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Review

Endothelial progenitor cells and cardiovascular homeostasis: Clinical implications

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Abstract

Emerging evidences indicate that endothelial progenitor cells (EPCs) actively contribute in regulating cardiovascular homeostasis, and interest is growing for possible future diagnostic and therapeutic applications in the cardiovascular arena. In the present clinically-oriented review, special attention was given to the clinical implications of the potential of EPCs to test and strengthen the capacity of the organism to challenge atherosclerosis, vascular remodelling and ischemia. Accumulating data suggest that the vasculo-protective functions of EPCs may be used as cellular biomarkers for endothelial damage, or may be pharmacologically modulated to enhance the body's defence to atherosclerosis. Furthermore, biomedical engineering and cell transplantation open new scenarios to reverse vascular and graft remodelling and achieve therapeutic angiogenesis in limb and heart ischemia. However, a number of unsolved issues remain to be exploited, such as the identification of the true identity of EPCs and a better characterization of their role in vascular homeostasis under normal and pathologic conditions.

Keywords: Endothelial progenitor cells; Cardiovascular homeostasis; Risk factors; Cell therapy

1. Introduction

The existence of stem cells originating in the bone marrow (BM) that can give rise to endothelial cells both in culture and in animal models of ischemic diseases, has come to the limelight with seminal reports by Asahara and colleagues [1–3], describing the presence in the bone marrow and in the peripheral blood of cells, termed endothelial progenitor cells (EPCs), capable of endothelial differentiation and contributing to neo-vascularization in ischemic tissues.

After the initial characterization of EPCs as putative cells to be used for cellular therapy in ischemic tissues, much work has been devoted to their biological characterization. Growing evidence suggests that EPCs may offer at the same time new therapeutic and diagnostic perspectives and become a valuable tool to monitor and interact with the endogenous healing capacity of the cardiovascular system [4]. In this article, we summarize the current body of knowledge about EPCs biology and pathophysiology, focusing thereafter on the impact that these findings may have in the daily practice of the cardiovascular clinician.

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2. Endothelial progenitor cells: from biology to the patient

2.1. EPCs antigen characterization and expansion in culture

Surface antigen expression is insufficient to distinguish EPCs from hematopoietic stem cells. In fact, EPCs have promiscuous antigens (CD133, CD34, c-kit/CD117, VEGFR2/KDR, CXCR4) in common with hematopoietic stem cells and share with these cells the ability to differentiate into hematopoietic cells [4]. Therefore, it depends largely on culture or *in vivo* conditions whether EPCs behave as hematopoietic or endothelial progenitors. Typical EPCs activity can be identified in culture in the c-kit$^+$(CD117$^+$), CD133$^+$ and CD34$^+$ fractions from the BM and the umbilical cord blood (UCB) [5,6], but also from peripheral blood mononuclear cells [7]. The endothelial outgrowths arising from EPCs in culture are typically shaped as round cluster of spindle-like cells adhering to fibronectin or gelatin and taking up Acetylated-LDL and positive to *Ulex europaeus* (UEA-1) lectin staining. Typically, two types of endothelial progenitor cell colonies can be obtained depending on the timing of their emergence in culture. Early and late colonies have been thus distinguished having different proliferation potential [8]. It has been suggested that the early colonies originate from partially committed progenitor populations while late colonies arise as a result of *in vitro* proliferation of rare "true" progenitors having wider proliferation ability [8]. Latest findings have added some complication to the picture. In fact, it has been shown that cells having monocyte features (for instance, CD14$^+$ cells) are also able to give rise to EPCs outgrowths (resembling early EPCs) in culture [9–12] and improve neo-vascularization *in vivo*. Furthermore, cells bearing immune cells features such as T-cells may sustain formation of endothelial colonies by cooperating with monocytes [13]. Conversely, the ability of CD34$^+$CD133$^+$KDR$^+$ cells to form endothelial cells has been questioned [14].

At present, three types of cells bearing angiogenic differentiation potential have been defined based on their surface antigen repertoire and their dynamics in culture. These cells are named respectively Colony Forming Units-Endothelial Cells (CFU-ECs), Circulating Angiogenic Cells (CACs) and Endothelial Colony Forming Cells (ECFCs) [7]. The term EPCs may therefore comprehend a group of cells that are in a variety of stages of differentiation from early precursors to mature endothelial cells. At present, there are no accepted standard criteria for defining "true" EPCs.

2.2. EPCs recruitment and differentiation in ischemia

The relative contribution of EPCs in mechanisms of neo-vascularization is complex and still under investigation (see Fig. 1). In animal models and patients, the occurrence of ischemic events at cardiac and peripheral levels is associated with mobilization of BM-derived EPCs into peripheral blood [8,15]. Different techniques to assess and quantify mobilization of BM-derived stem cells are currently in use for research purpose, such as the formation in culture of cluster of spindle-shaped cells [16] and more quantitative analyses such as flow cytometry [15].

Several markers of hematopoietic/endothelial cells have been used to define the EPCs mobilization dynamics following occurrence of ischemia, both in animal models and patients. In case of mice, the Sca-1 antigen, the c-kit the KDR and Tie-2 receptors have been utilized as markers to show mobilization of BM-derived stem cells [2,3,5,17]. In humans, CD133 and CD34 antigens as well as the CXCR4 are commonly used although, recently, c-kit CD117 and CD146 antigens were also considered [7,15,18] as EPCs markers. Recently, the hypothesis that commonly used EPCs markers may not define a true EPCs population has been raised, according to the evidence that CD34$^+$CD133$^+$KDR$^+$ cells do not form endothelial colonies *in vitro*, but hematopoietic colonies only [14]. This has challenged the concept that circulating KDR$^+$/CD34$^+$ cells represent in humans a population of true endothelial precursors, although their therapeutic potential into mouse models of limb and heart ischemia has been reported in previous experiments [19–21].

According to present knowledge, it has been shown that BM- and peripheral blood (PB)-derived circulating endothelial progenitor cells, defined as CD34/c-kit$^+$ and CD34/CXCR4$^+$ cells, may directly contribute to formation of new blood vessels by differentiating into fully mature endothelial cells, fibroblast and pericytes [1,3,12], and also exert a supportive paracrine effect on tissue resident endothelial cells by secreting a number of angiogenic factors including vascular endothelial growth factor (VEGF), angiopoietins and fibroblast growth factor-2 (FGF-2) [12,22]. However, mobilization into the peripheral circulation and homing in hypoxic sites seem insufficient to completely regenerate tissues lost with ischemia. Thus, much work has been dedicated to unraveling signals involved in this phenomenon. Several factors that are implicated in peripheral blood EPCs mobilization have been identified. These include the granulocyte (macrophage) colony stimulating factor G(M)-CSF [23], the stem cell factor (SCF) [17] and several others. The two most characterized players that have been found to act in EPCs mobilization and homing are, however, VEGF and the Stromal Derived Factor-1-alpha (SDF-1α) chemokine. Both these factors are under the transcriptional control of the hypoxia inducible factor-1-alpha (HIF-1α), a transcription factor that is specifically activated in hypoxic tissues [24,25]. Acting as potent chemotactic factors for EPCs, it has been proposed that VEGF and/or SDF-1α gradients are established from the periphery to the BM upon ischemia and that these gradients are responsible for EPCs mobilization into peripheral blood and their specific recruitment into ischemic sites [5,26]. These findings are also in agreement with preclinical studies showing that VEGF and SDF-1α genes delivery into ischemic tissues is