

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 toward vascular endothelial growth factor-A and fibroblast growth factor-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 ($P=0.04$), 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. (*Hypertension*. 2014;64:165-171.) • [Online Data Supplement](#)

Key Words: fetal blood ■ preeclampsia ■ pregnancy

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 mellitus, fetal bronchopulmonary dysplasia, and intrauterine growth restriction.^{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 35 (control) white mother-offspring pairs were included in this study. Preeclampsia was defined as high blood

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pressure (>140/90 mmHg) 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 α -methyl dopa; in addition, 5 patients also received labetalol. Intrauterine growth restriction was defined as a fetus with an individualized weight percentile <10% and with asymmetry in several ultrasound measurements, including a significant decrease in abdominal perimeter compared with 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 on obstetric factors are described in Materials and Methods in the online-only Data Supplement.

Enumeration and Characterization of ECFCs

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 performed following previously described methods^{17–19}; details can be found in expanded Materials and Methods in the online-only Data Supplement.

Statistical Analysis

Data from preeclampsia and control subjects were compared and analyzed with IBM SPSS v. 19.0 software (IBM Corp, Armonk, NY). Categorical variables were expressed by absolute frequencies and percentages (n, %). Noncategorical variables were expressed by mean $\pm$ SD or median and 25th to 75th interquartile range. Categorical variables were analyzed with Fisher exact tests except for tobacco use and offspring sex, which were analyzed with Pearson χ^2 tests. Noncategorical 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 the use of Spearman correlation coefficient. Data from experiments performed in vitro and in mice were analyzed using GraphPad Prism v. 5 software (GraphPad Software, La Jolla, CA). These data were expressed as mean $\pm$ SE and mean values were compared using unpaired Student *t* tests. For all analyses, *P*<0.05 was considered significant.

Results

Patient Demographics

We studied 15 (preeclampsia) and 35 (control) mother–offspring pairs (Table). Based on the severity of the pathology, the preeclampsia group included subjects with mild (n=6), severe (n=7), and hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome (n=2). In addition, 2 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*=0.001). Offspring born from mothers with preeclampsia had lower gestational age, birth weight, and birth weight percentile than those in the control group (*P*=0.001, *P*=0.004, and *P*=0.006, respectively). Preeclamptic mothers had higher pregestational diastolic blood pressure (*P*=0.003) than control. There were no statistical differences in the remainder of the obstetric characteristics analyzed (*P*>0.05).

Table. Obstetric Characteristics of Preeclampsia and Control Groups

	Control (n=35)	Preeclampsia (n=15)	<i>P</i> Value
Maternal			
Age, y	30.9 $\pm$ 5.7	30.4 $\pm$ 6.3	0.68
Primipara, n (%)	23 (65.7)	13 (86.7)	0.18
In vitro fertilization, n (%)	3 (8.6)	2 (13.3)	0.63
Cesarean delivery, n (%)	4 (11.4)	9 (60.0)	0.001
Tobacco use, n (%)	16 (45.7)	5 (33.3)	0.42
Gestational diabetes mellitus, n (%)	0 (0.0)	1 (6.7)	0.30
Pregestational BMI, kg/m ²	26.1 $\pm$ 5.1	26.4 $\pm$ 5.6	0.85*
<25, n (%)	15 (42.8)	5 (33.3)	...
25–30, n (%)	12 (34.3)	6 (40.0)	...
>30, n (%)	8 (22.9)	4 (26.7)	...
Gestational weight gain, kg	12.2 $\pm$ 6.6	12.5 $\pm$ 5.3	0.88*
Pregestational blood pressure,† mm Hg			
Diastolic	63.1 $\pm$ 7.7	73.1 $\pm$ 11.1	0.003
Systolic	106.7 $\pm$ 11.2	112.4 $\pm$ 16.6	0.20*
Blood pressure at onset of PE, mm Hg			
Diastolic	...	97.1 $\pm$ 4.6	...
Systolic	...	158.5 $\pm$ 9.8	...
Neonatal			
Male, n (%)	23 (65.7)	10 (66.7)	0.95
Gestation age, wk	39.1 $\pm$ 2.1	36.6 $\pm$ 2.6	0.001
Intrauterine growth restriction, n (%)	0 (0.0)	2 (13.3)	0.08
Preterm birth, n (%)	5 (14.3)	5 (33.3)	0.14
Birth weight, kg	3.3 $\pm$ 0.6	2.6 $\pm$ 0.8	0.004*
Birth weight percentile, %	53.4 $\pm$ 34.8	25.9 $\pm$ 34.1	0.006
Cord blood MNC level,‡ millions per 10 mL blood	36.7 $\pm$ 23.3	28.1 $\pm$ 18.6	0.28

Categorical variables are represented by absolute frequencies and percentages (n, %). Noncategorical variables are represented by mean $\pm$ SD. Categorical variables were analyzed with Fisher exact tests except for tobacco use and offspring sex that were analyzed with Pearson χ^2 tests. *P* values are from comparison of control and preeclampsia groups.

BMI indicates body mass index; MNC, mononuclear cell; and PE, preeclampsia.

*Noncategorical variables that were normally distributed were analyzed with Student *t* tests. Noncategorical variables that were not normally distributed were analyzed with Mann–Whitney *U* tests.

†Values for pregestational blood pressure are from n=27 control subjects.

‡Values for MNC level are from n=29 control subjects.

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 $\geq$ 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 *Ulex europaeus* agglutinin type 1 lectin (Figure 1B). Colonies in the control group emerged in culture as early as 1 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

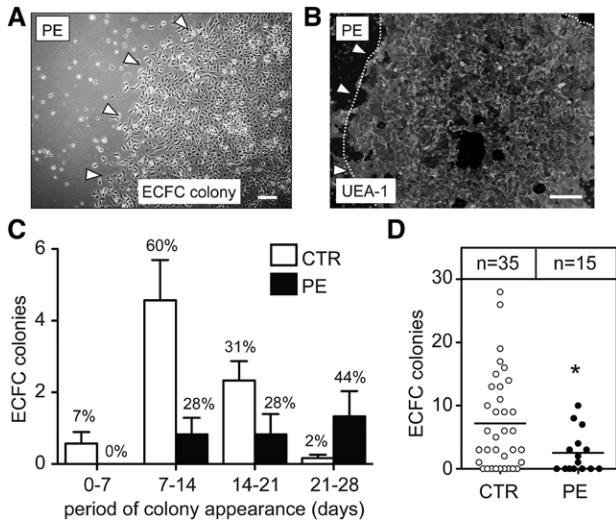


Figure 1. Cord blood levels of endothelial colony-forming cells (ECFCs) in preeclampsia. **A**, Phase contrast micrograph of a representative ECFC colony from a preeclamptic (PE) pregnancy. Arrowheads delineate the border of the colony (scale bar, 200 μ m). **B**, Binding of fluorescently labeled *Ulex europaeus* agglutinin type 1 lectin (UEA-1) to a colony of ECFCs (scale bar, 200 μ m). **C**, Weekly appearance of ECFC colonies in culture. Bars represent mean $\pm$ 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 PE (n=15) pregnancies. Lines represent mean ECFC abundance. n values are denoted on top of each group. * $P < 0.05$. CTR indicates control.

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 25th to 75th interquartile range of 0.5 to 13 colonies. Meanwhile, ECFC level in preeclampsia was statistically lower than in control ($P = 0.04$), with a median abundance of 1 colony per 10 mL of cord blood and a 25th to 75th interquartile of 0 to 4 colonies (Figure 1D). Moreover, a significant portion of the preeclamptic group in the study had no measurable ECFCs. Statistical analyses performed in both preeclampsia and control groups demonstrated that the level of ECFCs was independent ($P > 0.05$) of most obstetric factors (Tables S1 and S2 in the online-only Data Supplement), including maternal age ($P = 0.06$ and $P = 0.77$ in preeclampsia and control, respectively; Table S2), mode of delivery ($P = 0.53$ and $P = 0.83$; Table S1), offspring sex ($P = 0.40$ and $P = 0.64$; Table S1), offspring birth weight ($P = 0.27$ and $P = 0.87$; Table

S2), offspring birth weight percentile ($P = 0.26$ and $P = 0.71$; Table S2), gestational weight gain ($P = 0.31$ and $P = 0.08$; Table S2), and cord blood mononuclear cell level ($P = 0.95$ and $P = 0.97$; Table S2). Moreover, the level of cord blood ECFCs in preeclampsia was independent of both the severity of the pathology (mild/severe/hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome; $P = 0.06$), the time of onset of preeclampsia (early/late; $P = 0.42$), diastolic and systolic pregestational blood pressure ($P = 0.51$ and $P = 0.94$; Table S2), and diastolic and systolic blood pressure at the time of onset of preeclampsia ($P = 0.52$ and $P = 0.27$; Table S2).

Variation of Cord Blood ECFC Levels With Maternal Body Mass Index and Gestational Age

Previously, we demonstrated that maternal body mass index (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 prepregnancy maternal BMI < 25 kg/m² (normal weight; n=15/n=5 control/preeclampsia), 25 to 30 kg/m² (overweight; n=12/n=6), and > 30 kg/m² (obese; n=8/n=4; Figure 2A). ECFC levels in control subjects increased from normal prepregnancy 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 difference in ECFC levels between control and preeclampsia for maternal BMI 25 to 30 kg/m² was statistically significant ($P < 0.05$). 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/m²) 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 ($P > 0.05$), and it remained

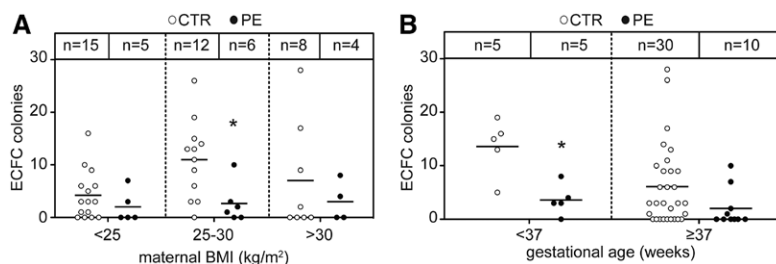


Figure 2. Variation of cord blood endothelial colony-forming cell (ECFC) levels with maternal body mass index (BMI) and gestational age. **A**, ECFC abundance in cord blood from subjects categorized by prepregnancy maternal BMI. **B**, Cord blood level of ECFCs from deliveries categorized by gestational 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. * $P < 0.05$ between control (CTR) and preeclamptic (PE) groups for maternal BMI 25 to 30 kg/m² and gestational age < 37 weeks.

significantly lower than the control for both preterm (4 ± 1 colonies in preeclampsia and 14 ± 2 colonies in control; $P=0.02$) and term deliveries (2 ± 1 colonies in preeclampsia and 6 ± 2 colonies in control; $P=0.06$; Figure 2B). In addition, the difference in ECFC levels between control and preeclampsia for gestational age <37 weeks was statistically significant ($P<0.05$). 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.

Phenotypic and Functional Characteristics of Cord Blood ECFCs in Preeclampsia

We then examined whether 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 vascular endothelial-cadherin at the cell–cell borders, and the expression of von Willebrand factor in a punctuate pattern in the cytoplasm (Figure 3B). Quantitative reverse transcription polymerase chain reaction analyses demonstrated similar levels of expression of endothelial cell markers (CD31, von Willebrand factor, vascular endothelial-cadherin, and endothelial nitric oxide synthase) and absence of mesenchymal cell markers (CD90 and platelet-derived growth factor receptor- β) in ECFCs from both preeclampsia and control ($P>0.05$; Figure 3C). We also observed that ECFCs from both groups expressed high levels of growth factor receptors vascular endothelial growth factor (VEGF) receptor-1, VEGF receptor-2, and fibroblast growth factor (FGF) receptor-1 and low levels of VEGF receptor-3, FGF receptor-2, and FGF receptor-3 (Figure 3D), which is consistent with a vascular endothelial phenotype.²⁰

To assess ECFC function, we randomly selected 6 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 intrauterine growth restriction. We observed no statistical difference between preeclampsia and control in ECFC cloning-forming

ability (Figure 4A; $P=0.48$ and Figure 4B; $P=0.60$) and in the capacity of ECFCs to assemble into capillary-like structures on Matrigel (Figure 4C and 4D; $P=0.95$ and Figure 4E; $P=0.81$). We observed a moderate decrease in the mitogenic and migratory response to VEGF-A (Figure 4F; $P=0.52$ and Figure 4H; $P=0.20$) and FGF-2 (Figure 4F; $P=0.15$ and Figure 4H; $P=0.29$) 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 hematoxylin and eosin-stained sections of the explants (Figure 5A) and confirmed by immunohistochemical staining of human-specific CD31 (Figure 5B). Microvessels also stained positively for *Ulex europaeus* agglutinin type 1 lectin, a lectin that specifically binds to 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 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; $P=0.71$).

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 examined systematically. A previous study by Hwang et al²² demonstrated a decrease of cord blood AC133⁺/KDR⁺/CD34⁺ endothelial progenitor cells and their progeny in pregnancies complicated by preeclampsia. However, there is increasing consensus on the distinction between cells that originate from AC133⁺/KDR⁺/CD34⁺ endothelial progenitor cells and those that are defined as ECFCs.^{23–25} Indeed, Yoder et al²³ demonstrated that early endothelial

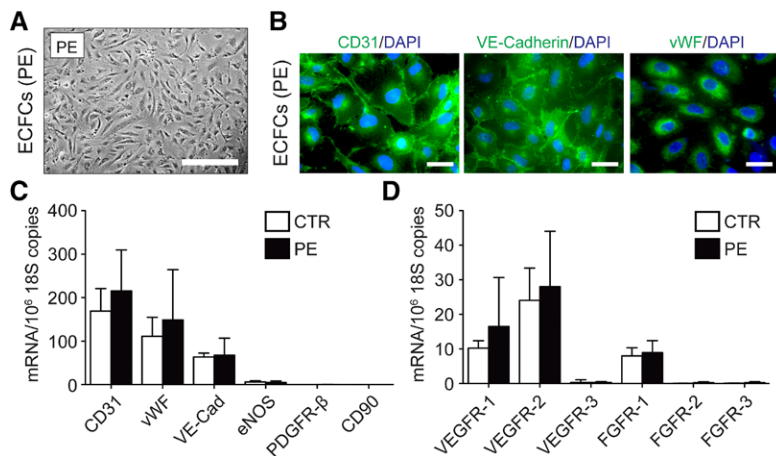


Figure 3. Phenotype of cord blood endothelial colony-forming cells (ECFCs) in preeclampsia. **A**, Phase contrast micrograph of CD31-selected culture-expanded ECFCs from preeclamptic (PE) cord blood (scale bar, 200 μ m). **B**, ECFC expression of CD31, vascular endothelial (VE)-cadherin, and von Willebrand factor (vWF) demonstrated by indirect immunofluorescence. Cell nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI; scale bar, 50 μ m). **C**, Quantitative reverse transcription polymerase chain reaction analyses of ECFCs for endothelial (CD31, vWF, VE-cadherin, and endothelial nitric oxide synthase [eNOS]) and mesenchymal (platelet-derived growth factor receptor [PDGFR]- β , CD90) cell markers and for **(D)** vascular endothelial growth factor receptors (VEGFRs; VEGFR-1, VEGFR-2, and VEGFR-3) and fibroblast growth factor receptors (FGFRs; FGFR-1, FGFR-2, and FGFR-3). Bars represent mean $\pm$ SE (n=6) number of mRNA transcripts normalized to 10⁶ copies of 18S ribosomal RNA. CTR indicates control.

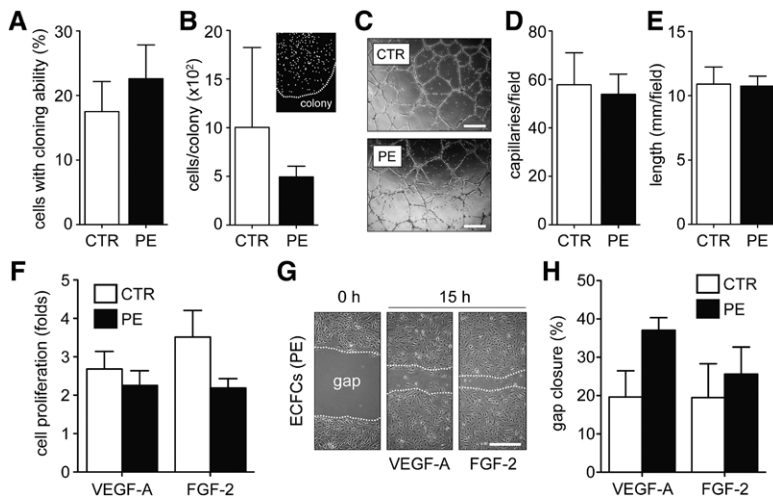


Figure 4. In vitro functional properties of cord blood endothelial colony-forming cells (ECFCs) in preeclampsia. **A**, Clonogenic properties of ECFCs expressed as (A) percentage of cells with cloning-forming ability and (B) mean number of cells per colony after 10 days in culture. Cell nuclei were identified by 4',6-diamidino-2-phenylindole (DAPI) staining (inset). **C**, Representative phase contrast micrographs of capillary-like networks formed by ECFCs on Matrigel (scale bar, 300 μ m). The ability to form capillary-like networks was quantified and expressed as (D) total number of capillaries per field and (E) total capillary length per field. **F**, Cell proliferation in response to vascular endothelial growth factor (VEGF)-A (10 ng/mL) and fibroblast growth factor (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 (scale bar, 200 μ m). 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 $\pm$ SE (n=6). CTR indicates control; and PE, preeclamptic.

progenitor cells that generate endothelial cell colony-forming units are hematopoietic in origin, fail to form perfused vessels in vivo, and are clonally distinct from ECFCs. Thus, in addition to variations in the number of AC133⁺/KDR⁺/CD34⁺ endothelial progenitor cells, 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 nonobese healthy mothers with nonpathological 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 prepregnancy 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 (gestational age, 28–35 weeks) than at term,⁸ although 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

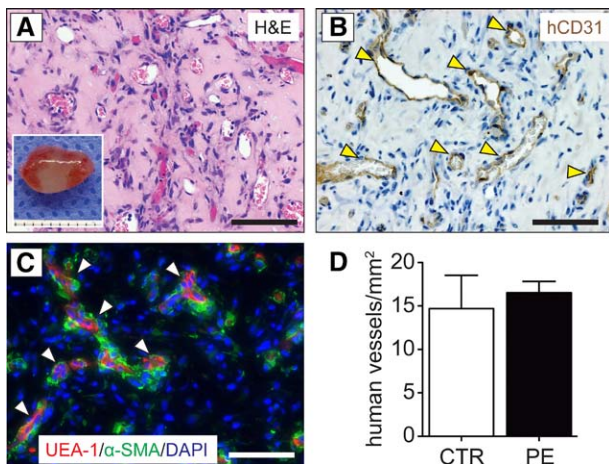


Figure 5. In vivo vasculogenic properties of cord blood endothelial colony-forming cells (ECFCs) in preeclampsia. ECFCs were combined with mesenchymal stem cells in Matrigel and the mixture subcutaneously injected into nude mice for 7 days. **A**, Hematoxylin and eosin (H&E)-stained section of a representative 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 blood vessel lumens (yellow arrowheads). Cell nuclei were counterstained with hematoxylin. **C**, Perivascular coverage was assessed by double immunofluorescence staining using *Ulex europaeus* agglutinin type 1 lectin (UEA-1; red) and an antibody against α -smooth muscle actin (α -SMA; green; white arrowheads indicate double positive lumens). Nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI). All images (A–C) are representative of implants that were seeded with ECFCs from preeclamptic (PE) pregnancies (scale bar, 100 μ m). **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 PE or control (CTR) pregnancies. Bars represent mean $\pm$ SE (n=6).

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 intrauterine growth restriction 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 nonpathological 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.

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.

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Disclosures

None.

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Novelty and Significance

What Is New?

- To our knowledge, this is the first prospective cohort study that examines cord blood endothelial colony-forming cell (ECFC) level and function in preeclampsia.

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 impaired 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 body mass index. 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.