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Brief Review

Endothelial progenitor cells in angiogenesis

XU Qing-Bo*

Department of Cardiac and Vascular Sciences, St George's Hospital Medical School, London, W17 0RE, UK

Abstract: Circulating blood contains a subtype of progenitor cells that have the capacity to differentiate into mature endothelial cells *in vitro* and *in vivo*. These cells have been termed endothelial progenitor cells (EPCs). The isolation of EPCs by adherence culture or magnetic microbeads has been described. EPCs are characterized by the expression of 3 markers, CD133, CD34, and the vascular endothelial growth factor receptor-2. After differentiation, EPCs express CD31, vascular endothelial cadherin, and von Willebrand factor. Evidence is accumulating that EPCs can facilitate endothelial repair and angiogenesis *in vivo*. We observed that EPCs can regenerate damaged endothelial cells in vascular grafts in apoE-deficient mice, and that abundant vascular progenitor cells are present in the adventitia of the vessel wall. It is not clear yet, however, whether these EPCs are essential for these angiogenic and atherogenic processes. Moreover, there are still many uncertainties about how cardiovascular risk factors alter EPC function. Thus, further studies on the mechanisms of EPC homing, releasing and attaching will be of help to explore areas of potential basic research and clinical application of EPCs.

Key words: EPC; bone marrow; stem cells; angiogenesis and atherosclerosis

血管再生中的内皮祖细胞

徐清波*

圣·乔治医院医学院心血管科学系，伦敦，英国

摘 要: 循环血液里存在一种被称为内皮祖细胞(endothelial progenitor cells, EPCs)的祖细胞亚群，具有在体内外分化为成熟内皮细胞的能力。根据内皮祖细胞与其他血液细胞的粘附能力的差异和内皮祖细胞的抗原特异性，内皮祖细胞可通过贴壁培养和免疫磁珠筛选而分离获得。内皮祖细胞可特异性表达三种祖细胞分子标志：CD133、CD34 和血管内皮生长因子受体 -2。当内皮祖细胞分化为成熟内皮细胞后，血小板内皮细胞粘附分子 -1 (CD31)、血管内皮粘附素(VE-cadherin，又称 CD144)和Ⅷ因子(vWF)表达将上调。越来越多的证据显示，内皮祖细胞有利于体内内皮损伤后修复和血管再生。我们的研究发现，内皮祖细胞可修复 apoE- 缺陷小鼠血管移植物中的损伤内皮并且在动脉血管外膜中存在大量的血管祖细胞。然而，在机体的血管再生和动脉硬化的形成进程中，这些内皮祖细胞的作用和机制还不太明确。另外，有关机体内相应心血管疾病危险因素是如何影响内皮祖细胞功能的机制也不清楚。因此，对内皮祖细胞的归巢、释放和粘附机制的进一步深入研究将有助于人们探索内皮祖细胞的基础理论和临床应用价值。

关键词: 内皮祖细胞；骨髓；干细胞；血管再生及动脉硬化
中图分类号: Q254; Q257

Introduction

In 1997, Asahara *et al.*[1] reported for the first time about endothelial progenitor cells (EPCs) that differentiated from CD34+-enriched mononuclear cells in peripheral blood and participated in vasculogenesis in the animal hindlimb ischemic model. In these studies, they used EPCs that was spindle-shaped, derived from peripheral blood, and cultured for less than 3 weeks. This EPC showed the limited

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*Corresponding author. Tel: +44-20-87252817; Fax: +44-20-87252812; E-mail: q.xu@sghms.ac.uk

proliferating potential for long-term culture and disappeared 4 to 6 weeks later in *in vitro* condition. But there were reports[2,3] suggesting the existence of another type of EPC that originated from bone marrow, circulated in peripheral blood, and showed different morphology and proliferation pattern from EPCs that Asahara[1] reported.

Accumulating evidence indicates the both types of EPCs in the blood have the capacity to proliferate, migrate, and differentiate into mature endothelial cells. EPCs are characterized by expressing CD34 and vascular endothelial growth factor receptor-2 (VEGFR-2), 2 antigens shared by embryonic endothelial progenitors, and hematopoietic stem cells[1]. Bone marrow-derived endothelial progenitors are largely responsible for generating endothelial cells of microvessels or neovascularization in ischemic or damaged tissues[4,5], while vascular progenitor cells might be important for replacement of dead endothelial cells in large vessels. Vascular progenitor cells mainly localize in the adventitial layer and few, if any, in the intima under the endothelium[6]. In addition, Planat *et al.*[7] reported that adipose tissues contain progenitor cells which serve as common progenitors for both adipocytes and endothelial cells. These vascular progenitor cells could be a source for repairing the endothelium via direct or indirect pathways.

Stem/progenitor cells

One of the most promising areas in basic research today involves the use of stem cells. These unique cells have the capability to transform and replenish the different tissue types that make up the body, and they also represent the fundamental building blocks of organ development. In general, stem cells can be divided into two broad categories: adult (somatic) stem cells and embryonic stem cells[8]. One of the most fascinating and important aspects of stem cells is their ability to differentiate *in vitro*, via "progenitor cells", into terminally differentiated somatic cells of all tissue types, including cardiomyocytes[9] and endothelial cells[10]. Because of the discovery that stem cells have a role in vascular repair, stem cell research could be important not only for understanding the pathogenesis of the disease, but also for development of cell-based therapies and tissue-engineering.

Most published papers in the field use the term *progenitor cells* to describe circulating and bone marrow-derived cells[11]. Evidence indicates that circulating "endothelial" progenitor cells differentiate into mature endothelial cells, macrophages, smooth muscle cells and cardiomyocytes[12,13], although recent papers demonstrated that bone marrow cells cannot differentiate into cardiomyocytes *in vivo*[14,15]. In addition, bone marrow progenitor cells also differentiate into other types of cells[16]. Importantly, recent reports from several groups demonstrated that abundant progenitor cells exist in the adventitia of the arterial wall[6] and fat tissues[7], which might be sources of circulating EPCs. At the present, it less clear how EPCs are released from tissues into blood (Fig. 1). Further study is needed to clarify the molecular mechanisms of progenitor cell release and whether other sources of progenitor cells contribute to EPC pool in circulation.

Isolation and culture of EPCs

EPCs can be isolated from bone marrow or peripheral blood. In addition, EPCs have also been isolated from fetal liver or umbilical cord blood[10]. To obtain two types of EPCs sequentially from the same donors, Hur *et al.*[17] cultured total mononuclear cells from human peripheral blood, called them early EPCs and late EPCs. Early EPCs with spindle shape showed peak growth at 2 to 3 weeks and died at 4 weeks, whereas late EPCs with cobblestone shape appeared late at 2 to 3 weeks, showed exponential growth at 4 to 8 weeks, and lived up to 12 weeks. Late EPCs was different from early EPCs in the expression of VE-cadherin, Flt-1, KDR, and CD45. Late EPCs produced more nitric oxide, incorporated more readily into human umbilical vein endothelial cells monolayer, and formed capillary tube better than early EPCs. Early EPCs secreted angiogenic cytokines (vascular endothelial growth factor, interleukin 8) more so than late EPCs during culture *in vitro*. Both types of EPCs showed comparable *in vivo* vasculogenic capacity. EPCs have the capacity to form capillary tubes in basement matrix gel, to incorporate acetylated LDL and to bind endothelial-specific lectin[17].

EPCs for angiogenesis.

EPCs may be involved in the regeneration of ischemic myocardium by modulation of angiogenesis and myogenesis, cardiomyocyte apoptosis, and remodeling in the ischemic cardiac tissue[12]. EPCs have also been reported to participate in cerebral neovascularization after ischemic stroke. The fluorescent-labelled EPC integrated into the vascular network and improved blood flow in the affected limb. Furthermore, in rats, after coronary ligation, injection of EPC improved vascularization, reduced infarct size and preserved cardiac function[12].

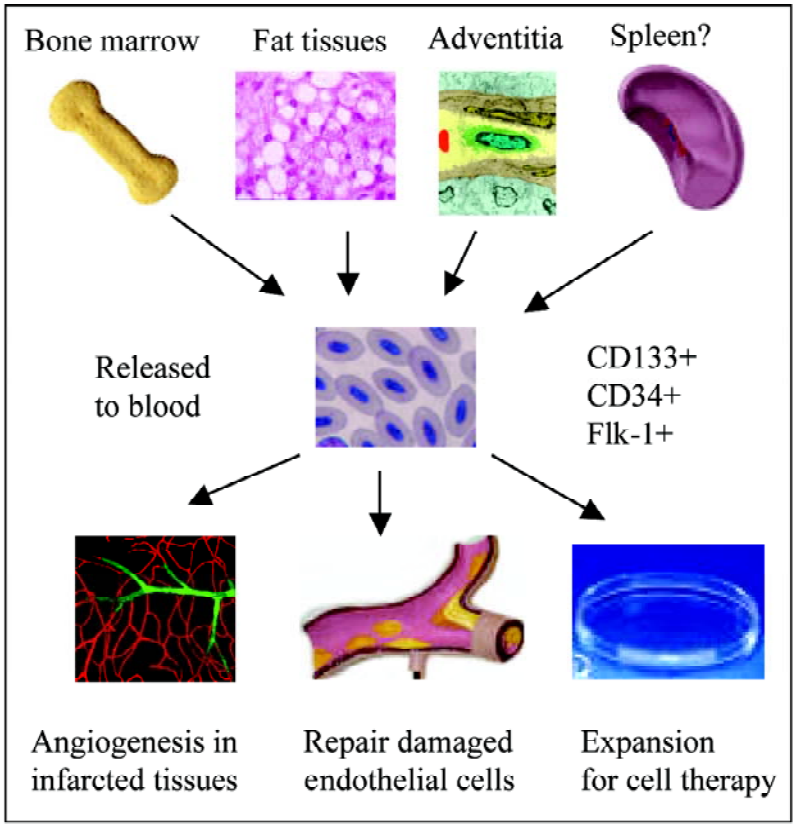

Fig. 1. EPC generation and angiogenesis. EPCs could be released from bone marrow, fat tissues, adventitia and possibly spleen into blood, where they express CD133 at the early stage, and then CD34-Flk-1. Circulating EPCs can form neovessels in infarcted tissues, repair damaged endothelial cells of large vessels, and also be expanded *in vitro* using for cell therapy.

Elegant experiments with bone marrow transplants in animal models further proved that donor marrow-derived endothelial cells (apparent from transgenic marker gene expression behind and dependent on endothelial promoters) are incorporated in new vasculature in tumours, healing wounds, the myometrium and ischaemia-damaged brain tissues (stroke) of the host[18,19]. From these combined data, it is likely that the EPC-dependent vasculogenesis facilitates both angiogenesis and arteriogenesis (Fig. 1).

Progenitor cells regenerate dying mature endothelial cells

Atherosclerosis is a slowly progressive disease that begins in childhood but does not become clinically manifest until middle age or later[20]. The atherosclerotic lesion is defined by intimal cell proliferation, lipid accumulation, and connective tissue deposition. Depending on their size and composition, the lesions are usually divided into fatty streaks, predominantly consisting of lipid-rich macrophages and T lymphocytes within the innermost layer of the artery wall, and plaques, advanced stages of the lesions, which are also called “atheroma”. The three major cellular components of human atherosclerotic plaques are the smooth muscle cells (SMCs), which dominate the fibrous cap, the macrophages, which are the most abundant cell type around the necrotic core, and the lymphocytes[21], which have been mainly ascribed to the fibrous cap[22].

It may be impossible to determine with certainty the lifespan of endothelial cells at different sites of the arterial wall in humans. Therefore, systematic examination of the sequence of endothelial turnover occurring in animal models that are like humans is particularly useful. There is evidence of structural and functional heterogeneities in the endothelium of large and middle-sized arteries[23,24]. While overall rates of cell turnover are very low[25], there are clusters of increased cell replication that are correlated with increased permeability to plasma proteins[26,27]. Thus, endothelial turnover occurs at a physiological state which is enhanced or promoted by risk factors, e.g. hyperlipidemia and hypertension.

To take advantage of transgenic animals, we recently developed and characterised a new animal model of vein graft atherosclerosis in wild-type[28] and apoE-deficient

mice[29]. The lesion displayed classical complex morphological features and a heterogeneous cellular composition. Furthermore, transgenic mice expressing LacZ genes controlled by specific endothelial, SMC or house keeping gene promoters are now available. When these mice are crossed with apoE knockout mice, which develop spontaneous atherosclerosis[30], then staining the tissue with X-gal enables the detection of endothelial origins in vein grafts. We demonstrated that circulating stem/progenitor cells cover the surface of neointimal and atherosclerotic lesions of vein grafts. Similarly, arterial endothelium is also replaced by stem/progenitor cells when an arterial segment is isografted in animal models. Furthermore, other groups have demonstrated that circulating stem/progenitor cells contribute to the endothelial regeneration of vein grafts[31] and injured arteries[32-36] in mouse models, which can be influenced by ageing, estrogen and drug treatment.

Concerning endothelial turnover and stem/progenitor cell replacement in the vessel wall in humans, a recent report demonstrated that significant endothelial cell replacement by circulating progenitor cells occurs in human transplant vessels[37]. In addition, it is well known that endothelial apoptosis occurs at the sites where the endothelium is exposed to lower or disturbed shear stress. Evidence from human study indirectly supports the notion that dead endothelial cells in arteries might be also replaced by progenitor cells, rather than mature endothelial cells neighbouring the lost cell.

Ageing influences progenitor cells

Atherosclerosis is an ageing-related disease, in which altered mature endothelial and progenitor cells in ageing could be responsible for the disease mechanism. The data have indicated that ageing cells are sensitive to stress-induced apoptosis *in vitro* and *in vivo*[38]. Arterial endothelium of young people are much more resistant to injuries like smoking or hyperlipidemia. It is conceivable that older people have a higher turnover rate of endothelial cells. Such continuous endothelial damage or dysfunction leads to an eventual depletion or exhaustion of a presumed finite supply of endothelial stem/progenitor cells[39]. Rauscher *et al.*[40] provided direct evidence that cells with vascular progenitor potential are decreased in the bone marrow of ageing apoE-/- mice. Chronic treatment with bone marrow-derived progenitor cells from young nonatherosclerotic apoE-/- mice prevents atherosclerosis progression in apoE-/- recipients despite persistent hypercholesterolemia. In contrast, treatment with bone marrow cells from older apoE-/- mice with atherosclerosis is much less effective. These suggest that progenitor cell numbers can be exhausted with ageing due to ongoing replacement of dead endothelial cells.

Summary and perspectives

Due to the introduction of new techniques, a great amount of information has become available at the cellular and molecular levels during last decade, which has expanded our understanding of the role of EPCs. EPCs obviously participate in the regeneration of injured endothelium and of ischemic organs. A standardization of the procedures used for the isolation, phenotypic characterization, and culture of these cells will be a prerequisite for the use of EPC quantification *in vivo* as a diagnostic or prognostic tool or as a surrogate marker in clinical or pharmacotherapeutical studies. Besides other open questions, the role of CD34-negative cells in the process of vessel wall or tissue remodelling needs to be clarified. In addition, additional experimental, clinical, and cell biological studies are needed to increase the understanding of the function of EPCs and of the factors that determine their number and turnover rate as well as the mechanisms that stimulate or inhibit their mobilization, differentiation, and homing *in vitro* and *in vivo*. Such investigations are required to explore areas of future basic and clinical research, particularly because the first clinical trials using progenitor cells have just been started.

Furthermore, progenitor cells have the unique properties for cell replacement and possible role in atherosclerosis. Several key issues within this concept have to be addressed in further studies. First, we need to know whether focal areas of arterial endothelial cells in humans are replaced by progenitor cells in the physiological state at the early life, e. g. infant, and how it is related to initiation of fatty streak. At the present, it might be difficult, if impossible, due to ethical and technical aspects. The second basic science issue to be determined is the molecular mechanisms of progenitor cells in homing, attachment, chemoattractant, differentiation *in vivo* and *in vitro*. Finally, the data of *in vivo* studies mostly come from investigations using animal models, especially mice. Although some results obtained from mice are verified by human studies showing a similar pattern[41], further confirmation on human subjects is needed.

In summary, much knowledge on stem/progenitor cells and vascular cell biology has been gained, which should permit us to develop new diagnostic tools and new strategies for prevention and therapy, by further testing the hy-

pothesis of angiogenesis and atherogenesis.

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