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DECREASED LEVEL OF CORD BLOOD CIRCULATING ENDOTHELIAL COLONY-FORMING CELLS IN PREECLAMPSIA

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Abstract

Preeclampsia is a pregnancy-related disorder associated with increased cardiovascular risk for the offspring. Endothelial colony-forming cells (ECFCs) are a subset of circulating endothelial progenitor cells that participate in the formation of vasculature during development. However, the effect of preeclampsia on fetal levels of ECFCs is largely unknown. In this study, we sought to determine whether cord blood ECFC abundance and function are altered in preeclampsia. We conducted a prospective cohort study that included women with normal (n=35) and preeclamptic (n=15) pregnancies. We measured ECFC levels in the umbilical cord blood of neonates and characterized ECFC phenotype, cloning-forming ability, proliferation and migration towards VEGF-A and FGF-2, in vitro formation of capillary-like structures, and in vivo vasculogenic ability in immunodeficient mice. We found that the level of cord blood ECFCs was statistically lower in preeclampsia than in control pregnancies (P = .04), a reduction that was independent of other obstetric factors. In addition, cord blood ECFCs from preeclamptic pregnancies required more time to emerge in culture than control ECFCs. However, once derived in culture, ECFC function was deemed normal and highly similar between preeclampsia and control, including the ability to form vascular networks in vivo. This study demonstrates that preeclampsia affects ECFC abundance in neonates. A reduced level of ECFCs during preeclamptic pregnancies may contribute to an increased risk of developing future cardiovascular events.

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Conflict(s) of Interest/Disclosure(s) None

Keywords

preeclampsia; endothelial colony-forming cells; endothelial progenitor cells; cord blood; pregnancy

Introduction

Preeclampsia is a multisystem syndrome affecting 2% to 8% of pregnancies, and it is a major cause of maternal and fetal morbidity and mortality [1,2]. Offspring of preeclamptic pregnancies have an increased risk of developing postnatal cardiovascular events, including hypertension and stroke [3,4]. Epidemiological studies have shown that several cardiovascular diseases have origins during development [5]. However, the effects of preeclampsia on the fetal cardiovascular system remain poorly understood.

Endothelial colony-forming cells (ECFCs) are circulating progenitor cells that give rise to highly vasculogenic endothelial cells [6,7]. ECFC levels in fetal blood are elevated during the third trimester of pregnancy [8-10], and these cells are postulated to contribute to the rapid formation of fetal vasculature and to the maintenance of vascular integrity [11,12]. Recent studies have shown that cord blood ECFC level and function are impaired in several pregnancy-related disorders associated with long-term cardiovascular risks, including gestational diabetes, fetal bronchopulmonary dysplasia, and intrauterine growth restriction (IUGR) [13-16]. However, it remains unclear whether cord blood levels of ECFCs are also altered during preeclampsia.

Here, we conducted a prospective cohort study to determine the umbilical cord blood levels of ECFCs in preeclampsia and analyzed the results in light of potential confounding obstetric factors. We also compared the functional properties of ECFCs derived from preeclamptic and normal pregnancies.

Methods

Study subjects

Fifteen (preeclampsia) and thirty-five (control) Caucasian mother-offspring pairs were included in this study. Preeclampsia was defined as high blood pressure (>140/90 mm Hg) and excess protein in the urine (>0.3 g in 24 hours) after 20 weeks of pregnancy. Pre-existing chronic hypertension was not an exclusion criterion for preeclampsia. All preeclamptic mothers were treated with alfa-methyldopa; in addition, five patients also received labetalol. IUGR was defined as a fetus with an individualized weight percentile smaller than 10% and with asymmetry in several ultrasound measurements, including a significant decrease in abdominal perimeter compared to long bone length and biparietal diameter. Exclusion criteria included multiple gestation, maternal infections, respiratory disease, and women who carried fetuses with chromosomal abnormalities or congenital malformations. In the control group, women with hypertensive disorders were excluded. The local ethics committee at the Hospital Universitario Virgen del Rocío approved this research, and all the parents gave written informed consent for extraction of data from their obstetric records and for the use of umbilical cord blood in accordance with the Declaration

of Helsinki. Methods regarding obstetric factors are described online in our expanded Material and Methods.

Enumeration and characterization of endothelial colony-forming cells

Umbilical cord blood samples (20–50 mL) were collected ex utero using heparinized tubes and processed within 2 hours. Enumeration and characterization of ECFCs were carried out following previously described methods [17-19]; details can be found online in our expanded Material and Methods.

Statistical analysis

Data from preeclampsia and control subjects were compared and analyzed with IBM SPSS v. 19.0 software (IBM Corp., Armonk, NY, USA). Categorical variables were expressed by absolute frequencies and percentages (n,%). Non-categorical variables were expressed by mean ± standard deviation (SD) or median and interquartile range 25^{th}-75^{th}. Categorical variables were analyzed with Fisher exact tests except for tobacco use and offspring sex that were analyzed with Pearson chi-squared tests. Non-categorical variables were analyzed with 2-tailed unpaired Student t tests, with the exception of maternal age and gestational age, which were not normally distributed and therefore analyzed with Mann-Whitney U tests. Shapiro-Wilk tests were used to determine normality. Univariate correlations were performed with use of Spearman correlation co-efficient. Data from experiments performed in vitro and in mice were analyzed using GraphPad Prism v. 5 software (GraphPad Software, La Jolla, CA, USA). These data were expressed as mean ± standard error (SE) and means were compared using unpaired Student t tests. For all analyses, $P < .05$ was considered significant.

Results

Patient demographics

We studied fifteen (preeclampsia) and thirty-five (control) mother-offspring pairs (Table 1). Based on the severity of the pathology, the preeclampsia group included subjects with mild (n=6), severe (n=7), and HELLP syndrome (n=2). In addition, two subjects in the preeclampsia group had pre-existing chronic hypertension. The prevalence of cesarean deliveries in the preeclampsia group was statistically higher than in control ($P = .001$). Offspring born from mothers with preeclampsia had lower gestational age, birth weight, and birth weight percentile than those in the control group ($P = .001$, $P = .004$, and $P = .006$, respectively). Preeclamptic mothers had higher pre-gestational diastolic blood pressure ($P = .003$) than control. There were no statistical differences in the remainder of the obstetric characteristics analyzed ($P > .05$).

Cord blood levels of ECFCs in preeclampsia

We quantified the number of ECFCs in the umbilical cord blood of neonates at the time of delivery. ECFCs were identified in culture as outgrown colonies containing ≥ 50 endothelial cells. The endothelial nature of the colonies was corroborated by the cobblestone-like morphology of the cells (Figure 1A) and by binding of fluorescently labeled UEA-1 (Figure 1B). Colonies in the control group emerged in culture as early as one week (7% of the

colonies), and most of the colonies emerged between 2 weeks (60%) and 3 weeks (31%) (Figure 1C), which is consistent with previous reports [18]. In contrast, the time needed for colony appearance in the preeclampsia group was higher, and a substantial proportion of colonies (44%) emerged in the fourth week of culture (Figure 1C). Total ECFC level in each group was determined after 4 weeks in culture. The median ECFC level in control was 5 colonies per 10 mL of cord blood with a broad 25^{th}-75^{th} interquartile range of 0.5-13 colonies. Meanwhile, ECFC level in preeclampsia was statistically lower than in control (P = .04), with a median abundance of 1 colony per 10 mL of cord blood and a 25^{th}-75^{th} interquartile of 0-4 colonies (Figure 1D). Moreover, a significant portion of the preeclamptic group in the study had no measurable ECFCs. Statistical analyses carried out in both preeclampsia and control groups demonstrated that the level of ECFCs was independent (P > .05) of most obstetric factors (Tables S1 and S2), including maternal age (P = .06 and P = .77 in preeclampsia and control, respectively; Table S2), mode of delivery (P = .53 and P = .83; Table S1), offspring sex (P = .40 and P = .64; Table S1), offspring birth weight (P = .27 and P = .87; Table S2), offspring birth weight percentile (P = .26 and P = .71; Table S2), gestational weight gain (P = .31 and P = .08; Table S2), and cord blood MNC level (P = .95 and P = .97; Table S2). Moreover, the level of cord blood ECFCs in preeclampsia was independent of both the severity of the pathology (mild/severe/HELLP; P = .06), the time of onset of preeclampsia (early/late; P = .42), diastolic and systolic pre-gestational blood pressure (P = .51 and P = .94; Table S2), and diastolic and systolic blood pressure at the time of onset of preeclampsia (P = .52 and P = .27; Table S2).

Variation of cord blood ECFC levels with maternal BMI and gestational age

Previously, we demonstrated that maternal BMI is a potential confounding factor for cord blood levels of ECFCs [18]. To address whether the difference in ECFC abundance between preeclampsia and control was confounded by maternal weight, we categorized the study into pre-pregnancy maternal BMI < 25 kg/m^2 (normal weight; n=15/n=5 control/preeclampsia), 25-30 kg/m^2 (overweight; n=12/n=6), and > 30 kg/m^2 (obese; n=8/n=4) (Figure 2A). ECFC levels in control subjects increased from normal pre-pregnancy maternal weight (mean of 4 colonies) to overweight (11 colonies) and obese (7 colonies) subjects, with statistically significant differences between these subgroups (Figure 2A). In contrast, the level of ECFCs in preeclampsia was consistently low, irrespective of the value of maternal BMI, with mean ECFC abundances of 2, 3, and 3 colonies in cord blood samples from normal weight, overweight, and obese mothers, respectively (Figure 2A). In addition, the different in ECFC levels between control and preeclampsia for maternal BMI 25-30 kg/m^2 was statistically significant (P < .05). Taken together, these results confirmed that maternal BMI is a confounding factor for ECFC level and demonstrated that the reduction in ECFC abundance observed in preeclampsia was more prominent among subjects in the overweight (BMI = 25-30 kg/m^2) group.

To address whether the difference in ECFC abundance between preeclampsia and control was influenced by gestational age, we categorized the study into premature (< 37 gestational weeks; n=5/n=5 control/preeclampsia) or term (≥ 37 weeks; n=30/n=10) deliveries (Figure 2B). Our study did not include extremely premature infants, and the lowest gestational age for both groups was 31 weeks. We observed that ECFC abundance in the control group was

increased in prematurity (Figure 2B), which is consistent with previous reports [8,18]. However, the level of ECFCs in preeclampsia did not change with gestational age ($P > .05$), and it remained significantly lower than the control for both preterm (4 ± 1 colonies in preeclampsia and 14 ± 2 colonies in control; $P = .02$) and term deliveries (2 ± 1 colonies in preeclampsia and 6 ± 2 colonies in control; $P = .06$) (Figure 2B). In addition, the different in ECFC levels between control and preeclampsia for gestational age <37 weeks was statistically significant ($P < .05$). These results confirmed that gestational age is a confounding factor for ECFC level (increased in prematurity) and demonstrated that the overall reduction in ECFC abundance observed in preeclampsia was more prominent among premature deliveries.

Phenotypical and functional characteristics of cord blood ECFCs in preeclampsia

We then examined if there were functional differences among ECFCs from the control and preeclampsia groups. To this aim, ECFCs were first expanded in culture and purified by virtue of CD31 expression (Figure 3A). The endothelial phenotype of CD31-selected cells was verified via expression of CD31 and VE-cadherin at the cell-cell borders, and expression of vWF in a punctuate pattern in the cytoplasm (Figure 3B). Quantitative RT-PCR analyses demonstrated similar levels of expression of endothelial cell markers (CD31, vWF, VE-Cadherin, and eNOS) and absence of mesenchymal cell markers (CD90 and PDGFR-β) in ECFCs from both preeclampsia and control ($P > .05$; Figure 3C). We also observed that ECFCs from both groups expressed high levels of growth factor receptors VEGFR-1, VEGFR-2, and FGFR-1, and low levels of VEGFR-3, FGFR-2, and FGFR-3 (Figure 3D), which is consistent with a vascular endothelial phenotype [20].

To assess ECFC function, we randomly selected six ECFC cultures from each group and performed several in vitro functional assays (Figure 4). In the preeclampsia group, one of the ECFC cultures selected corresponded to a subject with IUGR. We observed no statistical difference between preeclampsia and control in ECFC cloning forming ability (Figure 4A, $P = .48$; Figure 4B, $P = .60$) and in the capacity of ECFCs to assemble into capillary-like structures on Matrigel (Figure 4C; Figure 4D, $P = .95$; Figure 4E, $P = .81$). We observed a moderate decrease in the mitogenic and migratory response to VEGF-A (Figure 4F, $P = .52$; Figure 4H, $P = .20$) and FGF-2 (Figure 4F, $P = .15$; Figure 4H, $P = .29$) in cord blood ECFCs from preeclampsia, although these differences were not statistically significant for n=6. We also examined the in vivo vasculogenic ability of ECFCs after transplantation into immunodeficient mice (Figure 5). In both preeclampsia and control groups, transplanted ECFCs formed extensive networks of perfused microvessels by day 7, as revealed by H&E-stained sections of the explants (Figure 5A) and confirmed by immunohistochemical staining of human-specific CD31 (Figure 5B). Microvessels also stained positively for UEA-1, a lectin that specifically binds human (but not murine) endothelial cells (Figure 5C). In addition, ECFC-lined microvessels had extensive perivascular coverage at day 7, as revealed by positive α-smooth muscle actin (α-SMA) expression (Figure 5C), which indicated vascular stability. Importantly, quantitative histological evaluation of human-specific microvessel density demonstrated no statistical difference between ECFCs from preeclampsia and control (Figure 5D, $P = .71$).

Discussion

The mechanisms that govern the abundance of ECFCs in health and disease are insufficiently known. The maternal vascular pathophysiologic features of preeclampsia are well characterized and involve widespread endothelial dysfunction [21]. However, the effects of preeclampsia on fetal levels of circulating progenitor cells have not been systematically examined. A previous study by Hwang et al (2008) demonstrated a decrease of cord blood $AC133^+/KDR^+/CD34^+$ endothelial progenitor cells (EPCs) and their progeny in pregnancies complicated by preeclampsia [22]. However, there is increasing consensus on the distinction between cells that originate from $AC133^+/KDR^+/CD34^+$ EPCs and those that are defined as ECFCs [23-25]. Indeed, Yoder and colleagues demonstrated that early EPCs that generate endothelial cell colony-forming units (CFU-ECs) are hematopoietic in origin, fail to form perfused vessels in vivo, and are clonally distinct from ECFCs [23]. Thus, in addition to variations in the number of $AC133^+/KDR^+/CD34^+$ EPCs, it remains unclear whether preeclampsia alters baseline levels of cord blood ECFCs. Here, we unambiguously identified ECFCs based on well-known endothelial cell markers and functional properties, and demonstrated that the level of cord blood circulating ECFCs is decreased in preeclampsia. This reduction was statistically significant, independent of common obstetric factors, and was not associated with changes in cell phenotype or function.

Recent studies have emphasized the importance of several confounding factors on circulating levels of ECFCs, including maternal BMI and gestational age [8,18]. Previously, we demonstrated a positive correlation between maternal BMI and ECFC abundance in umbilical cord blood of neonates born from non-obese healthy mothers with non-pathologic pregnancies [18]. This association suggested a potential physiological adaptation that occurs in the rapidly growing fetus in response to intrauterine conditions imposed by maternal weight. In this study, we examined the influence of maternal pre-pregnancy weight and found that ECFCs levels were consistently lower in preeclampsia than in control pregnancies, irrespective of maternal BMI. Gestational age has also been recognized as a source of variation for ECFC levels. Previous studies have shown that levels of circulating ECFCs are more elevated in premature deliveries (28–35 weeks gestational age) than at term [8], even though extremely premature infants (<28 weeks) have been associated with fewer ECFCs [9,14]. We examined the influence of gestational age in an equal number of premature infants (<37 gestational weeks) and observed that independent of gestational age, ECFCs levels were consistently low in the pathological group. This implicated that the difference in ECFC abundance between preeclampsia and control was more significant in premature deliveries than at term. Taken together, our data suggest an impaired mobilization of ECFCs in preeclampsia that is more evident in preterm deliveries and is independent of common obstetric factors.

Emerging evidence indicates that besides inflicting variations in abundance, deleterious conditions during fetal life can also impair ECFCs function [13,16,26]. For instance, ECFCs from newborns of diabetic mothers display premature senescence and reduced proliferative and vasculogenic properties, including a decrease in the ability to form chimeric vessels after transplantation into immunodeficient mice [13]. Similarly, ECFCs derived from pregnancies complicated by IUGR exhibit altered vasculogenic potential [16]. In this study,

we observed a considerable delay in the average time of colony appearance in preeclampsia, with a significant proportion of ECFC colonies emerging during the fourth week of culture. However, with the exception of the delayed endothelial colony formation, ECFCs from preeclamptic pregnancies were otherwise deemed functionally normal. The ability to grow at clonal density and the capacity to form capillary-like networks were highly similar between ECFCs from the preeclamptic group and their non-pathologic counterparts. The proliferative and migratory responses to angiogenic factors VEGF-A and FGF-2 were reduced in ECFCs from the preeclamptic group, although the differences with control ECFCs were not statistically significant. More importantly, ECFCs from the preeclamptic group displayed full vasculogenic capacity after transplantation into immunodeficient mice, forming extensive networks of perfused blood vessels with complete perivascular coverage. Taken together, ECFC function was deemed similar between preeclampsia and control. Nevertheless, whether a larger sample size may reveal small functional differences not appreciated in our study remains a possibility.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Novelty and Significance

What Is New?

To our knowledge, this is the first prospective cohort study that examines cord blood ECFC level and function in preeclamsia.

What Is Relevant?

Preeclampsia is a pregnancy-related disorder associated with increased cardiovascular risk for the offspring. ECFCs participate in the formation of new vasculature and the maintenance of vascular integrity; thus, an impared ECFC level during pregnancy may contribute to an increased risk of developing postnatal cardiovascular events.

Summary

Cord blood ECFC function is normal and highly similar between preeclampsia and control. However, ECFC level is significantly decreased in preeclampsia. This reduction in ECFC abundance is independent of other obstetric characteristics, including gestational age and maternal BMI. Further studies should examine whether a reduced level of cord blood ECFCs correlates with elevated risk of developing subsequent cardiovascular events such as stroke and hypertension.

Perspectives

In this study, we demonstrated a decreased level of umbilical cord blood circulating ECFCs in preeclampsia. Cord blood ECFCs from preeclamptic pregnancies required more time to emerge in culture as endothelial colonies than control ECFCs, but they displayed otherwise normal vascular activity in vitro and in vivo. Epidemiological studies have indicated that several cardiovascular diseases originate during development, and thus there is increasing interest in understanding the relation between the activity of fetal progenitor cells and the appearance of cardiovascular pathologies in the offspring. To date, the pathophysiological implications of having reduced levels of circulating ECFCs during pregnancy are not well understood. Further studies should examine whether the reduced level of cord blood ECFCs observed in preeclampsia correlates with elevated risk of developing subsequent cardiovascular events such as stroke and hypertension.

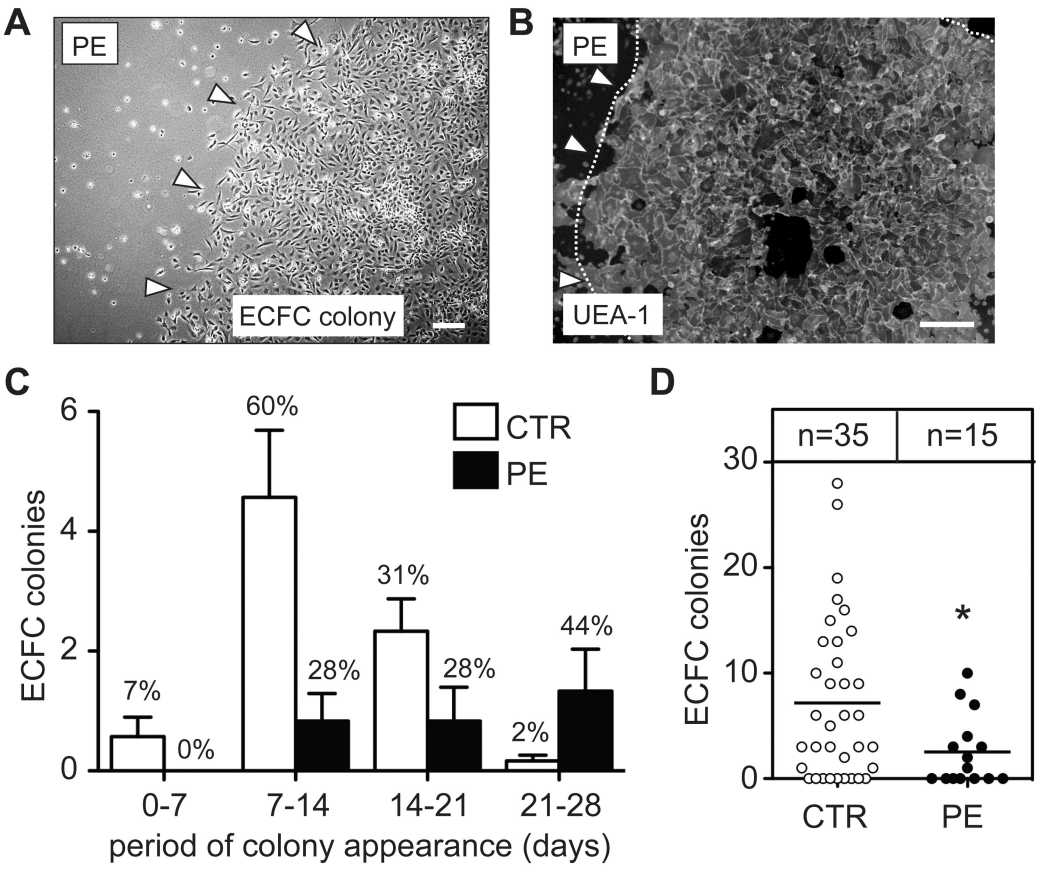

Figure 1. Cord blood levels of ECFCs in preeclampsia

(A) Phase contrast micrograph of a representative ECFC colony from a preeclamptic pregnancy. Arrowheads delimitate the border of the colony (scale bar: 200 μm). (B) Binding of fluorescently labeled *Ulex Europaeus Agglutinin* type 1 lectin (UEA-1) to a colony of ECFCs (scale bar: 200 μm). (C) Weekly appearance of ECFC colonies in culture. Bars represent mean ± SE levels of ECFCs in 10 mL of cord blood. Percentages represent the proportion of total ECFCs appeared each week. (D) Total number of ECFC colonies in 10 mL of cord blood from normal (n=35) and preeclamptic (n=15) pregnancies. Lines represent mean ECFC abundance. N values are denoted on top of each group. PE indicates preeclamptic pregnancy. CTR indicates control pregnancy. * $P < .05$.

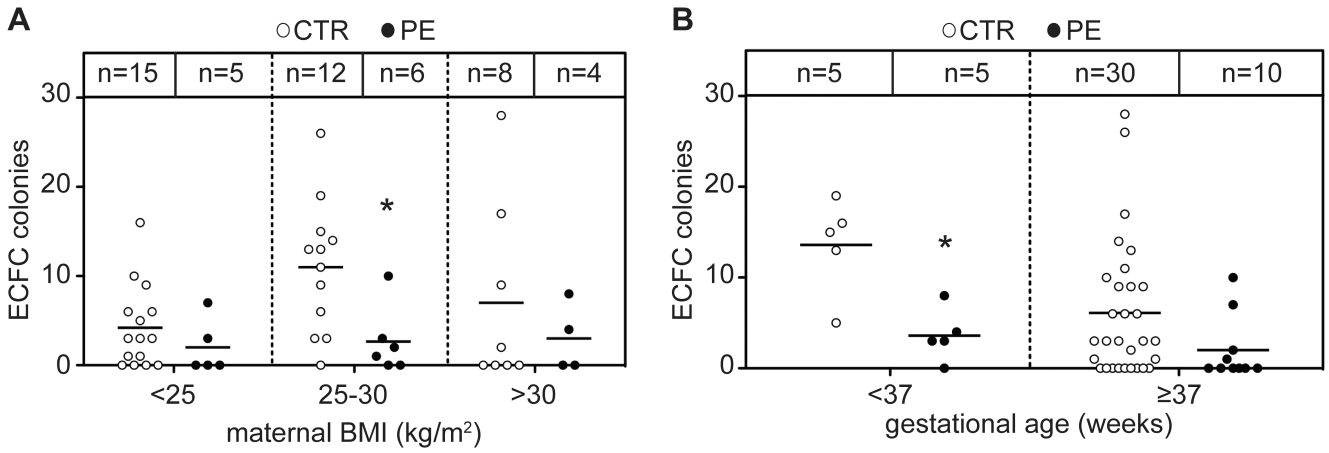

Figure 2. Variation of cord blood ECFC levels with maternal BMI and gestational age

(A) ECFC abundance in cord blood from subjects categorized by pre-pregnancy maternal BMI. (B) Cord blood level of ECFCs from deliveries categorized by gestational age as preterm (< 37 weeks) and term (≥ 37 weeks). Lines represent mean ECFC abundance in 10 mL of cord blood. N values are denoted on top of each group. PE indicates preeclamptic pregnancy. CTR indicates control pregnancy. * $P < .05$ between CTR and PE groups for maternal BMI 25-30 kg/m^2 and gestational age <37 weeks.

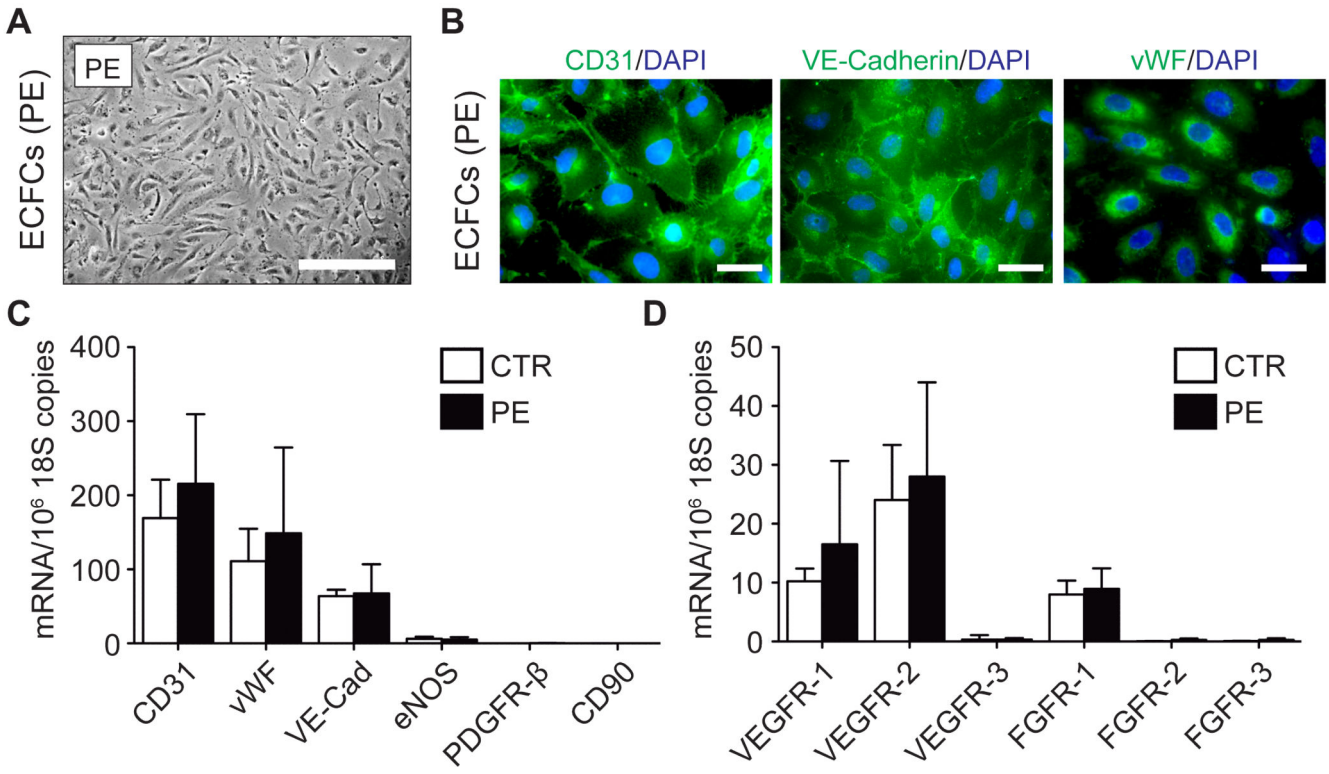

Figure 3. Phenotype of cord blood ECFCs in preeclampsia

(A) Phase contrast micrograph of CD31-selected culture-expanded ECFCs from preeclamptic cord blood (scale bar: 200 μm). (B) ECFC expression of CD31, VE-Cadherin, and vWF demonstrated by indirect immunofluorescence. Cell nuclei were counterstained with DAPI (scale bar: 50 μm). (C) Quantitative RT-PCR analyses of ECFCs for endothelial (CD31, vWF, VE-Cadherin, eNOS) and mesenchymal (PDGFR-β, CD90) cell markers and for (D) VEGF receptors (VEGFR-1, VEGFR-2, VEGFR-3) and FGF receptors (FGFR-1, FGFR-2, FGFR-3). Bars represent mean ± SE (n=6) number of mRNA transcripts normalized to 10^6 copies of 18S ribosomal RNA. PE indicates preeclamptic pregnancy. CTR indicates control pregnancy.

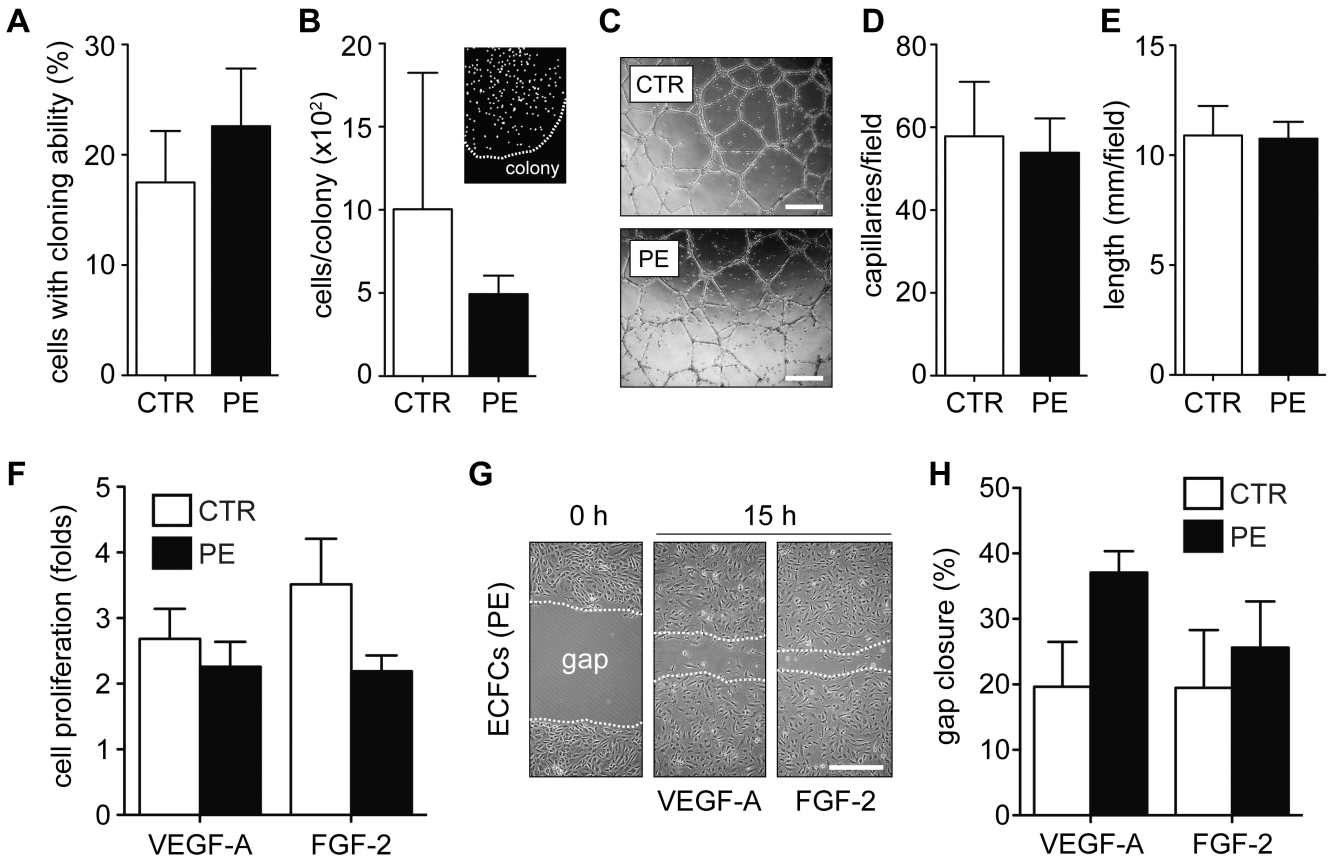

Figure 4. In vitro functional properties of cord blood ECFCs in preeclampsia

(A) Clonogenic properties of ECFCs expressed as (A) percentage of cells with cloning-forming ability and (B) mean number of cells per colony after 10 days in culture. Cell nuclei were identified by DAPI staining (inset). (C) Representative phase contrast micrographs of capillary-like networks formed by ECFCs on Matrigel. The ability to form capillary-like networks was quantified and expressed as (D) total number of capillaries per field and (E) mean capillary length per field. (F) Cell proliferation in response to VEFG-A (10 ng/mL) and FGF-2 (1 ng/mL) expressed as fold increase in cell number. (G) Representative phase contrast micrographs depicting the closure of a gap created in an ECFC monolayer. Gap closure was monitored in response to VEGF-A (10 ng/mL) and FGF-2 (1 ng/mL). (H) Migratory capacity of ECFC in response to VEGF-A and FGF-2 expressed as percentage of gap closure after 15 hours. Bars represent mean r SE (n=6). PE indicates preeclamptic pregnancy. CTR indicates control pregnancy.

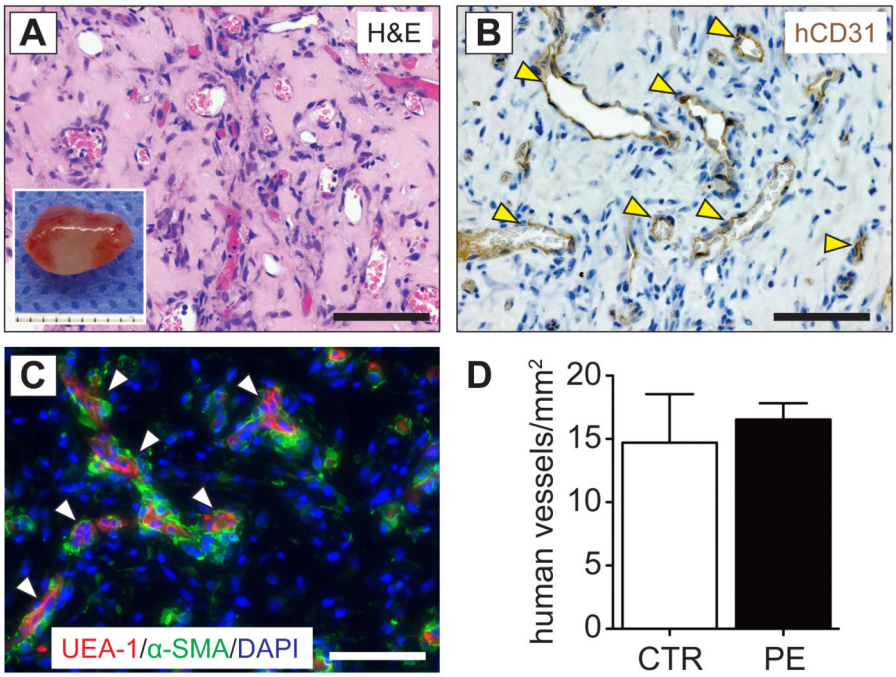

Figure 5. In vivo vasculogenic properties of cord blood ECFCs in preeclampsia

ECFCs were combined with MSCs in Matrigel and the mixture subcutaneously injected into nude mice for 7 days. (A) H&E-stained section of a representative explant revealing numerous perfused blood vessels at day 7. Macroscopic view of the explant is depicted in the inset (scale in mm). (B) Immunohistochemical staining with an antibody against human-specific CD31 (hCD31) revealing numerous human lumens (yellow arrowheads). Cell nuclei were counterstained with hematoxylin. (C) Perivascular coverage was assessed by double immunofluorescence staining using UEA-1 (red) and an antibody against α-SMA (green) (white arrowheads indicate double positive lumens). Nuclei were counterstained with DAPI. All images (A-C) are representative of implants that were seeded with ECFCs from preeclamptic pregnancies (scale bar: 100 μm). (D) Microvessel density determined as the number of ECFC-lined blood vessels per unit of area in implants that were seeded with ECFCs from either preeclamptic or control pregnancies. Bars represent mean r SE (n=6). PE indicates preeclamptic pregnancy. CTR indicates control pregnancy.

Table 1

Obstetric characteristics of preeclampsia and control groups

	Control (n=35)	Preeclampsia (n=15)	*P* Value
Maternal			
Age - years	30.9±5.7	30.4±6.3	.68
Primipara - n (%)	23 (65.7)	13 (86.7)	.18
In vitro fertilization - n (%)	3 (8.6)	2 (13.3)	.63
Cesarean delivery - n (%)	4 (11.4)	9 (60.0)	.001
Tobacco use - n (%)	16 (45.7)	5 (33.3)	.42
Gestational diabetes - n (%)	0 (0.0)	1 (6.7)	.30
Pre-gestational BMI - kg/m^2	26.1±5.1	26.4±5.6	.85 *
<25 kg/m^2 - n (%)	15 (42.8)	5 (33.3)	
25-30 kg/m^2 - n (%)	12 (34.3)	6 (40.0)	
>30 kg/m^2 - n (%)	8 (22.9)	4 (26.7)	
Gestational weight gain - kg	12.2±6.6	12.5±5.3	.88 *
Pre-gestational blood pressure (†)			
Diastolic - mmHg	63.1±7.7	73.1±11.1	.003
Systolic - mmHg	106.7±11.2	112.4±16.6	.20 *
Blood pressure at onset of PE			
Diastolic - mmHg	-	97.1±4.6	-
Systolic - mmHg	-	158.5±9.8	-
Neonatal			
Male - no. (%)	23 (65.7)	10 (66.7)	.95
Gestation age - weeks	39.1±2.1	36.6±2.6	.001
Intrauterine growth restriction - n (%)	0 (0.0)	2 (13.3)	.08
Preterm birth - no. (%)	5 (14.3)	5 (33.3)	.14
Birth weight - kg	3.3±0.6	2.6±0.8	.004 *
Birth weight percentile - %	53.4±34.8	25.9±34.1	.006
Cord blood MNC level (‡) - millions/10 mL blood	36,7±23.3	28.1±18.6	.28

Categorical variables are represented by absolute frequencies and percentages (n,%). Non-categorical variables are represented by mean ± SD. Categorical variables were analyzed with Fisher exact tests except for tobacco use and offspring sex that were analyzed with Pearson chi-squared tests.

(*) Non-categorical variables that were normally distributed were analyzed with Student *t* tests. Non categorical variables that were not normally distributed were analyzed with Mann-Whitney U tests.

(†) Values for pre-gestational blood pressure are from n = 27 control subjects.

(‡) Values for MNC level are from n = 29 control subjects. P values are from comparison of control and preeclampsia groups. BMI = body-mass index; PE = preeclampsia.