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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

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