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Review Article

Endothelial progenitor cell subsets and preeclampsia: Findings and controversies

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Abstract

Vascular remodeling is an essential component of gestation. Endothelial progenitor cells (EPCs) play an important role in the regulation of vascular homeostasis. The results of studies measuring the number of EPCs in normal pregnancies and in preeclampsia have been highly controversial or even contradictory because of some variations in technical issues and different methodologies enumerating three distinct subsets of EPCs: circulating angiogenic cells (CAC), colony forming unit endothelial cells (CFU-ECs), and endothelial colony-forming cells (ECFCs). In general, most studies have shown an increase in the number of CACs in the maternal circulation with a progression in the gestational age in normal pregnancies, while functional capacities measured by CFU-ECs and ECFCs remain intact. In the case of preeclampsia, mobilization of CACs and ECFCs occurs in the peripheral blood of pregnant women, but the functional capacities shown by culture of the derived colony-forming assays (CFU-EC and ECFC assays) are altered. Furthermore, the number of all EPC subsets will be reduced in umbilical cord blood in the case of preeclampsia. As EPCs play an important role in the homeostasis of vascular networks, the difference in their frequency and functionality in normal pregnancies and those with preeclampsia can be expected. In this review, there was an attempt to provide a justification for these controversies.

Keywords: Endothelial progenitor cell; Gestation; Hypertension; Preeclampsia; Pregnancy

1. Introduction

Traditional concept was that formation of new blood vessels after birth occurs only by proliferation and migration of mature endothelial cells (ECs), a process termed *angiogenesis*. Recently, this paradigm has been changed by the introduction of *endothelial progenitor cells* (EPCs). These cells are capable of differentiation into ECs and produce new vessels, a process called *vasculogenesis*. It seems that an interaction between ECs and EPCs is required for proper endothelial functioning.[1] As pregnancy is accompanied by formation of new blood vessels, it had been postulated that EPCs may play a role during pregnancy and its vascular complications such as preeclampsia. Several investigations in the field were conducted, and the results, although controversial, clarified the importance of EPCs during gestation. In this review we have tried to provide a justification for these controversies.

2. EPC definition

As the first explanation of EPCs, Asahara et al. isolated and characterized putative EPCs from human peripheral blood by a

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method of culturing on fibronectin.[2] Since then, many changes have been made in the assessment techniques and even the definitions of EPCs. Hill et al. modified their methods and introduced a cluster-forming assay (colony-forming unit hill [CFU-hill]). They showed that there was a significant inverse correlation between concentration of CFU-hill and Framingham cardiovascular risk score in human subjects.[3] Similar findings are depicted in a wide array of other diseases such as type 1 diabetes, chronic obstructive pulmonary disease, obesity chronic heart failure, acute cerebrovascular attacks, peripheral vascular diseases and even rheumatoid arthritis.[4]

In the year 2000, through indirect evidence, Peichev et al. demonstrated that cells co-expressing CD34, CD133 and CD309 markers could be putative EPCs.[5] This study became the basis for many other investigations enumerating EPCs with flow cytometry. However, this study had some design problems, and the conclusions were, therefore, not correct and were not completely based on its results.[6] Results from later investigations are convincing that the cells measured by the two aforementioned methods do not represent true EPCs. These cells express some endothelial lineage markers such as CD34 and CD309, and also express some macrophage/monocyte antigens such as CD14 and CD45. However, they cannot merge into vascular endothelium or differentiate into ECs *in vitro* and, by definition, cannot be true EPCs.[6]

Eventually, true EPCs were discovered by Ingram et al., cells which are now called *endothelial colony-forming cells* (ECFCs). These cells express CD34 and CD309 but lack CD14, CD45, and CD133 expression.[7] Despite these findings and changes in our understanding about EPCs, both CFU-*hills* and *circulating EPCs* measured by flow cytometry remained under the classification umbrella of EPCs in the literature,[4] but they were renamed as CFU-*endothelial cells* (CFU-ECs) and *circulating angiogenic cells* (CACs), respectively.[6] Nevertheless, the reverse correlation between CFU-EC and CAC numbers with cardiovascular disease risk and pathologic endothelial function cannot be ignored.[6]

Formation of new blood vessels is a necessary step in pregnancy, and proper endothelial function is the key. So, EPCs may play an important role in healthy pregnancies and preeclampsia, the most common vascular disorder of pregnancy. However, the few studies addressing this issue have often produced conflicting results.[8]

3. Pregnancy and vascular remodeling

During the normal menstruation cycle and even before a pregnancy occurs, extensive changes occur within the human endometrium. A fundamental part of proliferative and secretory phases of the menstrual cycle is angiogenesis. Expansion of vascular network within the endometrium begins in the proliferative phase and is continued in the secretory phase. This process is believed to happen by elongation and intussusception of the existing small vessels.[9] When pregnancy occurs, more extensive changes will happen in the uterine vasculature. The uterine artery undergoes vasodilatation and by an extensive remodeling, maternal spiral arteries provide a large vascular bed, supplying the placental intervillous space.[8] Angiogenesis is an essential part of placentation, too. Sprouting, a hallmark of angiogenesis in pathological situations such as ischemia, is also a key step in placentation.[8] This step is mediated by invasion of trophoblasts to the interstitial and endovascular spaces of the maternal vessels. During this wave, the endothelium of spiral arteries in the uterus is repetitively damaged and repaired. The result is a fresh layer of new endothelium.[10] Initially, it was thought that transdifferentiation of the trophoblasts helps repair these injuries, but current findings support re-endothelialization, a process in which EPCs may play a critical role.[11]

4. EPCs in normal pregnancies

Chan et al. for the first time suggested that the stem cells may play a critical role in the shedding and repair of the human endometrium during human menstruation cycles.[12] At that time, presence of EPCs in mouse endometrium was shown, but their exact role had not been investigated.[2] In a later study, it was shown that these cells do contribute to angiogenesis in the mouse endometrium.[13] In human studies, flow cytometric enumeration of EPCs in the menstrual cycle was performed and, although not definitive, an elevation in the secretory and follicular phases was noticed.[14,15]

Investigating the role of EPCs in healthy human pregnancies started with a study by Sugawara et al., who showed a significant increase in CFU-ECs as gestation progresses.[16] In a contrary report, Savvidou et al. showed a minor decrease in CFU-ECs with progression of pregnancy, although this number was higher in cases with twin pregnancies than those with singletons.[17] Also, Matsubara et al. showed a decrease in CFU-ECs with increase in the gestational age.[18] In our study, we saw a decrease in CFU-EC from the first to second trimesters. However, we could see some increase afterwards during the third trimester.[19]

Considering CACs as the target population, our data were in accordance with the reports by Buemi et al.[20] and Luppi et al.[21] which showed an increase in the number of CACs with progress in gestational ages but contradicted with the results of the study by Matsubara et al.,[18] who concluded that frequency of CACs decreased with the gestational age and postulated the cause a dilution by expanding the plasma volume.[8]

Our study is the only research which has also enumerated the number of CAC precursors (defined as $CD34^{-}CD133^{+}CD309^{+}$ cells[22]), and we have shown a significant increase in their frequency as gestational age increased.[19]

Estrogens are proposed to have protective effect on human cardiovascular system by increasing the production of nitric oxide and decreasing the reactive oxygen species.[23] Mobilization of EPCs in response to estrogens has been shown. Estrogens also can retard the senescence of EPCs and stimulate VEGF production.[24,25] Therefore, it may be possible that estrogen protects the vascular endothelium during pregnancy by mobilizing the EPCs. As local hormones, cytokines, and chemokines such as estradiol, TNF-α, IL-6, VEGF and ICAM-1 play an important role in the trafficking and migration of

EPCs, their contribution to menstruation can be expected. However, the role of sex hormones and circulating inflammatory cytokines in recruiting EPCs in the non-pregnant states has not been shown yet.[14] In one study, Gussin et al. cultured peripheral blood mononuclear cells from non-pregnant and pregnant women; both groups formed early-outgrowth colonies, but late-outgrowth cells, which have a higher proliferative potential, were only formed by the cells from the pregnant women. The authors initially hypothesized that these cells were of fetal origin, so they stained them for X and Y chromosomes and discovered that none of the colonies were from the fetal cells. Consequently, they concluded that maternal cells are the origin of EPCs in the pregnancy circulation.[26] The role of fetal EPCs in uterine adaptive changes have also been investigated.[27] Ingram et al. have shown that fetal EPCs are dramatically more efficient than adult counterparts. In a comparative study, they showed that fetal EPCs were more abundant and active than their adult counterparts and adult EPCs couldn't present the high proliferative phenotype.[7] Fetal ECFCs, in the mouse models, have been shown to migrate into the maternal circulation and are capable of homing into the micro-vessels of the gravid uterus.[27] This is not verified in humans yet, but it may be a potential alternative participant in restoration of the uterine vessel endothelium. On the other hand, as placenta is known to be a source of hematopoietic cell production,[28] it can be postulated that it is at least a supplementary source of EPCs found in the fetal circulation. In a recent study, it was shown that ECFCs and CACs are more frequent in the umbilical arteries than the umbilical veins. So it is doubtful that the placenta is a source of EPCs for the fetus, and that this organ retains EPCs on their passage.[29] Although these findings are not confirmative, current evidence suggests that EPCs may play a role in the placenta endothelium and vascular homeostasis, even in late stages of pregnancy.

5. Preeclampsia and vascular dysfunction

Pre-eclampsia is a vascular gestational disorder associated with maternal endothelial dysfunction. Pre-eclampsia occurs in 5–7% of first pregnancies, shows 20–25% recurrence, and is the most common medical complication of pregnancy. The disease is characterized by hypertension and proteinuria and is a significant cause of maternal and perinatal morbidity and mortality worldwide.[9]

At present, the initiating insult that causes pre-eclampsia is not defined. Nevertheless, deficient placentation is considered a causative agent for early-onset cases. Maternal vascular maladaptations are more responsible in late-onset cases. In both cases, there is evidence supporting that endothelial dysfunction precedes clinical presentations and a rise in soluble markers of endothelial dysfunction (such as intercellular adhesion molecule-1 [ICAM], vascular cellular adhesion molecule-1 [VCAM], cellular fibronectin, E-selectin, and endothelin-1) and a reduction in flow-mediated dilatation of the brachial artery at 23–25 weeks gestation is observed.[30–32] Some markers of endothelial dysfunction may rise even before the clinical features of the disease appear.[8] They include plasminogen activator inhibitor type 1 (PAI-1),[33] dimethylarginine (an endogenous inhibitor of nitric oxide synthesis),[34] and tissue plasminogen activator (t-PA), which correlates with the degree of proteinuria.[35] Women with pre-eclampsia are also more likely to have impaired uterine artery Doppler waveforms,[36] suggesting that endothelial dysfunction precedes pre-eclampsia.[34] Endothelial dysfunction continues even after delivery,[37] and preeclamptic women are at an increased risk of future hypertension, and coronary and cerebro-vascular disorders.[38] In addition, conditions known to be associated with endothelial dysfunction, such as renal disease, hypertension, and diabetes, can also increase the risk of developing pre-eclampsia during pregnancy. All of these predisposing maladies are known to have intrinsically lower numbers of EPCs.[30–32] Although there are extensive studies reporting decreased levels of EPCs or abnormal function of EPCs in men and non-pregnant women with these conditions, the available data about EPCs in pre-eclampsia is not still conclusive.

6. EPCs in preeclampsia

Investigations about the role of EPCs in preeclampsia started with the study of Sugawara et al. Sugawara et al.,[39] Lin et al.,[4] and Parsanezhad et al[19] obtained the same results, that there are fewer CFU-ECs in patients with preeclampsia compared with gestational-age-matched normal pregnancies. This, however, was in contrast with the findings of Matsubara et al.[18] In another study by Murphy et al., preeclampsia was associated with reduced number of CFU-ECs 2 and 6 months postpartum.[40] Luppi et al. have also shown a reduction in CFU-ECs in preeclampsia, and have demonstrated a reduction in CACs, too.[21] In contrast, in our study as well as the report by Buemi et al.[20] the number of CACs was seen to increase in maternal circulation of pre-eclamptic women, a finding in disagreement with the results reported by Matsubara et al.[18] Sakashita et al. have shown that this number is even more decreased in preeclamptic patients who finally have developed placental abruption.[41] In the study by Murphy et al., levels of $CD34^{+}CD309^{+}$ and $CD133^{+}CD309^{+}$ cells were elevated in preeclamptic subjects 2 months postpartum compared to healthy control subjects, although they were reduced by 6 months postpartum.[40] In our study, the number of precursors of CACs was significantly higher in the maternal circulation of the preeclampsia group.[19]

In cord blood, the findings are as follows: Monga et al. have shown that UCB of patients with preeclampsia or intrauterine growth restriction (IUGR) has a reduced number of CAC.[42] Hwang et al. demonstrated that the number and functional ability of the fetal CAC and CFU-EC from preeclampsia without IUGR are significantly decreased, and they are more senescent compared with those of normal pregnancy as well.[43] Xia et al. have shown a reduction in CACs and CFU-ECs in the cord blood of preeclamptic women, and the numbers of both cell types were inversely correlated with the cord blood level of soluble fms-like tyrosine kinase 1 (sFlt-1). In addition,

the EPCs from patients with pre-eclampsia were significantly impaired in their proliferation, migration and vasculogenic capacities.[44] These anomalies are specifically related to diminished cord plasma-free VEGF and overabundance of sVEGFR-1 (sFlt-1).[45] Angiogenic activities within the human placenta are organized by various interacting factors, including pro-and antiangiogenic mediators, such as placental growth factor (PlGF), VEGF and its neutralizing soluble receptor, sVEGFR-1, and oxygen tension. Significant variations of these molecules have been depicted in the placenta of preeclamptic patients.[46] Several studies have investigated the impact of these environmental changes on fetal endothelial cells. Inherent impairments in mechanical properties, cell permeability and morphology, and release of vasodilatory mediators of isolated HUVECs, i.e. mature endothelial cells, have been shown in vitro, and even in the absence of in vivo altered molecular niche in preeclamptic pregnancies.[47–50] Although their significance is yet unproven, the altered functionality of the endothelial cells from preeclamptic pregnancies may arise from intrinsic differences or environmental adaptations prior to cell isolation, i.e. the angiogenic feto-placental milieu.[9] Recently, in a study by Kanki et al., it was shown that systemic transfusion of EPCs significantly reduced the rate of miscarriage in a mouse model, and the placental vascular pattern in miscarriage tended to be normalized with increased vessel size up to that of normal gestation by EPC recruitment.[51]

7. Technical issues

Existent controversy in the literature lies in several issues. First of all, some studies have enumerated EPCs in the cord blood and others in the maternal blood, which clearly cannot be linked to each other, and the results should be interpreted separately. Furthermore, there is a lack of consensus on the definitive EPC phenotype and a variety of surface markers, and functional assays have been used to enumerate these cells, which makes the comparison between their results difficult. In fact, the antigen panel that is chosen to measure CACs with flow cytometry can affect the final results very strongly. The complete platform of antigen markers for detection of CACs is $CD34^+CD133^+CD309^+$. Unfortunately, some studies have used two-marker combinations such as $CD34^+CD133^+$ or $CD34^+CD309^+$. The number of CACs is shown to be moderately correlated with the number of $CD34^+CD309^+$ cells and very strongly to that of $CD133^+CD309^+$, but not to the number of $CD34^+CD133^+$ cells. Furthermore, all studies have assessed EPCs with a marker combination lacking CD309 are inaccurate and mostly have addressed hematopoietic progenitor/stem cells instead of EPCs.[52] Additionally, the resulting data are reported in two ways: numbers in the volume of blood (i.e. number/ml) and frequencies in a defined number of mononuclear cells (i.e. number/$10^{5\text{ or }6}$ MNCs), which makes the comparison of different clinical studies even more difficult. In one study, comparison of these two methods of data calculation revealed a significant difference in the final results.[19] Some other technical issues are also important to consider. Because of the low frequency of the target cells in the peripheral blood, flow cytometry protocols should follow rare cell analysis protocols.[53] In this regard, at least 10^6 cells should be stained and analyzed. Dead cells should be removed or excluded to decrease the chance of non-specific cell antibody bindings.[53] Many studies have only used 10^5 cells, which significantly decreases the accuracy of test results. Also, simply enumerating the gated events during flow cytometry can be misleading, and absolute counting techniques should be used for each sample analyzed. Again, this is a missed portion in many studies. Furthermore, in relatively many studies, both flow cytometry and culture techniques have not been used together to compare EPC subsets. Finally, most of these studies were cross-sectional, and absence of a prospective approach could explain at least in part, some of the existing variations among reported results. Also, many factors affecting the circulation of EPC numbers were not described for the study subjects in most investigations. See Tables 1 and 2 for a detailed analysis of technical points in studies evaluating the role of EPCs in normal and preeclamptic pregnancies.

8. Endothelial colony forming cells (ECFCs)

In the special case of ECFCs, technical points become even more important. First of all, these cells are very rare in the peripheral blood of adults, although they can be found in concentrations of up to 20 times higher in umbilical cord blood (UCB).[54] Consequently, when one intends to enumerate them, even higher cell numbers should be obtained and cultured than when CFU-ECs or CACs are being analyzed. A seeding density in the range of $3–5 \times 10^7$ MNCs into each well of a 6-well tissue culture plate pre-coated with collagen I is ideal for ECFC colony formation from UCB,[55] and this number must be increased at least 5–10 times if one needs to use adult peripheral blood. Currently, only our study has measured ECFCs in maternal circulation during normal pregnancy and preeclampsia, and we overlooked culturing techniques to measure ECFCs; instead, we only used flow cytometry.[56] This is because we should have taken large volumes of blood samples for accurate measurements and most of our participants refused to donate such volumes of blood during their pregnancy period, especially those with preeclampsia who had a fixed vascular volume status with a very low reserve to compensate the lost blood. For the same reason, Luppi et al. also skipped measuring ECFCs in their study.[21] Instead, we used a flow cytometry technique to enumerate them. In a landmark study by Mund et al., it was shown that flow-cytometric-enumerated $CD34^+CD45^-$ are the cells that can prospectively form ECFCs.[54] In addition, it was shown that the enumerated culture-derived ECFCs colonies had a strong correlation with flow cytometric enumerated $CD34^+CD45^-$ and $CD34^+CD309^+$ cells while having no correlation to the $CD34^+CD133^+$ ones as a marker of CACs.[57,58] We used the combination $CD34^+CD133^-CD309^+CD45^-$, which is a more exact definition for ECFCs. Also, we used some other marker

Table 1
Studies enumerating EPC subsets in the three trimesters of uncomplicated healthy pregnancies.

Study	Fetal or maternal circulation?	EPC subset analyzed	Flow or culture?	Marker panel used	Absolute cell counting	Number of cells analyzed[a]	Type of reporting the results[b]
Sugawara et al.[16]	Maternal	CFU-EC	Culture	N/A	N/A	1.5×10^6 MNCs	Number/MNCs
Savvidou et al.[17]	Maternal	CFU-EC	Culture derived cells underwent flow cytometry	DiI-Ac-LDL$^+$Lectin$^+$	N/A	2×10^4 Culture derived cells	Number/MNCs
Matsubara et al.[18]	Maternal	CFU-EC	Culture	N/A	N/A	At least 10^6 MNCs	Number/mL
		CAC	Flow	CD34$^+$CD133$^+$CD309$^+$	No	10^5 MNCs	Number/MNCs
Parsanezhad et al.[19]	Maternal	CFU-EC	Culture	N/A	N/A	At least 10^6 MNCs for all cells	Both: Number/MNCs, and Number/mL for all cells
		CAC	Flow	CD34$^+$CD133$^+$CD309$^+$	Yes		
		CAC Precursors	Flow	CD34$^-$CD133$^+$CD309$^+$	Yes		
		ECFC	Flow	CD34$^+$CD133$^-$CD309$^+$CD45$^-$	Yes		
Buemi et al.[20]	Maternal	CAC	Flow	CD34$^+$CD133$^+$CD309$^+$	Yes	10^5 events	Number/mL
Luppi et al.[21]	Maternal	CFU-EC	Culture	N/A	N/A	At least 10^6 MNCs	Number/MNCs
		CAC	Flow	CD34$^+$CD309$^+$ or CD133$^+$CD309$^+$	No	At least 10^5 events	Number/lymphocytes

CAC = Circulating angiogenic cell; CFU-EC = colony-forming unit endothelial cells; ECFC = endothelial colony-forming cell; EPC = endothelial progenitor cell; Flow = Flow-cytometry; N/A = Not applicable; MNC = Mononuclear cell.

[a] Some studies have not reported the exact number of cells analyzed but reported the blood volume from which MNCs were isolated. When at least 10 ml of blood was used, we considered that at least 10^6 MNCs were studied.

[b] Some studies seeded an exact number of cell (for example, 5×10^6 MNCs) per well for enumerating CFU-ECs or ECFCs, and they reported the results as numbers per well. Here, to have a better understanding, we have considered this type of reporting as numbers among MNCs.

Table 2
Studies enumerating EPC subsets in preeclampsia compared to uncomplicated healthy pregnancies.

Study	Fetal or maternal circulation?	EPC subset analyzed	Flow or culture?	Marker panel used	Absolute cell counting?	Number of cells analyzed[a]	Type of reporting the results[b]
Lin et al.[4]	Maternal	CFU-EC	Culture	N/A	N/A	At least 5×10^6	Number/MNCs
Matsubara et al.[18]	Maternal	CFU-EC	Culture	N/A	N/A	At least 10^6 MNCs	Number/ml
		CAC	Flow	CD34$^+$CD133$^+$CD309$^+$	No	10^5 MNCs	Number/MNCs
Parsanezhad et al.[19]	Maternal	CFU-EC	Culture	N/A	N/A	At least 10^6 MNCs for all cells	Both: Number/MNCs, and Number/mL for all cells
		CAC	Flow	CD34$^+$CD133$^+$CD309$^+$	Yes		
		CAC Precursors	Flow	CD34$^-$CD133$^+$CD309$^+$	Yes		
		ECFC	Flow	CD34$^+$CD133$^-$CD309$^+$CD45$^-$	Yes		
Buemi et al.[20]	Maternal	CAC	Flow	CD34$^+$CD133$^+$CD309$^+$	Yes	10^5 events	Number/ml
Luppi et al.[21]	Maternal	CFU-EC	Culture	N/A	N/A	At least 10^6 MNCs	Number/MNCs
		CAC	Flow	CD34$^+$CD309$^+$ or CD133$^+$CD309$^+$	No	At least 10^5 events	Number/lymphocytes
Sugawara et al.[39]	Maternal	CFU-EC	Culture	N/A	N/A	1.5×10^6	Number/MNCs
Sakashita et al.[41]	Maternal	CAC	Flow	CD45^{Low}CD34$^+$CD133$^+$	Yes	At least 10^6 MNCs	Number/mL
Monga et al.[42]	Fetal	CAC	Flow	CD45^{Low}CD34$^+$CD133$^+$	No	At least 10^5 events	Number/MNCs
Hwang et al.[43]	Fetal	CFU-EC	Culture	N/A	N/A	At least 10^6 MNCs	Number/MNCs
		CAC	Flow	CD34$^+$CD133$^+$CD309$^+$	No	At least 10^6 MNCs	Number/mL
Xia et al.[44]	Fetal	CFU-EC	Culture	N/A	N/A	At least 10^6 MNCs	Number/MNCs
		CAC	Flow	CD133$^+$CD309$^+$	Yes	10^4 MNCs	Number/mL
Muñoz-Hernandez et al.[58]	Fetal	ECFC	Culture	N/A	N/A	At least 10^7 MNCs	Number/mL
von Versen-Höynck et al.[59]	Fetal	ECFC	Culture	N/A	N/A	At least 10^7 MNCs	Number/mL
Brodowski et al.[60]	Fetal	ECFC	Culture	N/A	N/A	At least 10^7 MNCs	Number/mL

CAC = Circulating angiogenic cell; CFU-EC = colony-forming unit endothelial cells; ECFC = endothelial colony-forming cell; EPC = endothelial progenitor cell; Flow = Flow-cytometry; N/A = Not applicable; MNC = Mononuclear cell.

[a] Some studies have not reported the exact number of cells analyzed, but reported the blood volume from which MNCs were isolated. When at least 10 ml of blood was used we considered that at least 10^6 MNCs were studied.

[b] Some studies seeded an exact number of cell (for example, 5×10^6 MNCs) per well for enumerating CFU-ECs or ECFCs, and they reported the results as numbers per well. Here, to have a better understanding, we have considered this type of reporting as numbers among MNCs.

combinations suggested for measuring ECFCs and found a higher number of ECFCs measured with all possible combination markers in the maternal circulation as preeclampsia occurs, although only the difference in the number of $CD309^{+}CD45^{-}$ cells reached statistical significance. This finding is similar to those in the case of acute myocardial infarction, where, as endothelial damage occurs, the number of ECFCs rises in the peripheral blood.[57,58] Regarding the normal pregnancy, we did not find any significant differences in the number of putative ECFCs in the maternal circulation with changes in the gestational age.

In cord blood, Muñoz-Hernandez et al. have shown that the level of ECFCs was statistically lower in preeclampsia than in control pregnancies, a reduction that was independent of other obstetric factors. In addition, the cord blood ECFCs from preeclamptic pregnancies required more time to emerge in culture than did 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; also preeclampsia affected ECFC frequency in neonates.[59] Interestingly, two studies have shown that vitamin D supplementation can prevent ECFC reduction and dysfunction in UCB of patients with preeclampsia.[60,61] However, it has been shown that the level of ECFCs in UCB is affected by several other factors. For example, Moreno-Luna et al. found a positive correlation between pre-pregnancy maternal BMI and ECFC numbers. Despite this variation in frequencies, ECFC phenotype and functionality were deemed normal and highly similar between subjects with a maternal BMI below 25 kg/m^2 and between 25 and 30 kg/m^2. So, maternal BMI needs to be considered as a potential confounding factor for the cord blood levels of ECFCs in future studies comparing between healthy and pathologic pregnancies.[62] Also, Baker et al. have shown that the delivery age can affect ECFCs as well. They reported that preterm UCB (28/35-week gestation) yielded significantly more ECFC colonies than term ones. Preterm ECFCs appeared in increased numbers and proliferated more rapidly but had an increased susceptibility to hyperoxia compared with term ECFCs, and antioxidants protected preterm ECFCs from hyperoxia.[63] In addition, the presence of IUGR can affect ECFC numbers as well. A study by Sipos et al. has demonstrated that the cord-blood derived ECFCs of fetuses with IUGR formed fewer blood vessels and capillaries compared with normal pregnancy-derived ECFCs. In culture conditions, IUGR derived ECFCs had reduced proliferation and migration and diminished chemotactic abilities to stromal cell-derived factor 1 coupled with reduced hypoxia-induced matrix metalloproteinase-2 release. Finally, in IUGR pregnancies, the number of ECFCs was lower in the arterial cord blood and the placental uptake of the cells was reduced. This could be a cause of placental dysfunction in IUGR, leading to enhanced long-term postnatal cardiovascular risks.[64]

In conclusion, most studies have shown an increase in the number of CACs in the maternal circulation with a progression in the gestational age in normal pregnancies while functional capacities measured by CFU-ECs and ECFCs remain intact. In the case of preeclampsia, mobilization of CACs and ECFCs occurs in the peripheral blood of pregnant women but the functional capacities shown by culture the derived colony forming assays (CFU-EC and ECFC assays) are altered. Furthermore, the number of all EPC subsets will be reduced in UCB in the case of preeclampsia.

As EPCs play an important role in the homeostasis of vascular networks, the difference in their frequency and functionality in normal pregnancies and those with preeclampsia can be expected. It was known that during stress or endothelial injuries such as acute myocardial infarction, EPCs can be mobilized resulting in increased number of these cells in the peripheral blood.[65] In fact, this can be an attempt to promote re-endothelialization of the damaged vessels. The same scenario may be true about preeclampsia. However, chronic inflammation can affect the functionality of these cells.[3] Accordingly, the increased numbers of CACs and ECFCs in the blood of patients with preeclampsia is possibly a result of mobilization. However, due to inflammation, the cells were incapable of performing their normal function, which can be demonstrated by a lower ability for colony formation, 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. On the other hand, in higher gestational ages of a normal pregnancy, as the fetus grows, the demand for EPC rises and the number of CACs increases with normal functional capabilities. Also, it is possible that EPCs represent a common cellular pathway linking cardiovascular risk factors and endothelial dysfunctional states with preeclampsia, which may render the individuals more vulnerable to future cardiovascular disorders.[8]

These findings emphasize again the importance of EPCs as a target for biological diagnoses and therapies of preeclampsia and other vascular diseases. There is an urgent need to reach a global definition for EPCs and a standardized method of reporting experimented results. In future studies, several technical points should be considered. First of all, the exact EPC subset which is studied should be addressed properly, and only gold-standard phenotypes should be used for measurements. In addition, rare cell analysis protocols should be carefully considered in methodologies. Also, baseline confounding factors such as pre-pregnancy maternal BMI, gestational age at the time of sampling, and the presence or absence of IUGR and/or placental abruption should be carefully matched in study groups. Prospective studies are recommended to be conducted to determine whether measuring the EPC numbers in early pregnancy can help predict the occurrence of preeclampsia or not.

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