Placenta 31 (2010) 1–10

Contents lists available at ScienceDirect

Placenta

journal homepage: www.elsevier.com/locate/placenta

Current Topic

Endothelial Progenitor Cells: Their Potential in the Placental Vasculature and Related Complications

P.I. Sipos [a,*], I.P. Crocker [a], C.A. Hubel [b], P.N. Baker [a]

[a] Maternal and Fetal Health Research Group, The University of Manchester, Research Floor, St Mary's Hospital, Oxford Road, Manchester, M13 9WL, UK
[b] Magee-Womens Research Institute, 204 Craft Avenue, Pittsburgh, PA 15213, USA

ARTICLE INFO

Article history:
Accepted 12 October 2009

Keywords:
Endothelial progenitor cells
Embryogenesis
IUGR
Pre-eclampsia
Placenta

ABSTRACT

Endothelial progenitor cells (EPCs) have received significant attention in recent times. A role for EPCs has been suggested in a range of pathologies and some recent studies of EPCs in pregnancy have been-published. This review provides a guide to the confusing field of EPCs. Attention is paid to their phenotyping, as although elementary this remains a highly debated topic. The current understanding of different subtypes and physiological role of EPCs in the placenta, fetus and adult are also considered. An overview is given as to role of EPC's in the pathophysiology of different disease states and the possible therapeutic and diagnostic applications expected from EPC-related research in obstetrics.

1. Introduction

In 1997, Asahara et al. [1] proposed a new concept of neovasculogenesis and suggested that a cell type exists in the adult, which resides in the bone marrow and migrates to ischaemic sites to form new endothelium. They also proposed a culture technique for the isolation of such cells. These outgrowth cells were referred to as putative Endothelial Progenitor Cells (EPCs). Since then, more than 2000 EPC-related articles have been published and a relationship has been found between endothelial progenitors and dozens of disease states from pulmonary hypertension [2] to moya-moya disease [3]. EPCs are thought to be diagnostic of cardiovascular risk [4–10] and are therapeutic in animal ischaemia models [11–14]. In hoping to find a cure for atherosclerosis, the main stream of EPC-related research has been conducted in the field of adult cardiovascular medicine, despite the fact that the presence of these cells has been known for much longer in the fetus and that vessel-related complications represent a significant clinical problem in pregnancy. This article provides a critical overview of the current literature and discusses avenues for further obstetric research. In doing so, we propose a potential role for EPCs in the pathophysiology of pre-eclampsia, intrauterine growth restriction (IUGR), gestational diabetes and increased life-long cardiovascular risk associated with these conditions. The potential for EPCs to be used as diagnostic tools or therapeutic agents in pregnancy-related complications is also briefly considered.

2. Physiology of EPCs

Vascularisation is a new described model of vessel formation - as opposed to the previously recognised vasculogenesis and angiogenesis. This model was created once the presence of EPCs in the adult circulation was suspected. Vasculogenesis refers to the *de novo* formation of blood vessels from progenitor cells. Angiogenesis, in contrast, means the sprouting of new vessels or elongation from existing ones through the remodelling of differentiated endothelial cells. Vascularisation in many ways resembles angiogenesis; however, in this case, EPCs contribute to neovessel formation by their incorporation into vessel walls, their secretion of paracrine hormones and subsequent angiogenic stimulation.

Endothelial and blood cell precursors originate from the same ancestor: the haemangioblast [15,16]. EPCs were originally thought to be derivates of the haemangioblast and were defined as cells capable of proliferation, incorporation into forming blood vessels, and differentiation into mature endothelial cells. They were thought to reside in the bone marrow, leave after recruitment, and have the capacity to migrate and participate in vasculogenesis and vascularisation.

The expression "putative EPC" is rather historical, and the EPC population consists of two distinct sub-populations with very different phenotypes and functional capabilites, both closely involved in vessel formation. The first group is a haemopoietic sub

* Corresponding author. Tel.: +44 447931254176.
E-mail addresses: Peter.Sipos@manchester.ac.uk (P.I. Sipos), Ian.Crocker@manchester.ac.uk (I.P. Crocker), chubel@mwri.magee.edu (C.A. Hubel), Phil.Baker@manchester.ac.uk (P.N. Baker).

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doi:10.1016/j.placenta.2009.10.006

population, the so called Circulating Angiogenetic Cells (CACs), which have strong paracrine and hormonal activities and stimulate cell migration and proliferation [17–20]. The second, Endothelial Colony Forming Cells (ECFC), have more endothelial-like characteristics and are profoundly influenced by CACs. They are highly proliferative and migrate to sites of vessel formation, before differentiating into mature endothelial cells, thus forming *de novo* endothelium [21–23].

In isolating EPCs and defining these subtypes, cell culture techniques have been widely used. In some protocols, fibronectin coated dishes are employed and adherent cells are typically harvested within one week after seeding. These cells are frequently referred to as "early outgrowth cells". The most common approaches include: the Ashara technique [1], Hill-Colony Forming technique [4] and Vasa technique [24]. A different approach, using collagen-coated dishes and adherent cells harvested several weeks later, generates the so called "late outgrowth cells". This technique was orginally described by Lin et al. 2000 [21] (See Fig. 1).

As the phenotypes and functions of early and late outgrowth cells are now clarified, cells obtained with early outgrowth techniques are practically considered CACs, while those produced with late outgrowth protocols are deemed ECFCs. In accord with current opinion, these terms will be used throughout the rest of this article: using the term EPC, where the actual sub-population is not determined, or where the comment applies equally to both CAC and ECFC phenotypes.

3. EPC-phenotypes

Although ECFCs and CACs can be isolated by the above culture techniques and by flow cytometry, no standardised and accepted means of phenotyping has yet been defined, and there are several different, partly contradictory, approaches found in the literature. Consequently, confirming cell-phenotype is a core agenda and remains a major issue. The identity of outgrowth cells or those acquired by flow cytometry is determined by the expression of tissue-specific surface markers. In addition, functional assays can contribute to the verification of endothelial or progenitor progeny.

3.1. Flow cytometry techniques

To date no EPC-specific antigen has been defined. Instead, various combinations of haematopoietic and endothelial stem-markers are proposed. It has been postulated that at the angioblast stage of development, i.e. before the loss of haematopoietic markers, cells start expressing endothelial antigens. The three antigenes most commonly considered are CD34, CD133 and KDR/VEGFR-2.

- CD34 is a single pass transmembrane sialomucin. As a cell-cell adhesion factor, it may mediate the attachment of stem cells to bone marrow extracellular matrix or directly to stromal cells. It is expressed on numerous cell types of mesodermal progeny, including blood cells, endothelial and fibroblast cells, epithelial and cancer stem cell populations. It is expressed by all haematopoietic stem cells, but it is subsequently lost upon differentiation [25,26]. It is also expressed by many cells of endothelial progeny, including differentiated endothelial cells in the adult [27].
- CD133 or prominin-1 is a 5-transmembrane domain cell-surface glycoprotein. It is localised on membrane protrusions of epithelial, haematopoietic and cancer stem cells. It is strongly expressed on haematopoietic stem cells, but also disappears during haematopoietic differentiation [28].
- KDR or VEGFR-2 (Human) is a thyrosine-kinase transmembrane receptor of vascular endothelial growth factor (VEGF). It is expressed by both early haemopoietic stem cells and endothelial cell-lines, but its expression also ceases with haematopoietic differentiation [29,30].

It was Asahara et al. that showed that CD34 enriched KDR^+ cells localise to sites of vasculogenesis [1], and Peichev et al. that suggested the addition of CD133 to differentiate EPCs from mature endothelial cells. They concluded that circulating $CD34^+/KDR^+/CD133^+$ cells represent a distinct population with a role in neo-angiogenesis and $CD34^+/KDR^+/CD133^-$ represent a more mature population of progenitors [31]. In 2006, Friedrich et al. identified a new $CD34^-/CD133^+/KDR_+$ EPC sub-population which differentiated into $CD34^+/133^+/KDR^+$-defined EPCs in culture [32]. Currently, most available research is based on these phenotypes; however direct clonal evidence is not usually provided and these techniques notably fail to distinguish CACs and ECFCs.

More recently the use of Fluorescence Activated Cell Sorting (FACS) and PCR has shown that ECFC from peripheral or umbilical blood are inevitably CD34 positive and CD45 negative, and that they also express KDR but not CD133 [33]. This population represents approximately 2% of all $CD34^+$ mononuclear cells. Timmermans et al. also showed that CACs belong to the $CD14^+$ monocytic sub-population of $CD34^+/CD45^+$ cells and although they co-express CD133 they never express KDR [33]. This $CD34^+/KDR^+/CD133^+$ triple-positive population has since been shown to be neither CAC, nor ECFC in origin, but nevertheless they are primitive haematopoietic progenitors [34], and some suggest they fit with a more broader definition of CACs [35,36].

In summary, ECFCs can be characterised by the surface marker-combination of $CD31^+/CD34^+/CD45^-/KDR^+/CD133^-$, but it must be noted that this combination does not differentiate ECFCs from circulating endothelial cells. Alternatively, CACs can be

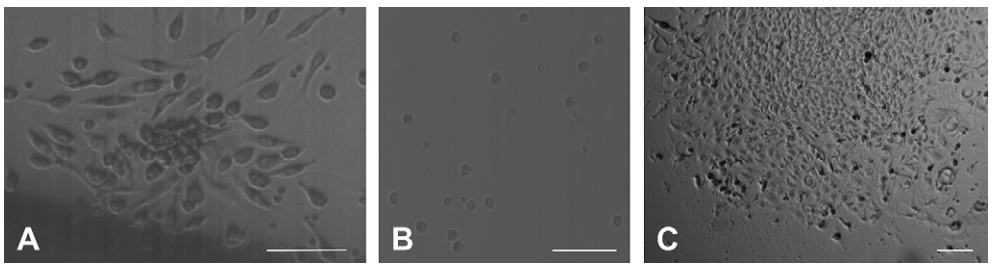

Fig. 1. Bright-field images of outgrowth cells from human fetal peripheral blood mononuclear cells (PBMNCs). A: CFU-Hill technique. Round cells in the centrum of the colony, spindle shaped cells in the periphery. The colonies appear to be smaller than in adult and spindle shaped cells migrate away from the centre rapidly. (Scale bar: 50 μm) B: Vasa technique: monolayer of round adherent cells. (Scale bar: 100 μm) C: Lin-technique: Colony formed by a monolayer of cells with cobble stone appearance. The colonies are larger and appear earlier than those in the adult. (Scale bar: 100 μm) Unpublished images of the authors.