horseradish peroxidase-conjugated secondary antibody was used to visualize the antibody signal with diaminobenidine using an immunohistochemistry detection kit (DAB, NeoBioscience). The sections were counterstained with diluted hematoxylin and examined using bright-field microscopy. Quantitative analysis was performed using Image-Pro Plus software.

Detection of protein expression using immunoblotting

Soluble proteins were collected from decidual tissues or cultured cells, disrupted using a reducing lysis buffer, resolved using SDS-PAGE, and electroblotted onto polyvinylidene difluoride membranes. Subsequently, the membranes were blocked using 5% bovine serum albumin and were exposed to primary and secondary antibodies. The antigen-antibody complexes were detected using enhanced chemiluminescence and visualized using a charge-coupled device camera-gel imaging system (Bio-Rad, ChemiDoc XRS+). Anti-E-cadherin, anti-vimentin, anti-PKGI, and anti-GAPDH were purchased from Proteintech Inc. (Chicago, USA). Anti-sGCα1, anti-eNOS and anti-beta-actin were purchased from Abcam Inc. (Cambridge, UK). p-VASP (Ser239) antibody was purchased from Cell Signaling Technology, Inc (Danvers, USA). The data were analyzed using the computer software ImageJ. Quantitative band densitometry was conducted in ImageJ software. The relative protein expression ratio was determined by comparing the target protein densities with that of the reference protein, GAPDH or beta-actin.

Statistical analysis

All experiments were repeated at least three times, yielding similar results. Representative images and summative data are shown. The results were analyzed by SPSS software (version 19.0, SPSS Inc., Chicago, IL, USA), and the quantitative data were reported as the mean ± standard deviation (SD). Comparisons between the control group and PE group were estimated by using the independent samples t-test. For multiple subgroup comparisons, one-way analysis of variance (ANOVA) was performed. When indicated, Tukey's range test was performed post hoc. In all cases, P values ≤ 0.05 were considered significant.

Results

Clinical characteristics of study pregnancies

The clinical characteristics of our studies are shown in Table 1. There was no statistically significant difference in maternal age weeks between the normal group and PE group ($P = 0.64$). Compared to 32.6 ± 0.7 weeks in the PE group, the average gestational age at delivery of the control group was 39.1 ± 0.2 weeks ($P < 0.0001$). The systolic blood pressure (SBP) and diastolic blood pressure (DBP) in the PE group were higher than those in the control group ($P < 0.0001$). The proteinuria was negative in the normal group, whereas the urine protein per 24 h was 3 ± 4.56 g in the PE group.

Measurement of PKGI expression in the decidua of preeclampsia

To identify differences in the expression levels of genes thought to play a primary role in causing MET-like changes in preeclampsia decidua, RNA microarray was performed. First, we detected a wide variation in the expression of MET candidate mRNAs in the tissues within the clinical groups. The mRNA microarray data suggested that *PRKG1* was expressed at a lower level in the PE group than in the control group (Fig. 1a). Using the probe ENST00000373976 in mRNA microarry, we detected the PKGIβ isoform of PKGI. Second, we tested whether the decreased mRNA was associated with decreased PKGI protein expression using immunoblot (Fig. 1b) and immunohistochemistry (Fig. 1c). To verify this result via IB quantification analysis and IHC, the protein levels of PKGI were decreased in the PE groups compared with the control group ($P \le 0.05$). The staining intensities of PKGI were higher in the control group than in the PE group, which was consistent with the IB results. The reduced PKGI levels were specifically mapped to stromal cells by immunohistochemistry.

Table 1 Clinical characteristics of patients

	Normal group ($n = 28$)	PE group ($n = 28$)	P
Age (years)	29.9 ± 0.6	29.5 ± 0.8	0.64
Gestational weeks (weeks)	39.1 ± 0.2	32.6 ± 0.7	<0.0001
Systolic pressure (mmHg)	119.3 ± 1.74	165.7 ± 2.4	<0.0001
Diastolic pressure (mmHg)	77.7 ± 1.4	105.7 ± 2.0	<0.0001
Urine protein/24 h (g)	Not detected	3 ± 4.56	–

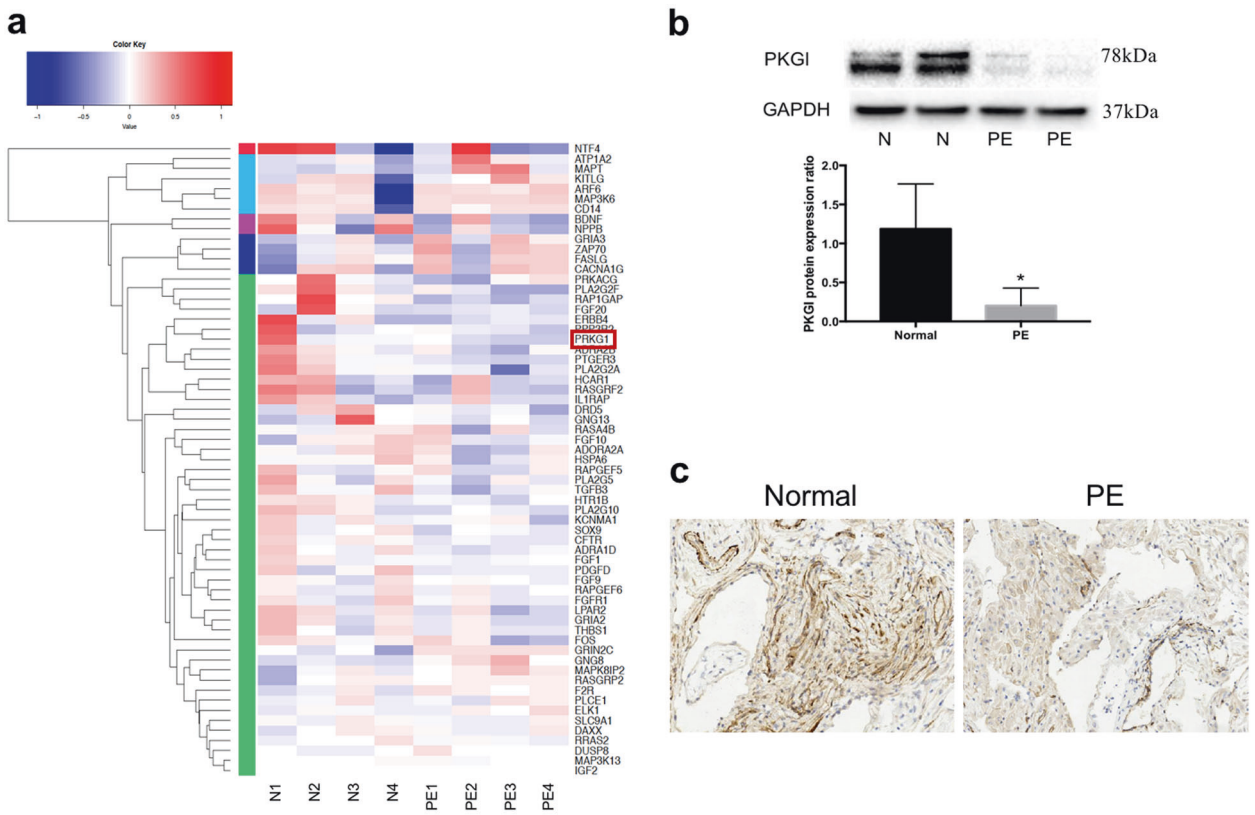

Fig. 1 PKGI expression in decidua: **a** Heatmap plot of genes differentially expressed in normal groups (N) and preeclampsia groups (PE) decidua tissue by detection of RNA microarray. There were four cases in normal groups and four cases in PE group; **b** The protein level of PKGI in each group. The bar graph showed mean PKGI protein expression (±SD), relative to GAPDH. were determined by densitometry quantification of immunoblots. Representative blots from two random samples in each group. $^*P < 0.05$, $n = 24$; **c** Location of PKGI in the preeclampsia and normal group decidua tissue

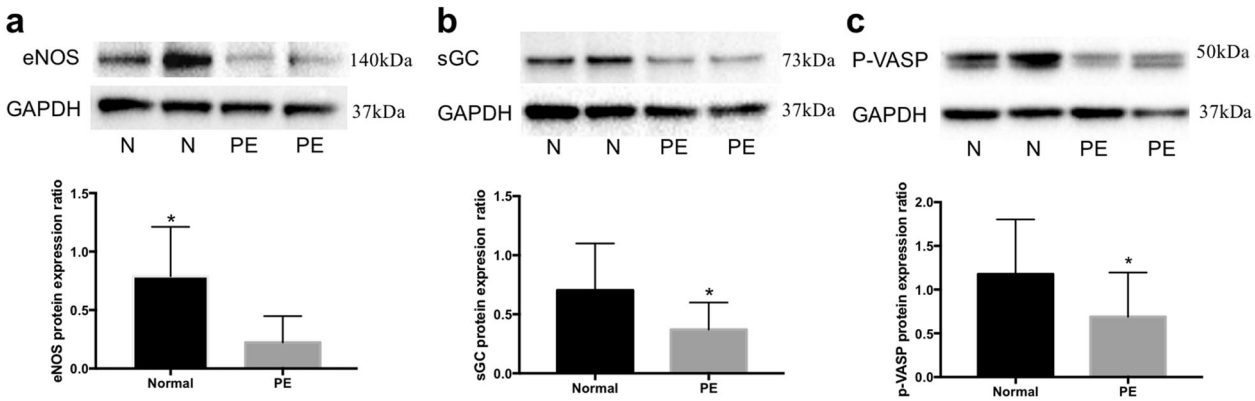

Fig. 2 Immunoblotting analyses of eNOS, sGCα1 and p-VASP in decidua tissues from normotensive pregnancy women and preeclampsia women. **a** Analysis of eNOS protein expression. Values are mean ± SD. $^*P < 0.05$, $n = 24$ each. **b** Measurements of sGCα1 protein expression. Values are mean ± SD. $^*P < 0.05$, $n = 24$ each. **c** Detection of phosphorylated VASP level. Values are mean ± SD. $^*P < 0.05$, $n = 24$ each

Determination of eNOS, sGC, and p-VASP expression

To study whether dysregulated PKGI expression could be associated with decreased proximal NO/cGMP signaling elements and result in decreased downstream NO/cGMP activity, as determined by VASP phosphorylation, eNOS, and sGCα1 protein expression and phosphorylated VASP were detected by immunoblotting. The results are shown in Fig. 2. Compared with the control group, eNOS and sGCα1 protein expression were decreased in the preeclampsia

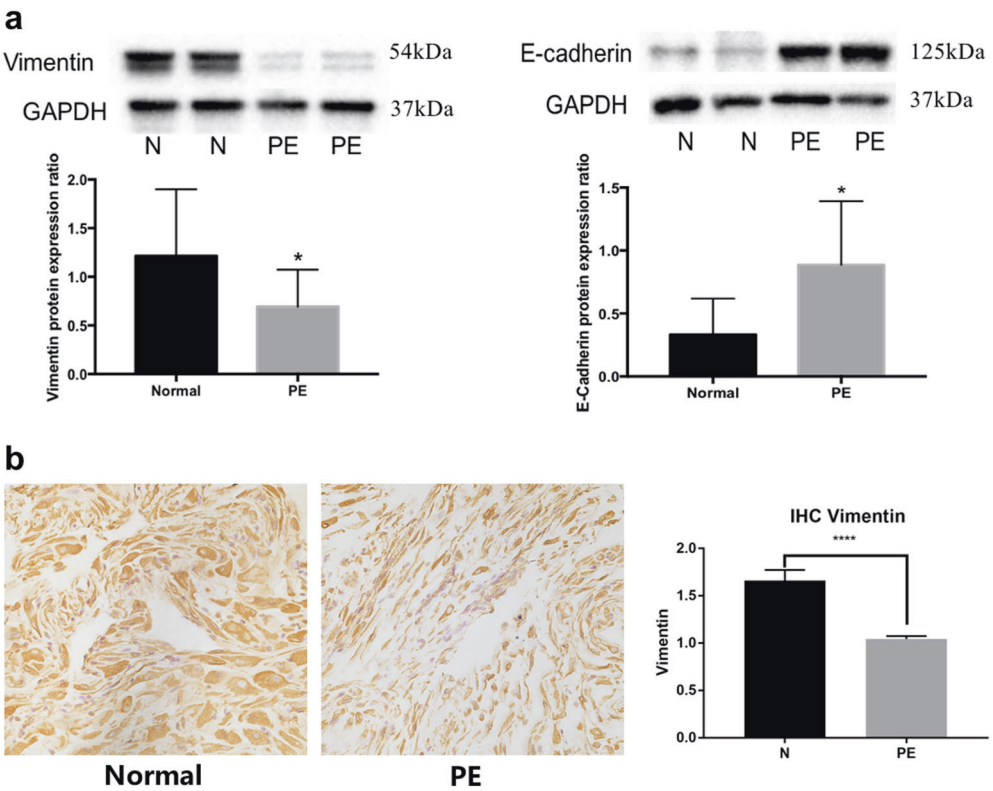

Fig. 3 Analyses of MET-like change in decidua tissues. **a** Measurement of vimentin and E-cadherin protein expression in decidua tissues from normotensive pregnancy women and preeclampsia women by immunoblotting. Values are mean ± SD. $*P < 0.05$, $n = 24$ each. **b** Immunohistochemical staining and quantitative analysis of vimentin. Values are mean ± SD. $*P < 0.05$, $n = 16$ each

group ($P \le 0.05$). Importantly, this downregulation of NO/cGMP signaling enzyme expression was associated with decreased downstream function. This is because the levels of phosphorylated VASP (p-VASP), a prime target of cGMP-activated PKGI, were decreased in the PE group compared with the control group ($P \le 0.05$).

MET-like change in decidual tissue

To detect MET-like changes in the decidual tissue, we measured MET markers by using immunoblotting and immunohistochemistry. As shown in Fig. 3a, vimentin protein expression was decreased in the PE group compared to the normal group ($P \le 0.05$). In contrast, E-cadherin protein expression was increased in the PE group compared with the control group ($P \le 0.05$). As shown in Fig. 3b, immunohistochemistry showed stronger reactivity for vimentin in PE decidual tissue than in the control ($P \le 0.05$).

PKGI mediated p-VASP to regulate MET-like changes in endometrial stromal cells

We next tested whether the disruption of PKGI/VASP signaling observed in stromal cells from preeclampsia patients might have a direct role in regulating MET. We used a standard model of endometrial stromal cell MET [18]. Before decidualization induction by dbcAMP and MPA, HESCs were treated with or without 8-Br-cGMP or Rp-8-pCPT-cGMP, which are membrane-permeable PKGI stimulators and inhibitors, respectively. Subsequently, the protein levels of the MET marker vimentin in the cells were analyzed using immunoblotting analysis. From Fig. 4a, the results showed that vimentin protein expression and p-VASP levels were decreased in the group treated with the PKGI inhibitor Rp-8-pCPT-cGMP compared with the decidualization control group and the PKGI activator 8-Br-cGMP. Additionally, IGFBP1 expression levels decreased significantly under treatment with the PKGI inhibitor (Fig. 4b).

Discussion

Aberrant placentation is known as the main cause of preeclampsia [19]. Although defective decidualization contributing to downregulated trophoblast invasion in preeclampsia has been recently published elsewhere [4], its mechanism remains largely unknown. Our studies indicated that PKGI mediated p-VASP to regulate EMT in the decidualization of preeclampsia.

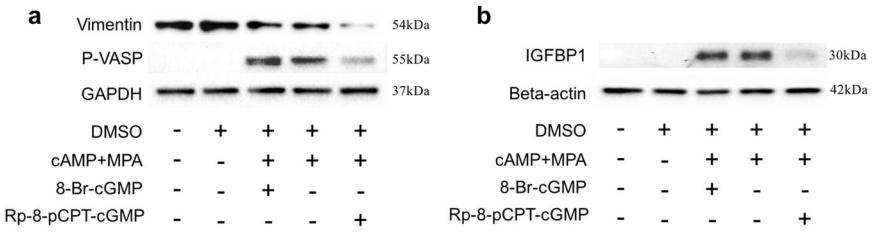

Fig. 4 In vitro, PKGI mediated p-VASP to regulate MET-like change in endometrial stromal cells. HESCs were treated with 8-Br-cGMP or Rp-8-pCPT-cGMP before decidualization induced by MPA and dbcAMP. **a** Expression of vimentin protein level and phosphorylation level. **b** Expression of IGFBP1 protein level. All experiments were repeated six times

NO/cGMP signaling plays an important role in regulating several cell phenotypes [20, 21]. PKGI, a target substrate of NO/cGMP signaling, is a serine/threonine kinase that phosphorylates substrates and is important in tissue contractility, cell motility proliferation, and differentiation [22]. However, little work has been done to determine its role in preeclampsia. Our studies showed decreased PKGI expression in the decidual tissue of preeclampsia compared to healthy pregnancies by using RNA sequencing, immunohistochemistry, and immunoblotting. PKGI expression is classically regulated by cGMP, which is generated by NO activating sGC. NO was generated by different nitric oxide synthase isoforms. The study showed placental reduction of nitric oxide synthase activity in preeclampsia [23]. In our studies, we also found that eNOS and sGCα1 expression was lower in preeclampsia than in healthy pregnancies. We speculated that PKGI expression was downregulated by eNOS- and sGC-mediated NO/cGMP signaling in the decidua of preeclampsia.

VASP has been reported to be phosphorylated by PKGI, which participates in cell adhesion and motility. VASP was found to be involved in trophoblast invasion and placentation [24, 25]. However, few studies on VASP have been carried out in the decidua. In our studies, we found that phosphorylated VASP was lower in the decidua of preeclampsia than in the decidua of healthy pregnancies. In in vitro decidualization experiments, when stromal cells were treated with the PKGI inhibitor Rp-8-pCPT-cGMP followed by decidualization, phosphorylated VASP was decreased compared with or without treatment with the PKGI activator 8-Br-cGMP. These results were consistent with the results of preeclampsia decidua experiments.

Vimentin is a substrate of PKGI [26] and mesenchymal markers of decidual stromal cells. Once implantation begins, the epithelial phenotype of decidualized endometrial stromal cells appears to give way to a more mesenchymal phenotype to allow for sufficient expansion of invading trophoblastic cells [27]. Multiple studies have shown that endometrial stromal cells undergo morphologic and functional changes consistent with the process of MET [28, 29]. In an in vitro decidualization experiment, vimentin, a marker of mesenchymal cells, was downregulated, suggesting that MET existed during decidualization [28]. Our data showed that vimentin was decreased in the decidual tissue of preeclampsia pregnancies compared with healthy pregnancies. In vitro, vimentin expression was decreased after treatment with the PKGI inhibitor compared with or without treatment with the PKGI activator 8-Br-cGMP. This result indicated that excessive MET exists in endometrial stromal cells of preeclampsia, which may induce insufficient room for trophoblast invasion. These results were consistent with the results of the decidualization biomarker IGFBP1, which suggested defective decidualization defects.

Although p-VASP and vimentin were not different with or without PKGI activator, due to insufficient PKGI activator to induce p-VASP and vimentin, our data suggested that PKGI induced MET in the decidua of preeclampsia through phosphorylated VASP.

In conclusion, our study demonstrated that excessive MET exists in endometrial stromal cells of preeclampsia patients, which might be the cause of insufficient trophoblast invasion. Our findings indicated that abnormal NO/cGMP signaling downregulated PKGI expression. PKGI modulated excessive MET by mediating phosphorylated VASP, which might constitute the mechanism of abnormal placentation in preeclampsia.

Acknowledgements This work was supported by the National Key R&D Program of China (No. 2017YFC1001402 and 2018YFC10029002), the National Natural Science Foundation (No. 81830045, 81671533 and 82071652), and the General Program of Guangdong Province Natural Science Foundation (No. 2020A1515010273).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Knight M, Tuffnell D. A view from the UK: the UK and Ireland confidential enquiry into maternal deaths and morbidity. Clin Obstet Gynecol. 2018;61:347–58.

2. Juan L, Xiaohong L, Chuyun K, Yanping W, Rachel KX, CM M, et al. Maternal mortality ratios in 2852 Chinese counties, 1996–2015, and achievement of Millennium Development Goal 5 in China: a subnational analysis of the Global Burden of Disease Study 2016. Lancet. 2019;393:241–52.
3. Haram K, Mortensen JH, Myking O, Roald B, Magann EF, Morrison JC. Early development of the human placenta and pregnancy complications. J Maternal Fetal Neonatal Med. 2020;33:3538–45.
4. Garrido-Gomez T, Dominguez F, Quinonero A, Diaz-Gimeno P, Kapidzic M, Gormley M, et al. Defective decidualization during and after severe preeclampsia reveals a possible maternal contribution to the etiology. Proc Natl Acad Sci USA. 2017;114: E8468–77.
5. Tong J, Zhao W, Lv H, Li W-P, Chen Z, Zhang C. Transcriptomic profiling in human decidua of severe preeclampsia detected by RNA sequencing. J Cell Biochem. 2017;119:607–15.
6. Mori M, Bogdan A, Balassa T, Csabai T, Szekeres-Bartho J. The decidua-the maternal bed embracing the embryo-maintains the pregnancy. Semin Immunopathol. 2016;38:635–49.
7. Patterson AL, Zhang L, Arango NA, Teixeira J, Pru JK. Mesenchymal-to-epithelial transition contributes to endometrial regeneration following natural and artificial decidualization. Stem Cells Dev. 2013;22:964–74.
8. Rutherford RA, McCarthy A, Sullivan MH, Elder MG, Polak JM, Wharton J. Nitric oxide synthase in human placenta and umbilical cord from normal, intrauterine growth-retarded and pre-eclamptic pregnancies. Br J Pharmacol. 1995;116:3099–109.
9. Sladek SM, Magness RR, Conrad KP. Nitric oxide and pregnancy. Am J Physiol. 1997;272:R441–63.
10. Bolnick JM, Kilburn BA, Bolnick AD, Diamond MP, Singh M, Hertz M, et al. Sildenafil stimulates human trophoblast invasion through nitric oxide and guanosine 3',5'-cyclic monophosphate signaling. Fertil Steril. 2015;103:1587–95. e1581–82.
11. Dordea AC, Sweeney M, Taggart J, Lartey J, Wessel H, Robson SC, et al. Differential vasodilation of human placental and myometrial arteries related to myofilament Ca(2+)-desensitization and the expression of Hsp20 but not MYPT1. Mol Hum Reprod. 2013;19:727–36.
12. Lund N, Henrion D, Tiede P, Ziche M, Schunkert H, Ito WD. Vimentin expression influences flow dependent VASP phosphorylation and regulates cell migration and proliferation. Biochemical biophysical Res Commun. 2010;395:401–6.
13. Kayisli UA, Demir R, Erguler G, Arici A. Vasodilator-stimulated phosphoprotein expression and its cytokine-mediated regulation in vasculogenesis during human placental development. Mol Hum Reprod. 2002;8:1023–30.
14. Chu S, Zhang X, Sun Y, Liang Y, Sun J, Lu M, et al. Atrial natriuretic peptide inhibits epithelial-mesenchymal transition (EMT) of bronchial epithelial cells through cGMP/PKG signaling by targeting Smad3 in a murine model of allergic asthma. Exp lung Res. 2019;45:245–54.
15. Vyas-Read S, Shaul PW, Yuhanna IS, Willis BC. Nitric oxide attenuates epithelial-mesenchymal transition in alveolar epithelial cells. Am J Physiol Lung Cell Mol Physiol. 2007;293:L212–21.
16. Duran-Reyes G, Gomez-Melendez MR, Morali-de la Brena G, Mercado-Pichardo E, Medina-Navarro R, Hicks-Gomez JJ. Nitric oxide synthesis inhibition suppresses implantation and decreases cGMP concentration and protein peroxidation. Life Sci. 1999;65:2259–68.
17. ACOG Practice Bulletin No. 202: gestational hypertension and preeclampsia. Obstet Gynecol. 2019;133:e1–25.
18. Teklenburg G, Salker M, Molokhia M, Lavery S, Trew G, Aojanepong T, et al. Natural selection of human embryos: decidualizing endometrial stromal cells serve as sensors of embryo quality upon implantation. PloS ONE. 2010;5:e10258.
19. Burton GJ, Redman CW, Roberts JM, Moffett A. Pre-eclampsia: pathophysiology and clinical implications. BMJ. 2019;366:l2381.
20. McCauley SD, Gilchrist M, Befus AD. Nitric oxide: a major determinant of mast cell phenotype and function. Mem Inst Oswaldo Cruz. 2005;100:11–14.
21. Cokic VP, Schechter AN. Effects of nitric oxide on red blood cell development and phenotype. Curr Top Dev Biol. 2008;82: 169–215.
22. Wolfertstetter S, Huettner JP, Schlossmann J. cGMP-dependent protein kinase inhibitors in health and disease. Pharmaceuticals. 2013;6:269–86.
23. Brennecke SP, Gude NM, Di Iulio JL, King RG. Reduction of placental nitric oxide synthase activity in pre-eclampsia. Clin Sci. 1997;93:51–55.
24. Kayisli UA, Selam B, Demir R, Arici A. Expression of vasodilator-stimulated phosphoprotein in human placenta: possible implications in trophoblast invasion. Mol Hum Reprod. 2002;8:88–94.
25. Ali M, Rogers LK, Heyob KM, Buhimschi CS, Buhimschi IA. Changes in vasodilator-stimulated phosphoprotein phosphorylation, Profilin-1, and Cofilin-1 in accreta and protection by DHA. Reprod Sci. 2019;26:757–65.
26. TML TAWyatt, Pryzwansky KB. Vimentin is transiently co-localized with and phosphorylated by cyclic GMP-dependent protein kinase in formylpeptide-stimulated neutrophils. J Biol Chem. 1991;266:21274–80.
27. Owusu-Akyaw A, Krishnamoorthy K, Goldsmith LT, Morelli SS. The role of mesenchymal-epithelial transition in endometrial function. Hum Reprod Update. 2019;25:114–33.
28. Zhang XH, Liang X, Liang XH, Wang TS, Qi QR, Deng WB, et al. The mesenchymal-epithelial transition during in vitro decidualization. Reprod Sci. 2013;20:354–60.
29. Patterson AL, Pirochta J, Tufano SY, Teixeira JM. Gain-of-function beta-catenin in the uterine mesenchyme leads to impaired implantation and decidualization. J Endocrinol. 2017;233:119–30.