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Review: Endothelial progenitor cells in pregnancy and obstetric pathologies

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ABSTRACT

Since their discovery, endothelial progenitor cells (EPCs) have generated considerable interest in vascular biology. They are a heterogeneous population of cells that exist in both the fetus and adult, and are mobilized to support *de novo* vessel formation or encourage vascular health. This review summarizes our understanding of these cells in pregnancy, paying particular attention to their physiological role in placental development and the uterus, alongside their involvement in related obstetric pathologies.

1. Introduction

Vasculogenesis and angiogenesis are processes of new blood vessel generation. Before 1997, vasculogenesis, *de novo* vessel formation through differentiation of angioblasts, was believed to be restricted to embryonic development, with angiogenesis, formation from pre-existing vessels, superseding postnatally. In 1997 Asahara et al. [1] published the first report of circulating bone-marrow derived cells in humans capable of differentiation into mature endothelia and participation in neovascularisation. Shi et al., then showed comparable cells endothelialising intra-aortic Dracon grafts in dogs [2]. These two studies established the existence of endothelial progenitor cells (EPCs) in the adult, with functional similarities to embryonic angioblasts. With this discovery came a paradigm shift in vascular biology, with the recognition that neovascularisation in adults could depend, at least in part, on putative postnatal vasculogenesis. Subsequent studies confirmed that EPCs are mobilized from the bone-marrow (and possibly from other organs) and home to sites of tissue ischemia or endothelial damage, adopting a more mature endothelial phenotype and contributing to *de novo* vessel formation. Thus, EPCs play an integral role in endothelium regeneration, vessel repair and maintenance.

Since 1997, the number of investigations into human EPCs has grown exponentially. The bulk of this research has focused on adult cardiovascular disease, with scant attention to the fetus, placenta or pregnancy. The fact that vessel formation and vascular expansion are implicit to pregnancy, and that the presence and activity of these cells is greater in the fetus compared to adults, has generally gone unnoticed. This article reviews current evidence of EPC involvement in human pregnancy. It appraises the current literature, introduces some preliminary findings and discusses possibilities for further obstetric research and intervention. The involvement of EPCs in the pathophysiology of pre-eclampsia, intrauterine growth restriction (IUGR) and fetal programming is considered.

2. Origins of EPCs

In recent years EPCs, and more specifically their absence, have emerged as risk factors for cardiovascular disease, with good evidence for a role in endothelial homeostasis and repair. Independent of more classic risk factors, the levels of these cells can predict endothelial dysfunction in healthy individuals, and cardiovascular events in patients with coronary artery disease [3,4]. A similar reduction in circulating EPCs is noted in a variety of other vascular-related conditions, including diabetes [5], peripheral vascular disease, rheumatoid arthritis [6] and obesity [7], but the mechanisms underlying these associations are uncertain.

Although not restricted to bone marrow, the majority of EPCs reside within its microenvironment, termed the stromal or osteoblast niche. In this state they are tethered by integrins to the stromal

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cells until they are transferred to the vascular niche for subsequent egress to the peripheral circulation. All classes of EPCs are derived from hemangioblasts, embryonic or adult, which differentiate to endothelial cells or myeloid progenitors (Fig. 1). Ultimately, whether all endothelial cell progenitors proceed through a "monocytic" phase is unclear. However, at least two fundamentally different types of EPCs may be mobilized, CD45 and CD34 expressing hematopoietic-like cells and CD34 positive CD45 negative "endothelial-like" equivalents [8]. These cells play a critical and some say complementary role in blood vessel formation and repair [9].

The proliferation and mobilization of EPCs from the bone-marrow involve a complex interplay of adhesion molecules, chemokines, cytokines and proteinases. In vascular injury and tissue ischemia, vascular endothelial growth factor (VEGF) and stromal cell-derived factor-1 (SDF-1) are thought to be particularly important, upregulated in the circulation by hypoxia-inducible factor 1 (HIF-1) [10]. Nitric oxide (NO) is also a key intermediary, as the activation of endothelial nitric oxide synthase (eNOS) in the bone marrow is considered a common mobilizing reaction of EPCs to VEGF, estrogen, insulin-like growth factor I (IGF-I) and erythropoietin [11]. In liberating EPCs, SDF-1 and NO activate matrix metalloproteinase 9 (MMP-9), which in turn catalyzes the proteolysis of Kit Ligand (KitL), severing its adhesion to c-kit receptor and freeing the cells from their bone marrow niche. In mice, acute administration of this receptor, in its soluble form, sc-kit, increases mobilization of EPCs, theoretically disrupting these c-kit - KitL interactions [12].

3. Characterization of EPCs

The characterization of EPCs is highly contentious. In cell cultures of unfractionated mononuclear cells, at least two fundamentally different types of EPCs have been generated, early- and late-outgrowth cells, respectively. The early outgrowth cells are referred to as colony-forming unit endothelial cells (CFU-ECs) or circulating angiogenic cells (CACs), whilst alternative conditions yield endothelial colony forming cells (ECFCs) (Fig. 1). These cells appear late in culture, are highly proliferative, can be clonally expanded, but are otherwise indistinguishable from mature endothelial cells. CACS and ECFCs may work synergistically *in vivo*, CACs primarily enhancing the survival and function of ECFCs by providing copious angiogenic factors (NO, VEGF, IGF-I etc), and ECFCs participating in blood vessel and endothelium formation per se [9]. Flow cytometry is also used to enumerate these groups. These approaches most often use CD34 or CD133 in combination with VEGFR-2 (KDR), but again a consensus is far from reached. In general, ECFCs are often characterized as $CD31^+/CD34^+/CD45^-/VEGFR\text{-}2^+/CD133^-$, CACs as $CD31^+/CD34^+/CD45^{+/dim}/CD14^+/CD133^{+/-}$ [13]. These techniques are improved by excluding false positive events, such as dead cells and $CD3^+$ T cells.

4. EPCs and the uterus

Extensive remodeling and growth within the human endometrium precedes implantation. Physiological angiogenesis is fundamental to the proliferative and secretory phases of the menstrual cycle. The involvement of adult stem cells in the cyclic regeneration and shedding of the endometrium was first suggested by Chan et al., in 2004, following the clonogenic derivation of human endometrial epithelial and stromal cells [14]. Although, EPCs had already been defined in the endometrium in the mouse at this time, their role had not been confirmed [15]. In 2007, bone-marrow derived EPCs were shown to contribute to *de novo* blood vessel formation in the mouse endometrium [16]. In verifying EPC participation in the menstrual cycle, flow cytometric determinations of circulating human cells were performed. Although not definitive, these studies showed elevations in the secretory and follicular phases, implying steroid regulation and endometrial regenerative contributions [17,18]. Given that local factors such as estradiol, tumor necrosis factor-α (TNF-α), interleukin (IL)-6, VEGF and intercellular adhesion molecule (ICAM)-1, participate in EPC migration and trafficking, their contribution to the human cycle was anticipated, but sex steroids and circulating inflammatory mediators have yet to be correlated with peripheral EPCs in the non-pregnant state [17].

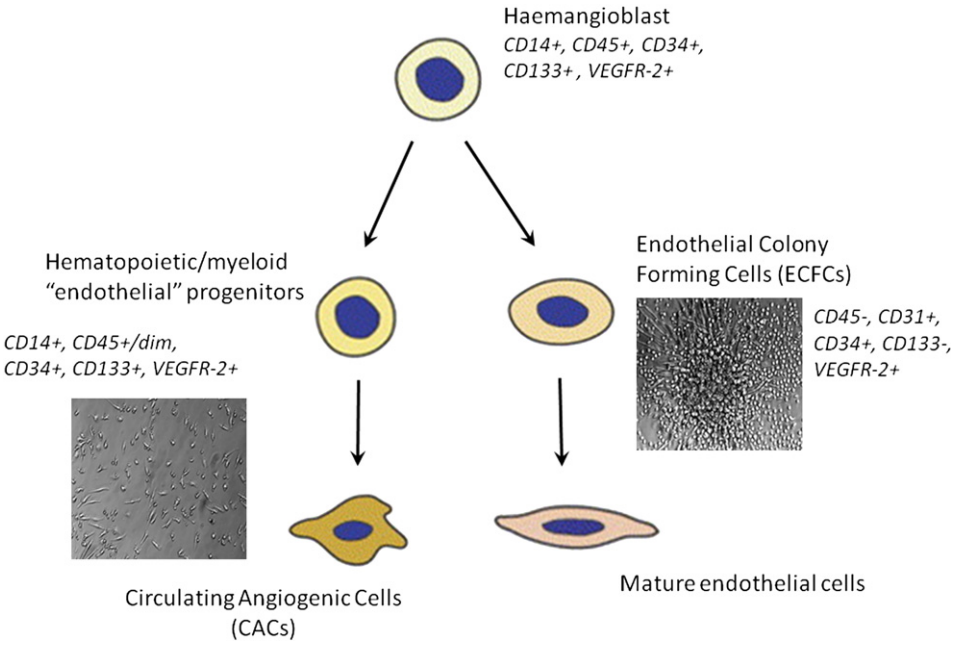

Fig. 1. Pathways to EPC ontogeny. Primitive haemangioblasts in the bone marrow give rise to myeloid and endothelial progenitors which yield early outgrowth, 'endothelial-like' colony-forming unit cells (ECFCs) or spindle-shaped circulating angiogenic cells (CACs). The relative expression of cell surface markers identifies these differentiation pathways. ECFCs eventually form mature endothelium, whilst CACs orchestrate neovascularisation.