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CD133+/C-kit+Lin$^-$ endothelial progenitor cells in fetal circulation demonstrate impaired differentiation potency in severe preeclampsia

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ABSTRACT

Objectives: Individuals delivered from preeclamptic pregnancies exhibit a long-term increased risk of developing cardiovascular and metabolic diseases, likely caused by aberrant fetal cell reprogramming incurred *in utero*. The present study investigated the functional impairment and epigenetic changes exhibited by endothelial progenitor cells derived from offspring born to preeclamptic pregnancies.

Study design: The capacity of CD133$^+$/C-kit$^+$/Lin$^-$ (CKL$^-$) human umbilical cord blood endothelial progenitor cells (EPCs) derived from gestationally matched normal and preeclamptic (n = 10 each) pregnancies to differentiate to form outgrowth endothelial cells (OECs) was assessed by observing both their morphology, and the number and size of generated OECs colonies. Likewise, OECs angiogenic function was evaluated via migration, adhesion, and tube-formation assays. EPCs from preeclampsia were cultured in normal-, and preeclampsia-derived serum-conditioned media to assess the effects of environmental factors on EPC differentiation potency and OEC angiogenic function, and finally, EPCs H3K4, H3K9, and H3K27 trimethylation levels were assayed.

Results: The preeclampsia-derived CKL$^-$ EPCs exhibited decreased H3K4 and H3K9 trimethylation levels, significantly delayed differentiation times, and a significant reduction in both their number of generated OECs colonies, and exhibited **reduced** OECs migration, adhesion, and tube formation activities compared to those achieved by the normal-derived EPCs. Interestingly, the reduced differentiation potency of the preeclampsia-derived EPCs was not rescued via exposure to normal serum.

Conclusions: Exposure to preeclampsia significantly and irreversibly reduced CKL$^-$ EPC differentiation potency and OEC angiogenic function, likely reflecting incurred irreversible epigenetic changes.

1. Introduction

The results of recent studies investigating the potential long-term health impacts of exposure to preeclamptic gestational conditions suggest that individuals exposed to maternal preeclampsia *in utero* exhibit an increased risk of developing cardiovascular and/or metabolic diseases such as hypertension, type 2 diabetes mellitus (DM), and metabolic syndrome [1–3]. The increased levels of inflammation, anti-angiogenic factor production, and autoimmune response stimulation-characteristics of preeclampsia influence placental permeability, and thus also disrupt normal levels of serum-soluble factors, such as cytokines, soluble fms-like tyrosine kinase-1 (sFLT1), and reactive oxygen species (ROS) [4,5]. It has been suggested that these effects of preeclampsia likely disrupt normal epigenetic changes and/or cellular remodeling processes during embryogenesis, and thereby incur the observed increase in long-term disease risk [3].

Endothelial progenitor cells (EPCs) differentiate from hemangioblast, and express CD133 and C-kit cell-surface markers [6]. They have the potential to differentiate to form the outgrowth endothelial cells (OECs) that direct neovascularization during embryogenesis; however, circulating EPCs are also capable of differentiating to form fibroblast-mimic cells and thus enable vascular damage repair [7]. Many studies

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have reported decreased circulating EPC levels in the context of human cardiovascular diseases such as coronary artery disease, diabetic vasculopathy, atherosclerosis, systemic lupus erythematosus, and metabolic syndrome [8–12]. Moreover, levels of cord blood-derived EPCs have also been shown to be decreased and increased in association with preeclampsia and senescence, respectively [13–15]. The changes in the function of EPCs in preeclampsia is not well understood, and likewise, very few studies have investigated the angiogenic or vasculogenic function of OECs that differentiated from EPCs under preeclamptic conditions [15,16]. Nevertheless, the current literature suggests that maternal vasculopathies including preeclampsia impact fetal circulation; thus, it is reasonable to hypothesize that preeclampsia may affect fetal EPCs function during *in utero* period. Furthermore, since EPCs direct *in utero* neovascularization and repair, their differentiation capacity and overall function is likely to be indicative of fetal vascular health.

The aim of the present study was to firstly compare the function of EPCs derived from normal and severely preeclamptic pregnancies by evaluating both their differentiation potency, and the angiogenic function of the OECs into which they differentiate. The study also investigated the effect(s) of environmental factors and histone methylation levels on the differentiation potency of EPCs derived from both normal and preeclamptic pregnancies.

2. Methods

2.1. Study population and sample collection

The study enrolled patients delivered either vaginally or by cesarean section at 36–41 weeks gestation at the Severance Hospital between September 2016–July 2017. Umbilical cord blood for $CD133^+$/C-kit^+/Lin^- (CKL^-) cell isolation was obtained at the time of delivery after fetal expulsion from women experiencing preeclamptic (n = 10) and normal pregnancies (n = 10) (Table 1). All women assigned to the 'preeclampsia' group exhibited severe preeclampsia, comprising hypertension (defined as the exhibition of a blood pressure level > 160/110 mmHg on two occasions spaced at least 4 h apart during bed rest) in association with thrombocytopenia (indicated by a platelet count < 100,000/μL), impaired liver function (indicated by a 2× elevation of the blood concentrations of liver enzymes above normal levels), severe persistent right-upper quadrant or epigastric pain that was unresponsive to medication and/or not accounted for by an alternative diagnosis, progressive renal insufficiency (indicated by a serum creatinine concentration >1.1 mg/dL, or a 2× elevation of the serum creatinine concentration in the absence of any other renal disease), pulmonary edema, and/or new-onset cerebral and/or visual disturbances, as stipulated by the American College of Obstetricians and Gynecologists guidelines [17,18]. Pregnancies associated with premature membrane rupture, fetal malformation, chromosome anomalies, multiple pregnancies, and/or renal or endocrine diseases other than diabetes mellitus were excluded from the study. All patients provided informed consent for their participation in the study, the design of which was approved by the Institute Review Board of Yonsei University (4-2016-0607).

Table 1
Patients' characteristics.

	Normal pregnancy (n = 10)	Preeclampsia (n = 10)	*P*-value
Maternal age (years)	34.0 ± 5.5	32.7 ± 2.8	0.44
Gestational age at delivery (weeks)	38.2 ± 0.5	36.8 ± 2.0	0.19
Gravida	2.9 ± 2.3	1.3 ± 1.0	0.05
Pre-pregnancy BMI (kg/m2)	19.9 ± 4.4	22.0 ± 6.9	0.40
Mode of Delivery			0.53
Cesarean section	9	8	
Vaginal delivery	1	2	
Blood pressure (mmHg)			
Systolic blood pressure	115.6 ± 10.8	150.7 ± 12.5	0.03
Diastolic blood pressure	73.4 ± 12.5	96.8 ± 12.0	0.03
Gender of baby			0.65
Female	6	5	
Male	4	5	
Birth weight (g)	3167.0 ± 282.5	2606.4 ± 1425	0.40
Small gestational age (n)	0	4	0.03
APGAR score at 1 min	6.7 ± 1.3	5.7 ± 2.0	0.28
APGAR score at 5 min	8.4 ± 1.0	7.6 ± 1.0	0.19

2.2. Isolation and cultivation of CKL^- EPCs

Blood samples (~50 mL) were collected via gravity flow from umbilical cords prior to placental expulsion during delivery. EPCs were isolated via density gradient centrifugation (30 min, 400*g*) using Biocoll solution (Biochrom, Berlin, Germany), and washed three times in phosphate buffered saline (PBS; Biochrom). CKL^- EPCs were purified via positive and negative selection using anti-CD133/C-kit/Lin^- microbeads (Miltenyi Biotec, Bergisch-Gladbach, Germany) and a magnetic cell sorter device (Miltenyi Biotec). Briefly, the cord-blood EPCs were incubated with anti-CD133 microbeads, and unbound antibodies were removed via washing. EPCs captured by the anti-CD133 microbeads were selected according to the manufacturer's instructions. Likewise, the generated $CD133^+$ EPC fraction was then incubated with anti-C-kit microbeads and processed via the manufacturer's protocol for sensitive positive selection, and the resultant $CD133^+$/C-kit^+ EPC fraction subsequently incubated with anti-Lin microbeads and column filtered to deplete of Lin^+ fraction. The purity of the CKL^- EPC fraction was confirmed to > 98% via fluorescence-activated cell-sorting analysis, and the CKL^- cells then seeded onto 6-well plates coated with human fibronectin (Sigma, St. Louis, MO), and maintained in endothelial basal medium-2 (EBM; Clonetics, Cell Systems, St. Katharinen, Germany). Then, the medium was supplemented with endothelial growth medium-2 (EGM2; Clonetics, Cell Systems) containing fetal bovine serum, human VEGF-A, human fibroblast growth factor-B, human epidermal growth factor, IGF1, and ascorbic acid in appropriate amounts. CKL^- cells were identified via staining with phycoerythrin-(PE-) conjugated human CD133-PE and C-kit-PE antibodies (BD Biosciences, Bedford, MA).

2.3. CKL^- EPC differentiation assay

CKL^- EPCs (5×10^5 cells/well) from preeclamptic and normal pregnancies were seeded on 6-well plates, and cultured in EGM2 culture medium that was replaced every 2 days. The 'day of differentiation' was defined as the first day on which a differentiated colony was observed after seeding. Differentiation days and the number of colonies formed in each set were determined by light microscopy.

2.4. Migration assay

Cell migration was assayed using the Transwell system (Corning Costar, Acton, MA) with 6.5-mm diameter polycarbonate filters (8 mm pores). Briefly, the lower surface of the filter was coated with 0.1% gelatin, and 3rd passage OECs (10^5 cells) were seeded onto chemotaxis filters in EBM supplemented with 0.5% FBS. Non-migrating cells were removed from the top surface of the membrane after 4 h. Migrating cells that adhered to the undersurface of the filters were identified via hematoxylin and eosin (H&E) staining, and quantified using Kodak 1D software (Eastman Kodak, Rochester, NY). Results are representative of four independent experiments.

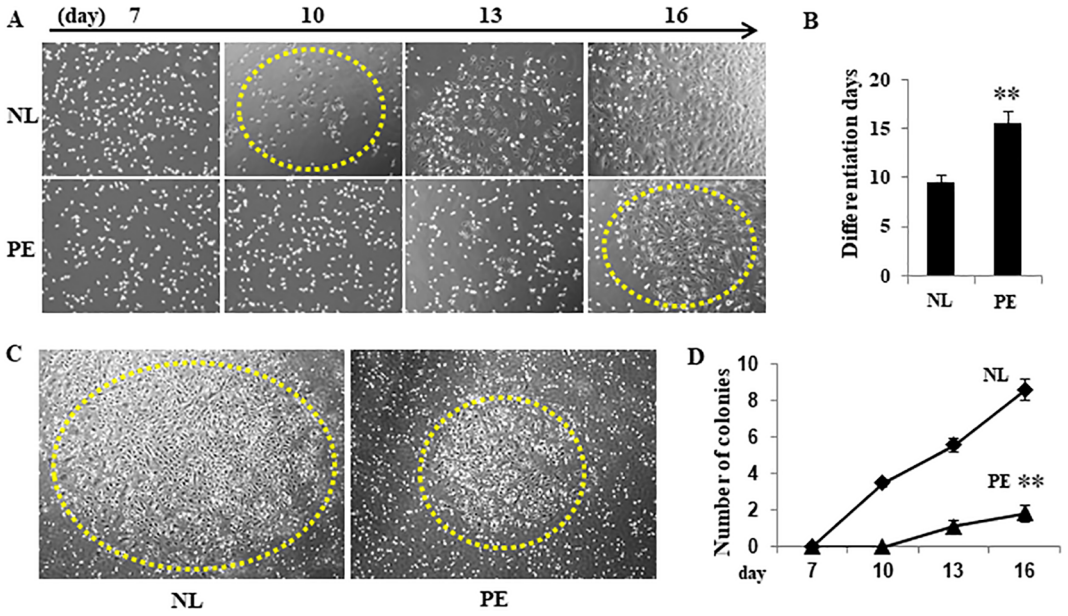

Fig. 1. Preeclamptic (PE) CD133^{+}/C-kit^{+}/Lin2^{-} (CKL^{-}) endothelial progenitor cells (EPCs) exhibit decreased differentiation potency. A, Cord blood-derived CKL^{-} EPCs from PE pregnancies exhibited delayed differentiation compared to those from derived from normal (NL) pregnancies. Differentiation days are indicated by yellow dotted circles. B, The time until differentiation was significantly delayed for PE (16 days) compared to NL (10 days) CKL^{-} EPCs. C, D, On the same differentiation day, the size and number of outgrowth endothelial cell (OEC) colonies generated by the PE CKL^{-} EPCs was reduced compared to that achieved by the NL CKL^{-} EPCs. CKL^{-} EPCs from both groups were cultured in EGM2 (Clonetics, Cell Systems, St. Katharinen, Germany) culture media. Colony boundaries are indicated by yellow dotted circles. ($^{**}P < 0.01$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.5. Adhesion assay

Cell-matrix adhesion assays were performed as previously described [19]. Briefly, 3rd passage OECs were seeded (10^5 cells/well, in 100 μL adhesion buffer comprising serum-free media and EBM) and incubated (30 min, 37 °C) on 96-well plates coated overnight (4 °C) with 10 μg/ml human fibronectin. After two washes with PBS to remove nonadherent cells, the remaining adherent cells were measured via H&E staining, and quantified in triplicate by counting adherent cells in five randomly selected fields per well (Axiovert 100; Carl Zeiss Micro-Imaging, Thornwood, NY). Results are representative of three separate experiments performed in duplicate.

2.6. Tube formation assay

Tube formation was assayed as previously described [20]. Briefly, 250 μL Matrigel solution (BD Biosciences, Bedford, MA) was added to a 16-mm diameter tissue culture well, and allowed to polymerize (30 min, 37 °C). After trypsinization, harvested OECs were resuspended in EBM, and plated onto the Matrigel (1.2×10^5 cells/well). Matrigel cultures were incubated at 37 °C, and photographed at 6, 12, 24 h of incubation (200× magnification). The area covered by the mature tube network was determined by scanning photographs of the tubes into Adobe Photoshop, and using ImageJ software (National Institute of Health) to quantify the identified area. Tube length was defined as the average of total lengths in five microscopic fields which was obtained as the sum of all the mature branch in each field.

2.7. Analysis of H3K4, H3K9, and H3K27 trimethylation levels

H3K4, H3K9, and H3K27 trimethylation levels were detected using specific antibodies. CKL^{-} EPCs were washed twice with phosphate-based saline, and then lysed with radioimmunoprecipitation assay buffer (RIPA buffer). The resulting cell lysates were resolved via SDS-polyacrylamide gel electrophoresis (PAGE), and the captured proteins transferred onto polyvinylidene difluoride membranes. The blocked membranes were then incubated with the anti-H3K4me3 (1:1000; Millipore, Millipore Corp., Bedford, MA, USA), anti-H3K9 (1:1000), and/or anti-H3K27me3 (1:1000; Abcam, Cambridge, MA, USA) antibodies. The resulting immunoreactive bands were visualized using a chemiluminescent reagent (Amersham Biosciences Inc., Piscataway, NJ, USA).

2.8. CKL^{-}EPC differentiation assay conducted during to serum-conditioned media

Preeclampsia-derived CKL^{-} EPCs (5×10^5 cells/well) were seeded on 6-well plates, and cultured for 3 days in EGM2 media. After 3 days in culture, the cell culture media were then changed into each conditioned media; EBM (negative control), EGM2 (20% FBS, positive control), normal maternal serum media (20% maternal serum), preeclamptic maternal serum media (20% maternal serum), normal cord blood serum media (20% cord blood serum), and preeclamptic fetal cord blood serum media (20% cord blood serum). The medium was changed every 2 days. The 'day of differentiation' was defined as the first day on which a differentiated colony was observed after seeding.

Serum for each type of serum-conditioned media was separated via centrifugation from normal maternal blood, preeclamptic maternal blood, normal cord blood, and preeclamptic cord blood. At least three assays were performed for each sample.

2.9. Statistical analyses

Data are shown as the mean ± the standard error of the mean (SE). Statistical comparisons between groups were performed using a *t*-test, one-way analysis of variance (ANOVA) and a subsequent Tukey's post hoc test.

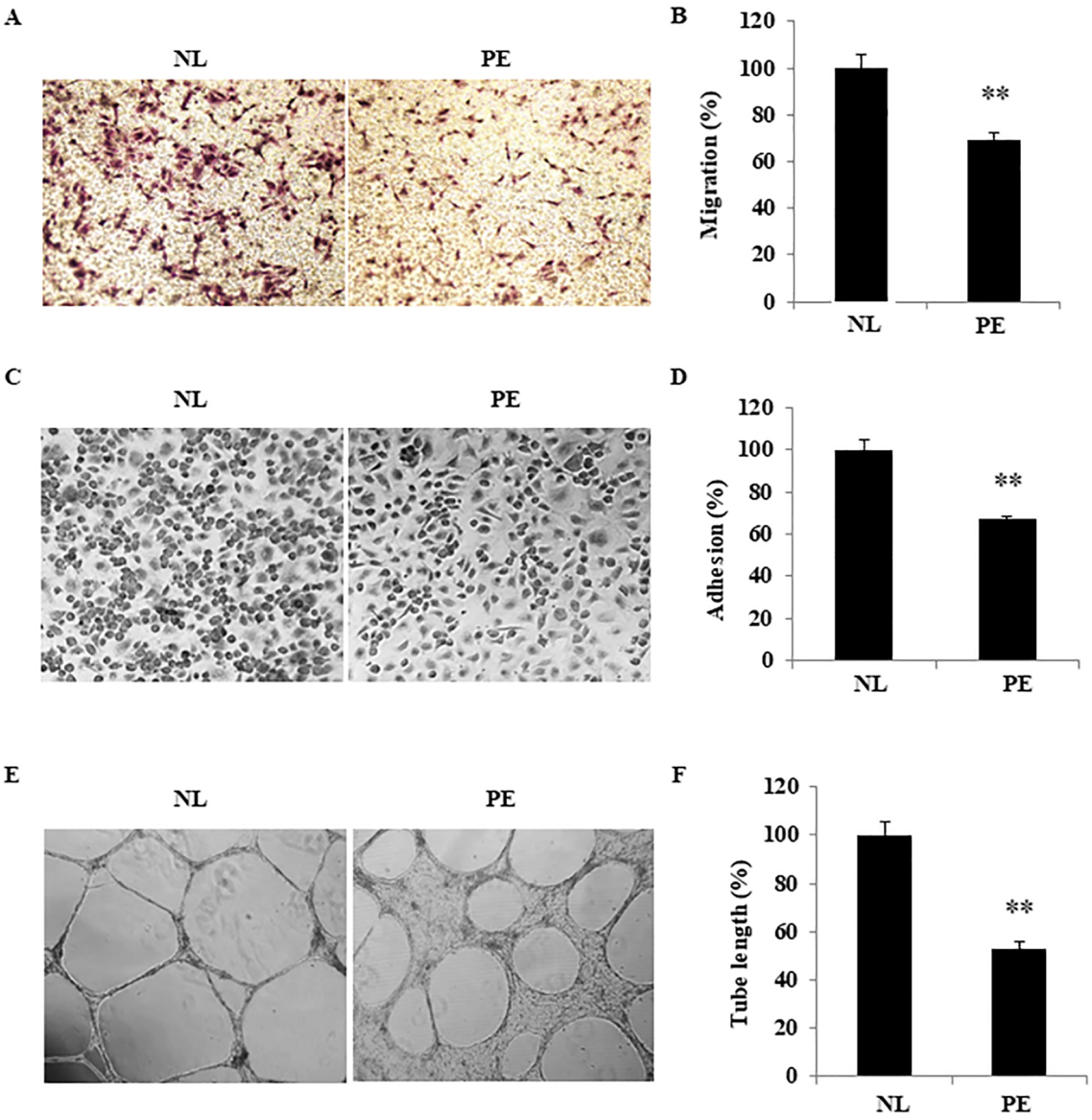

Fig. 2. Outgrowth endothelial cell (OEC) angiogenic function of in preeclamptic (PE) and normal (NL) pregnancies. A, B, A reduced number of PE compared to NL OECs migrated though the transwell chamber during the conducted transwell chamber assay. C, D, A reduced number of PE compared to NL OECs adhered to the well during the conducted adhesion assay. E, PE OECs cultured on Matrigel exhibited a reduced capacity to form capillary-like structures compared to NL OECs. Photographs took after 24 h of incubation in both groups. F, The tube length achieved by the PE OECs was significantly shorter than that achieved by the NL OECs. ($^{**}P < 0.01$).

3. Results

3.1. Decreased differentiation of human cord blood-derived CKL$^-$EPCs to OECs in preeclamptic compared to normal pregnancies

To assess their differentiation potential, the CKL$^-$ EPCs isolated from normal and preeclamptic pregnancies were cultured on fibronectin-coated plates. After 10 days of culture, the CKL$^-$EPCs isolated from normal pregnancies spontaneously differentiated to form OECs, as indicated by both their morphology, and their expression of lineage-specific markers (Fig. 1A, B). In contrast, the CKL$^-$EPCs isolated from preeclamptic pregnancies took 16 days to differentiate to form OECs (Fig. 1A, B). Moreover, both the number and size of OEC colonies formed by the CKL$^-$ EPCs isolated from preeclamptic pregnancies was reduced compared to those produced by CKL$^-$ EPCs derived from normal pregnancies on the same differentiation day (Fig. 1C, D).

Thus, the preeclamptic gestational conditions both delayed CKL$^-$EPC differentiation, and reduced the number and size of the produced OEC colonies, and thereby caused an overall decrease in the CKL$^-$EPC differentiation potency achieved under normal gestational conditions.

3.2. Diminished angiogenic potential of OECs differentiated from EPCs from preeclamptic compared to normal pregnancies

Migration, adhesion, and tube-formation assays were performed to investigate whether preeclampsia impacts OECs angiogenic function. The results of these analyses showed that the preeclampsia-derived OECs migrated poorly through the transwell chamber compared to the normal-derived OECs (Fig. 2A), resulting in a significantly reduced number of migrated preeclampsia-compared to normal-derived OECs (Fig. 2B). Similarly, the preeclampsia-derived OECs exhibited decreased adhesion (Fig. 2C, D), and a reduced capacity to form capillary-like tubes (Fig. 2E) that resulted in a shorter achieved tube length than was achieved by the normal-derived OECs (Fig. 2F). Moreover, preeclampsia-derived OECs exhibited broader tube-like structure than that

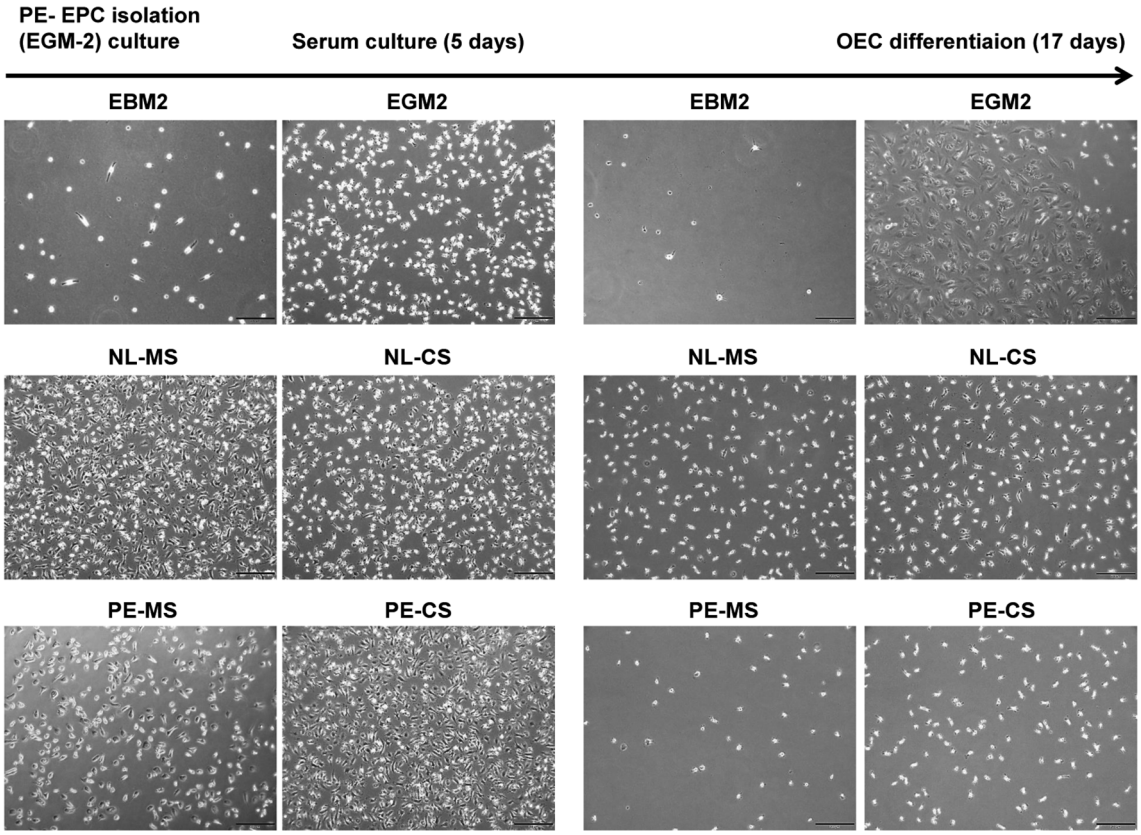

Fig. 3. Differentiation of preeclampsia-derived (PE) endothelial progenitor cells (EPCs) during exposure to serum-conditioned media. To determine whether the decreased differentiation potency the PE EPCs could be rescued, they were cultured in serum-conditioned media following 5-days of growth in EGM2 media. The CKL$^-$EPCs were resultantly shown to exhibit delayed differentiation during exposure to positive control (EGM2, Clonetics, Cell Systems, St. Katharinen, Germany) media, but failed to differentiate during exposure to either normal (NL) or PE serum-conditioned media. (NL-MS: Normal maternal serum; NL-CS: Normal cord blood serum; PE-MS: Preeclamptic maternal serum; PE-CS: Preeclamptic cord blood serum).

of normal OEC. These data demonstrate that although some preeclampsia-derived EPCs successfully differentiate to form OECs, these exhibit disrupted migration, adhesion, and tube formation function.

3.3. The decreased differentiation potency of the preeclampsia-derived CKL$^-$EPCs was not rescued via exposure to normal serum-conditioned media

To investigate whether the decreased differentiation potency exhibited by the preeclampsia-derived CKL$^-$ EPCs was able to be rescued via exposure to a normal gestational environment, they were cultured in human serum-conditioned media containing either maternal or cord-blood serum. (Serum-free and EGM2 media were used as negative and positive controls, respectively). The preeclampsia-derived CKL$^-$ EPCs continued to exhibit delayed differentiation during exposure to the positive control (EGM2) media, and failed to differentiate when maintained in either normal- or preeclamptic serum media (Fig. 3). Thus, the preeclampsia-derived CKL$^-$ EPCs exhibited defective differentiation activity that was not rescued via correction of the serum environment.

3.4. Preeclampsia derived-CKL$^-$ EPCs showed decreased histone methylation levels

Together, the results of the conducted analyses suggest that the preeclampsia derived-CKL$^-$ EPCs incurred semi- or permanent, rather than temporary functional changes. Thus, their histone methylation levels were analyzed, and their histone H3K4 and H3K9 trimethylation level resultantly shown to be significantly decreased compared to those exhibited by normal-derived EPCs. (No significant difference in H3K27me3 levels were observed between the two groups of EPCs) (Fig. 4). Moreover, the H3K4 and H3K9 trimethylation levels were further reduced in preeclampsia-derived EPCs that were cultured with preeclamptic maternal serum compared to EGM2 media (Fig. 4). This data suggests that the preeclampsia-derived EPCs incurred epigenetic changes, such as reduced histone methylation levels, via their exposure to soluble factors contained in the maternal-fetal circulation during preeclampsia.

4. Discussion

The results of the present study suggest that maternal preeclampsia may cause not only the immediate effect to fetus such as prematurity, or fetal growth restriction, but also the potential dysfunction on vascular health of a fetus via effects incurred *in utero*. This is supported by the results of various other studies that have previously reported an increased incidence of adult-onset cardiovascular and metabolic diseases, such as hypertension, cardiovascular disorders, cerebrovascular disorders, impaired neural development, type 2 diabetes, metabolic syndrome, and hypercortisolism, among individuals born to preeclamptic women [1,2,21–23]. Increased pulmonary and peripheral vascular stiffness has also been reported in school-aged children exposed to a preeclamptic environment *in utero* [24]. While the pathophysiological mechanisms underlying the fetal impacts of preeclampsia have not yet been confirmed, Barker *et al.* proposed that they may be the result of intrauterine environmental changes that stimulate epigenetic changes, and/or disrupt normal cellular remodeling at critical timepoints during development [25,3].

Previous studies investigating preeclamptic epigenetic changes

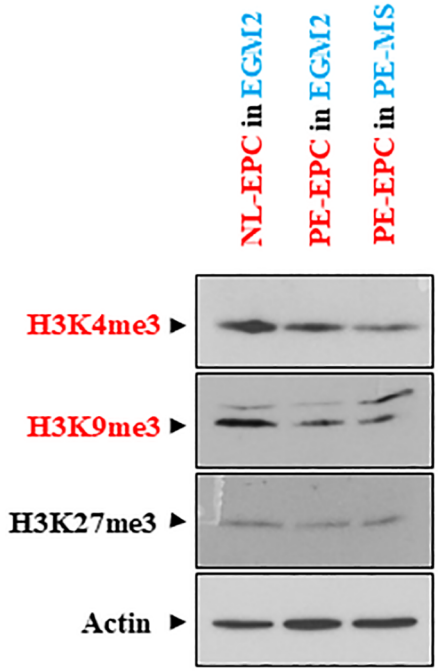

Fig. 4. H3K4me3, H3K9me3 and H3K27me3 levels exhibited by preeclampsia (PE)-derived CD133$^+$/C-kit$^+$/Lin2$^-$ endothelial progenitor cells (CKL$^-$ EPCs). The H3K4me3 and H3K9me3 levels exhibited by the PE CKL$^-$EPCs was decreased compared to those exhibited by the normal (NL)-derived CKL$^-$EPCs, and the H3K4me3 levels further reduced in PE CKL$^-$ EPCs that were cultured in maternal serum compared to EGM2 media. The H3K27me3 levels were not significantly different between the two groups.

mainly focused on the role that the placenta plays in the pathogenesis of the disease, rather than the maternal or fetal impact of preeclampsia [26]. They thus provided insights into the effects of DNA methylation, (e.g. genomic imprinting changes such as *STOX1* overexpression [27], promotor-region changes that modulate placental *SERPINA3* expression [28], hypomethylation of regulators of inflammatory modulators such as *RXRα/PPARγ* [29], and epigenetics impacts on *HIF1α* expression [30]) on pathologic changes of preeclampsia such as shallow placental implantation, endothelial dysfunction, and inflammation. Various studies have also been conducted which analyzed preeclampsia-associated changes to the expression of placental miRNAs, and their effect on the expression and function of related genes; however, they predominantly aimed to identify novel diagnostic markers or elucidate the mechanisms underlying preeclampsia pathophysiology [31]. Finally, recent studies that reported on the immediate and ongoing fetal impacts of preeclampsia-associated epigenetic changes focused on the way in which altered placental genetic imprinting induce hypoxia, and thus affect fetal neural development *in utero* [32].

EPCs are endothelial cell precursors, and as such, have the potential to direct vasculogenesis, angiogenesis, and neovascularization after birth [6,33]; thus, their dysfunction likely underlies various vascular diseases. In fact, previous studies have shown the number of circulating EPCs to be decreased in vascular and metabolic disorders such as coronary artery disease, diabetic vasculopathy, atherosclerosis, systemic lupus erythematosus, and metabolic syndrome [8–12]. Preeclampsia is also considered to be a hypertensive disorder, and has been shown to be associated with a reduced concentration of circulating EPCs in peripheral blood vessels [34].

Cord blood is included in fetal circulation, and thus, the angiogenic potential of cord blood CKL$^-$ EPCs likely reflect the overall angiogenic potential of the fetus. This is supported by the results of a previously published study that showed OECs differentiated from cord blood CKL$^-$EPCs to exhibit angiogenic potential [35]. While only limited research has been conducted to investigate the function of fetal EPCs exposed to preeclampsia to date, it has been established that the number of cord blood-derived EPCs is reduced in preeclamptic pregnancies, and furthermore, that the small number of identified EPCs are predominantly senescent [13–16]. The results of the present study showed that preeclampsia-derived cord-blood CKL$^-$ EPCs and resultant differentiated OECs exhibited reduced function and angiogenic potential, respectively, compared to those observed for normal-derived CKL$^-$ EPCs. Diminished migration, adhesion, and tube formation function of preeclampsia derived OECs compared to normal OECs means immature differentiation of OEC for angiogenesis. Furthermore, the reduced differentiation potency of the preeclampsia-derived CKL$^-$EPCs was not recovered via exposure to optimal serum conditions, suggesting that their decreased function may be irreversible. On contrary, normal-derived CKL-EPCs were successfully differentiated even in the culture media with preeclamptic serum (data was not shown). Thus, the preeclamptic intrauterine environment appears to permanently modify pathways that critically regulate and maintenance fetal vascular health by affecting fetal vasculogenic/angiogenic function.

Epigenetic changes are a common mechanism by which environmental conditions can stimulate irreversible changes to gene expression and/or protein function. Stem and progenitor cells are characterized by a high prevalence of transcriptionally competent but altered genes that are marked by both active and repressive histone modifications associated with important differentiation and developmental processes [36]. In the present study, the H3K4me3 and H3K9me3 levels in the preeclampsia-derived CKL$^-$ EPCs were found to be reduced. It is possible that such histone modifications may facilitate the activation of pro-angiogenic signaling (H3K4me3) and/or repressive signaling pathways (H3K27me3), which might indicate a shift in the proangiogenic/antiangiogenic balance that favors anti-angiogenesis. In addition, studies in endothelial cells have previously identified several signal transduction pathways (e.g., *VEGFR*, *CXCR4*, *VEGF*, *NOTCH*, and *WNT*) that coordinate cell survival, cell differentiation, arterial/venous specification, and blood-vessel morphogenesis [37], and are essential for angiogenesis and/or post-natal vasculogenesis. Interestingly, the placental expression of many of the genes associated with these angiogenic signaling pathways have been shown to be altered in preeclampsia via whole genome or RNA sequencing analyses [38,39].

Conversely, increasing H3K9me3 have been shown to be associated with reduce *HIF1α* transcription [40]. *HIF1α* is known to regulate *sFLT1* expression, and the production of soluble endoglin. In fact, *HIF1α* expression is generally upregulated in preeclampsia [41], and the resulting increased placental secretion of sFLT1 impacts the vascular health and angiogenic homeostasis of preeclamptic women by causing a decrease in free serum VEGF and PlGF, the disruption of angiogenic homeostasis, increased systemic inflammation, and altered TGF-β levels via changing soluble endoglin levels [42]. Thus, observed changes to the levels of soluble fetal serum factors in preeclamptic pregnancies are likely indicative of overall fetal vascular health. Staff et al. previously reported increased sFLT1, and decreased VEGF and PlGF levels in the cord blood and amniotic fluid of preeclamptic women compared to those experiencing a normal pregnancy [43]. Given that cord blood is considered to be included in fetal circulation, changes in the levels of these soluble factors may result in endothelial dysfunction and/or an increased systemic inflammatory response in fetal blood vessels, in the same way as they induce these effects in maternal blood vessels. Another recent study reported that the secretion of maternal placental VEGF is required to stimulate an human umbilical vein endothelial cells (HUVEC) angiogenic potential [44]. Thus, continued research is needed to investigate the impacts of preeclamptic epigenetic changes on angiogenic signaling pathway (e.g. sFLT1, VEGF, or PlGF) activity on fetal vascular health.

Furthermore, decreased H3K9me3 levels in stem cells have also been shown to be associated with cell senescence [45]. Considering that preeclampsia-derived cord blood EPCs have been shown to exhibit

increased senescence [14], it is possible that the decreased H3K9me3 levels observed in the present study may contribute to the decreased function of the preeclampsia-derived EPCs. Further study is required to confirm this hypothesis, and to elucidate the mechanisms by which decreased H3K9me3 levels affect CKL$^-$ EPC differentiation and angiogenesis during development.

The present study showed the differentiation potency of preeclampsia-derived cord blood CKL$^-$ EPCs to be irreversibly reduced compared to that exhibited by normal-derived CKL$^-$ EPCs. Moreover, the angiogenic function of OECs that differentiated from the preeclampsia-derived CKL$^-$ EPCs was also significantly diminished. Further study is needed, firstly to establish whether the decreased function of preeclampsia-derived cord blood CKL$^-$EPCs persists after birth, and secondly, to elucidate the mechanisms via which preeclampsia-induced effects on CKL$^-$ EPCs affect the long-term vascular health of the exposed fetus. The results of the present study suggest that preeclamptic EPCs likely undergo irreversible epigenetic changes that disrupt their angiogenic capacity.

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Conflict of interest

The authors report no conflict of interest.

References

[1] D.L. Gumina, E.J. Su, Endothelial progenitor cells of the human placenta and fetoplacental circulation: a potential link to fetal, neonatal, and long-term health, Front. Pediatr. 5 (2017) 41.
[2] E. Kajantie, C. Osmond, J.G. Eriksson, Gestational hypertension is associated with increased risk of type 2 diabetes in adult offspring: the Helsinki Birth Cohort Study, Am. J. Obstet. Gynecol. 216 (3) (2017) 281 e1-281 e7.
[3] V. Stojanovska, S.A. Scherjon, T. Plosch, Preeclampsia as modulator of offspring health, Biol. Reprod. 94 (3) (2016) 53.
[4] C.W. Redman, I.L. Sargent, Placental stress and pre-eclampsia: a revised view, Placenta 30 (Suppl. A) (2009) S38–S42.
[5] M. Furuya, K. Kurasawa, K. Nagahama, K. Kawachi, A. Nozawa, T. Takahashi, I. Aoki, Disrupted balance of angiogenic and antiangiogenic signalings in preeclampsia, J. Pregnancy 2011 (2011) 123717.
[6] M. Eguchi, H. Masuda, T. Asahara, Endothelial progenitor cells for postnatal vasculogenesis, Clin. Exp. Nephrol. 11 (1) (2007) 18–25.
[7] V.L. Bautch, Stem cells and the vasculature, Nat. Med. 17 (11) (2011) 1437–1443.
[8] G.A. Kunz, G. Liang, F. Cuculi, D. Gregg, K.C. Vata, L.K. Shaw, P.J. Goldschmidt-Clermont, C. Dong, D.A. Taylor, E.D. Peterson, Circulating endothelial progenitor cells predict coronary artery disease severity, Am. Heart J. 152 (1) (2006) 190–195.
[9] G.P. Fadini, S. Sartore, M. Albiero, I. Baesso, E. Murphy, M. Menegolo, F. Grego, S. Vigili de Kreutzenberg, A. Tiengo, C. Agostini, A. Avogaro, Number and function of endothelial progenitor cells as a marker of severity for diabetic vasculopathy, Arterioscler. Thromb. Vasc. Biol. 26 (9) (2006) 2140–2146.
[10] G.P. Fadini, A. Coracina, I. Baesso, C. Agostini, A. Tiengo, A. Avogaro, S.V. de Kreutzenberg, Peripheral blood CD34+KDR+ endothelial progenitor cells are determinants of subclinical atherosclerosis in a middle-aged general population, Stroke 37 (9) (2006) 2277–2282.
[11] P. Ebner, F. Picard, J. Richter, E. Darrelmann, M. Schneider, B.E. Strauer, M. Brehm, Accumulation of VEGFR-2+/CD133+ cells and decreased number and impaired functionality of CD34+/VEGFR-2+ cells in patients with SLE, Rheumatology 49 (1) (2010) 63–72.
[12] I. Jialal, S. Devaraj, U. Singh, B.A. Huet, Decreased number and impaired functionality of endothelial progenitor cells in subjects with metabolic syndrome: implications for increased cardiovascular risk, Atherosclerosis 211 (1) (2010) 297–302.
[13] J.Y. Kwon, Y.S. Maeng, Y.G. Kwon, Y.H. Kim, M.H. Kang, Y.W. Park, Decreased endothelial progenitor cells in umbilical cord blood in severe preeclampsia, Gynecol. Obstet. Invest. 64 (2) (2007) 103–108.
[14] H.S. Hwang, Y.S. Maeng, Y.W. Park, B.J. Koos, Y.G. Kwon, Y.H. Kim, Increased senescence and reduced functional ability of fetal endothelial progenitor cells in pregnancies complicated by preeclampsia without intrauterine growth restriction, Am. J. Obstet. Gynecol. 199 (3) (2008) 259 e1-7.
[15] R. Munoz-Hernandez, M.L. Miranda, P. Stiefel, R.Z. Lin, J.M. Praena-Fernandez, M.J. Dominguez-Simeon, J. Villar, R. Moreno-Luna, J.M. Melero-Martin, Decreased level of cord blood circulating endothelial colony-forming cells in preeclampsia, Hypertension 64 (1) (2014) 165–171.
[16] D.L. Gumina, C.P. Black, V. Balasubramaniam, V.D. Winn, C.D. Baker, Umbilical cord blood circulating progenitor cells and endothelial colony-forming cells are decreased in preeclampsia, Reprod. Sci. 24 (7) (2017) 1088–1096.
[17] A.C.o.P. Bulletins–Obstetrics, ACOG practice bulletin. Diagnosis and management of preeclampsia and eclampsia. Number 33, January 2002, Obstet. Gynecol. 99 (1) (2002) 159–167.
[18] O. American College of, Gynecologists, P. Task Force on Hypertension in, Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy, Obstet. Gynecol. 122(5) (2013) 1122–1131.
[19] Y.S. Maeng, Y.J. Choi, E.K. Kim, TGFBIp regulates differentiation of EPC (CD133(+) C-kit(+) Lin(−) cells) to EC through activation of the Notch signaling pathway, Stem Cells 33 (6) (2015) 2052–2062.
[20] J.K. Min, Y.L. Cho, J.H. Choi, Y. Kim, J.H. Kim, Y.S. Yu, J. Rho, N. Mochizuki, Y.M. Kim, G.T. Oh, Y.G. Kwon, Receptor activator of nuclear factor (NF)-kappaB ligand (RANKL) increases vascular permeability: impaired permeability and angiogenesis in eNOS-deficient mice, Blood 109 (4) (2007) 1495–1502.
[21] E. Kajantie, J.G. Eriksson, C. Osmond, K. Thornburg, D.J. Barker, Pre-eclampsia is associated with increased risk of stroke in the adult offspring: the Helsinki birth cohort study, Stroke 40 (4) (2009) 1176–1180.
[22] C. Warshafsky, J. Pudwell, M. Walker, S.W. Wen, G.N. Smith, T. Preeclampsia New Emerging, Prospective assessment of neurodevelopment in children following a pregnancy complicated by severe pre-eclampsia, BMJ Open 6 (7) (2016) e010884.
[23] D. Henley, S. Brown, C. Pennell, S. Lye, D.J. Torpy, Evidence for central hypercortisolism and elevated blood pressure in adolescent offspring of mothers with pre-eclampsia, Clin. Endocrinol. (Oxf) 85 (4) (2016) 583–589.
[24] P.Y. Jayet, S.F. Rimoldi, T. Stuber, C.S. Salmon, D. Hutter, E. Rexhaj, S. Thalmann, M. Schwab, P. Turini, C. Sartori-Cucchia, P. Nicod, M. Villena, Y. Allemann, U. Scherrer, C. Sartori, Pulmonary and systemic vascular dysfunction in young offspring of mothers with preeclampsia, Circulation 122 (5) (2010) 488–494.
[25] P. Bateson, D. Barker, T. Clutton-Brock, D. Deb, B. D'Udine, R.A. Foley, P. Gluckman, K. Godfrey, T. Kirkwood, M.M. Lahr, J. McNamara, N.B. Metcalfe, P. Monaghan, H.G. Spencer, S.E. Sultan, Developmental plasticity and human health, Nature 430 (6998) (2004) 419–421.
[26] V. Januar, G. Desoye, B. Novakovic, S. Cvitic, R. Saffery, Epigenetic regulation of human placental function and pregnancy outcome: considerations for causal inference, Am. J. Obstet. Gynecol. 213 (4 Suppl.) (2015) S182–S196.
[27] A. Ducat, L. Doridot, R. Calicchio, C. Mehats, J.L. Vilotte, J. Castille, S. Barbaux, B. Couderc, S. Jacques, F. Letourneur, C. Buffat, F. Le Grand, P. Laissue, F. Miralles, D. Vaiman, Endothelial cell dysfunction and cardiac hypertrophy in the STOX1 model of preeclampsia, Sci. Rep. 6 (2016) 19196.
[28] S.T. Chelbi, F. Mondon, H. Jammes, C. Buffat, T.M. Mignot, J. Tost, F. Busato, I. Gut, R. Rebourcet, P. Laissue, V. Tsatsaris, F. Goffinet, V. Rigourd, B. Carbonne, F. Ferre, D. Vaiman, Expressional and epigenetic alterations of placental serine protease inhibitors: SERPINA3 is a potential marker of preeclampsia, Hypertension 49 (1) (2007) 76–83.
[29] M. Ruebner, M. Langbein, P.L. Strissel, C. Henke, D. Schmidt, T.W. Goecke, F. Faschingbauer, R.L. Schild, M.W. Beckmann, R. Strick, Regulation of the human endogenous retroviral Syncytin-1 and cell-cell fusion by the nuclear hormone receptors PPARgamma/RXRalpha in placentogenesis, J. Cell. Biochem. 113 (7) (2012) 2383–2396.
[30] E. Maltepe, G.W. Krampitz, K.M. Okazaki, K. Red-Horse, W. Mak, M.C. Simon, S.J. Fisher, Hypoxia-inducible factor-dependent histone deacetylase activity determines stem cell fate in the placenta, Development 132 (15) (2005) 3393–3403.
[31] T. Gunel, M.K. Hosseini, E. Gumusoglu, H.I. Kisakesen, A. Benian, K. Aydinli, Expression profiling of maternal plasma and placenta microRNAs in preeclamptic pregnancies by microarray technology, Placenta 52 (2017) 77–85.
[32] Y. Nomura, R.M. John, A.B. Janssen, C. Davey, J. Finik, J. Buthmann, V. Glover, L. Lambertini, Neurodevelopmental consequences in offspring of mothers with preeclampsia during pregnancy: underlying biological mechanism via imprinting genes, Arch. Gynecol. Obstet. 295 (6) (2017) 1319–1329.
[33] T. Takahashi, C. Kalka, H. Masuda, D. Chen, M. Silver, M. Kearney, M. Magner, J.M. Isner, T. Asahara, Ischemia- and cytokine-induced mobilization of bone marrow-derived endothelial progenitor cells for neovascularization, Nat. Med. 5 (4) (1999) 434–438.
[34] K.M. Beasley, A.T. Lovering, J.S. Gilbert, Decreased endothelial progenitor cells in preeclampsia and consequences for developmental programming, Hypertension 64 (1) (2014) 23–25.
[35] C. Cardenas, J.Y. Kwon, Y.S. Maeng, Human cord blood-derived CD133(+)/C-Kit(+)/Lin(−) cells have bipotential ability to differentiate into mesenchymal stem cells and outgrowth endothelial cells, Stem Cells Int. 2016 (2016) 7162160.
[36] P. Voigt, W.W. Tee, D. Reinberg, A double take on bivalent promoters, Genes Dev. 27 (12) (2013) 1318–1338.
[37] P. Carmeliet, R.K. Jain, Molecular mechanisms and clinical applications of angiogenesis, Nature 473 (7347) (2011) 298–307.
[38] X. Liu, Q. Luo, Y. Zheng, X. Liu, Y. Hu, W. Liu, M. Luo, Y. Zhao, L. Zou, NOTCH4 signaling controls EFNB2-induced endothelial progenitor cell dysfunction in

preeclampsia, Reproduction 152 (1) (2016) 47–55.
[39] S. Karakus, B. Bagci, G. Bagci, E. Sancakdar, C. Yildiz, O. Akkar, A. Cetin, SDF-1/CXCL12 and CXCR4 gene variants, and elevated serum SDF-1 levels are associated with preeclampsia, Hypertens. Pregnancy 36 (2) (2017) 124–130.
[40] G. Dobrynin, T.E. McAllister, K.B. Leszczynska, S. Ramachandran, A.J. Krieg, A. Kawamura, E.M. Hammond, KDM4A regulates HIF-1 levels through H3K9me3, Sci. Rep. 7 (1) (2017) 11094.
[41] R. Tal, The role of hypoxia and hypoxia-inducible factor-1alpha in preeclampsia pathogenesis, Biol. Reprod. 87 (6) (2012) 134.
[42] S.T. Chelbi, D. Vaiman, Genetic and epigenetic factors contribute to the onset of preeclampsia, Mol. Cell. Endocrinol. 282 (1–2) (2008) 120–129.
[43] A.C. Staff, K. Braekke, N.K. Harsem, T. Lyberg, M.R. Holthe, Circulating concentrations of sFlt1 (soluble fms-like tyrosine kinase 1) in fetal and maternal serum during pre-eclampsia, Eur. J. Obstet. Gynecol. Reprod. Biol. 122 (1) (2005) 33–39.
[44] H. Zhao, L. Wu, Y. Wang, J. Zhou, R. Li, J. Zhou, Z. Wang, C. Xu, Nicotine promotes vascular endothelial growth factor secretion by human trophoblast cells under hypoxic conditions and improves the proliferation and tube formation capacity of human umbilical endothelial cells, Reprod. Biomed. Online 34 (4) (2017) 406–413.
[45] A.R. Mendelsohn, J.W. Larrick, Stem cell depletion by global disorganization of the H3K9me3 epigenetic marker in aging, Rejuvenation Res. 18 (4) (2015) 371–375.