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Endothelial Progenitor Cell Identification, Classification and Nomenclature: A Review

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Abstract

Endothelial integrity depends on a balance between the extent of endothelial cell injury and the capacity for endogenous repair. An imbalance is thought to be a contributory factor for development of atherosclerosis. In healthy individuals mature neighboring endothelial cells may replicate and therefore replace damaged cells. This mechanism has limited potential and therefore an alternative mechanism is also required. One that is proposed mechanism is of undifferentiated cells migrating to sites of vascular injury and then differentiate into mature endothelial cells. These cells are also thought to perpetuate the mechanism by secreting pro-angiogenic cytokines. However, there currently remains a lack of consensus on phenotypic and functional definition of endothelial precursor cells.

This review summarizes the current controversies surround the identification, nomenclature and classification of EPCs.

Introduction

Endothelial integrity depends on a balance between the extent of endothelial cell injury and the capacity for endogenous repair. Loss of endothelial integrity may cause atherosclerosis leading to coronary heart disease and stroke [1-4]. In healthy individuals, neighboring mature endothelial cells are capable to replicate locally and replace damaged cells [1]. However, local replication has limited potential and may be insufficient if the injurious stimuli remains prolonged and or repeated [5]. Therefore an alternative mechanism is required. One proposed mechanism is dependent on undifferentiated cells migrating to sites of vascular injury [6-8] then differentiating into mature endothelial cells [9-16]. These undifferentiated cells are thought to have a central role in vascular repair by their ability to proliferate, migrate to site of vascular injury and then differentiate into mature vascular endothelium [16,17]. They are also capable of augmenting this cycle by secreting pro-angiogenic cytokines [18-20]. Intense research has followed since the first reported observation of a bone marrow derived circulating progenitor cells termed endothelial progenitor cell (EPC) by Asahara et al. [21,22] EPCs are thought to be derived from pluri-potential stem cells within the bone marrow and accounting for only 0.001-0.0001% of peripheral blood cells [23]. Circulating EPCs are then thought to migrate to areas of vascular damage and differentiate into mature endothelial cells [21]. EPCs can be isolated from bone marrow or the circulation as a sub population of mononuclear cells [21,24,25] expressing a variety of endothelial surface markers [26].

However, there currently remains a lack of consensus EPC identification and function [27-32]. This may be confounding this, some studies have utilized EPC samples made of heterogenous cell population without recognition of the possible synergistic effect of different cell populations [33]. Furthermore EPCs are often referred to as a diverse group of cells lineages having angiogenic potential despite some of these cell populations being unable to differentiate into functional endothelial cells [27]. Consequently the current EPC nomenclature proposed over a decade ago is widely regarded as suboptimal [34]. This has made it difficult to clarify the role of EPCs in health and disease [35].

The review below highlights current controversies on a general consensus on a working definition on identification of EPCs [31]. The review will approach the current controversies on identification and function of EPCs by considering limitations of the commonly used laboratory methods used in EPC identification.

Clinical applications

EPC are currently not measured routinely in clinical practice but understanding the role and EPCs in both health and disease are a focus of recent research. They remain very much a research tool at present. However it is now generally accepted that cardiovascular risk correlates with EPC number, highlighting the integral relationship between endothelial integrity and atherosclerosis [36-40]. With impaired EPC function being associated with cardiovascular events in several studies [6,38-40]. Decreased numbers have been found in patients with traditional risk factors for coronary artery disease including smoking, hypertension, [41] diabetes mellitus, [42-47] elevated low-density lipoprotein cholesterol [48,49]and hypercholesterolemia [48-53]. Disruption of endothelial integrity by endothelial cell injury has been shown to be a stimulus for the development of atherosclerosis [4] as well as augmentation of EPC number and function [12,54,55]. Continued endothelial damage [56] may eventually lead to a reduction of the number of EPCs resulting in deficient endothelial repair and progression of atherosclerosis and increased risk of myocardial ischaemia [12,48]. This has led to research examining the effect of EPC being infused in patients with intractable angina, post myocardial infarction left ventricular recovery and in chronic heart failure patients with some studies showing beneficial outcomes [57-66]. The REPAIR-AMI trial found at 12 months the end points were significantly reduced in the bone marrow-derived progenitor cells group compared with placebo but also that the bone marrow-derived progenitor cells were independent predictor of favorable clinical outcome [67].

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The identification and classification of endothelial progenitor cells (EPCs)

There are currently two laboratory methods commonly used for identification and classification of EPCs'. The first a blood based assays quantified by a number of specific cell surface markers using flow cytometry. The second by the number of colonies of adherent cells that can be obtained from circulating mononuclear cells (MNCs) expressing mature endothelial cell markers *in-vitro* by cell culture isolation [68].

Flow cytometric analysis

Flow cytometry can be used to identify and quantify the density of cells of interest through fluorimetric analysis of specific cell surface markers. There remains controversy regarding which specific markers identify EPCs. EPCs, thought to be derived from CD34 hematopoietic progenitor cells, [9,21,24,26] with co-expression of specific endothelial marker proteins [9,24,26]. With certain cell surface markers thought to be related to the stage of maturations of the EPC, such as, the cell surface marker CD133, a 120-kDa trans-membrane polypeptide. CD133 found to be expressed on bone marrow derived hematopoietic stem and progenitor cells in peripheral blood [69]. Interestingly expression of CD133 decreases to a complete absence in mature EPCs within the peripheral circulation. The timing of the loss of expression of CD133 from EPCs remains unclear [70]. However, the loss of CD133 indicates the transformation to more mature endothelial like cells [69]. The converse is true for the expression of CD34 a cell surface marker found on immature pluri-potential stem cells [26]. CD34 gradually increases as the CD133 decreases as the EPC matures [69]. The value of using CD133 as a marker of EPC remains contentious firstly due to the rarity of cells expressing CD133 and more importantly studies suggest that CD133 are haematopoietic cell lines and therefore unable to form endothelial phenotypic EPCs [71,72].

Certain authors suggest a minimal antigenic profile should include at least 1 marker of immature cells, commonly CD34 and/or CD133 plus at least 1 marker of endothelial cells commonly VEGFR2 (KDR/Flk-1). CD133 either alone or in combination with CD34/VEGFR2 have been used for identification of EPCs in some studies [26,73]. Whereas as other studies suggest expression of CD34, CD133, and/or VEGF2 [23,27,71,74,75]. Table 1 summarizes and compares the distinct expression of three commonly used markers within bone marrow and EPCs. Some authors propose EPCs being derived from CD45-lineage [23]. Interestingly CD34, VEGFR2 and diminished CD45 (CD45dim) cells have been found to have greater correlation to coronary heart disease and response to statins when compared to healthy individuals [76,77]. With the combination of CD133, CD34 and VEGFR-2 associated with early functional EPCs [10,26].

Therefore EPCs may express markers of both hematopoietic stem cells (CD34 and CD133) and endothelial cells (CD146, vWF, and VEGFR2) [23,24,26,69,72,78-82] amongst other proposed markers [21,69,70,83]. Hence current flow cytometric identification of EPCs remains controversial.

	Bone marrow	Circulation	
		Early EPCs	Mature EPCs
$CD133^+$	+	+/-	-
$CD34^+$	+	+	+
$VEGFR2^+$	+	++	+++

Table 1: Cell surface markers during course of maturation of EPCs (Adapted from Sandhu et al.) [32].

Cell culture analysis

Cell culture allows identification by formation of colonies of cells that have a pattern of immunofluorescence identifying functioning endothelial cell lines [84]. Asahara et al first isolated and defined EPCs as circulating mononuclear cells expressing CD34 and Flk-1 with further cell culture identification by CD31, uptake of acetylated LDL, and lectin binding [21]. Characteristics that are still commonly used to define EPC in cell culture.

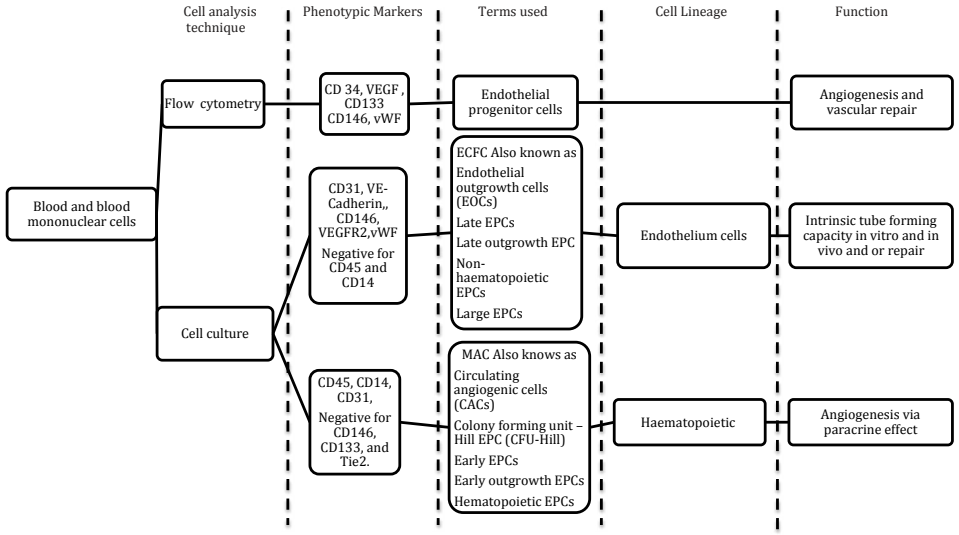
Cell culture definitions of EPCs also lack phenotypic specificity for a number of reasons. Firstly, micro-particles from platelets may transfer CD31 to haematopoietic cells [85]. Secondly CD31 and vascular endothelial growth factor receptor 2 (VEGFR2) may also be found in some monocytic subpopulations [86]. Finally AcLDL uptake and lectin binding have been found in both macrophages and mature endothelial cells [29].

A number of studies have described two types of time-dependent cell colonies with distinct properties. The spindle shaped early outgrowth EPC (EO-EPC) seen in the early period of culture and late outgrowth EPC (LO-EPC) that produce colonies and tube formation in latter period of culture [12,21,33,87-90]. These two populations have very different phenotypes, EO-EPC is thought of as haematopoietic and LO-EPC as endothelial cell lines [91]. This has led to populations being named as "hematopoietic EPCs" and "non-hematopoietic EPCs" [92]. Thereby supporting the hypothesis of hematopoietic EPCs giving rise to non-hematopoietic EPCs and ultimately endothelial cells.

Early outgrowth EPC (EO-EPC) are thought to be short-lived cells (<2 weeks) and do not differentiate into endothelial cells in vivo but have the ability to restore endothelial function and enhance angiogenesis after tissue ischaemia through a paracrine mechanism [21,80,93]. However, they are thought to be a heterogeneous population of hematopoietic cells [93-95] and often referred to as circulating angiogenic cells (CACs) [96]. CACs have been produced in-vitro in cell culture conditions, however there is little evidence to suggest that this occurs *in-vivo*. Leading to some authors to suggesting that this cell population be termed as myeloid angiogenic cells (MACs) based on their lineage and function [29]. MACs are characterized by cell culture immune-phenotyping with CD45, CD14, CD31, and negative for CD146, CD133, and Tie2 [97,98]. These cells have potent pro-angiogenic and vaso-reparative effect by a paracrine mechanism [15,99-101]. Importantly, they are not capable of becoming endothelial or progenitor cells [31,102]. Therefore the terms MACs/CACs should not be used interchangeably with EPCs [29].

In contrast LO-EPCs, are thought to be homogeneous endothelial-like progenitor cell population that possess a high proliferative potential, differentiate into vascular endothelial cells and form networks in vitro and in vivo. Furthermore LO-EPCs are also capable of augmenting the process by auto paracrine mechanism [29,33,103-106]. A mechanism noted in patients with cardiovascular risk factors [104,107]. New recommendations have suggested that this population of cells perhaps should be referred to as endothelial colony forming cells (ECFCs) [25,29]. ECFCs derived from peripheral blood mononuclear cells, or umbilical cord blood grown in endothelial cell culture conditions are characterized by immunophenotype positive for CD31, VE-Cadherin, von Willebrand factor, CD146, VEGFR2, and negative for CD45 and CD14. CD34 expression may also be expressed however may decline during in-vitro expansion [103,108,109] as mentioned above under flow cytometric analysis.

Interestingly, the proliferative, differentiation and tube forming



Abbreviations: EPC: Endothelial Progenitor Cell; ECFC: Endothelial Colony Forming Cells; MAC : Myeloid Angiogenic Cells; vWF : von Willebrand Factor.

Figure 1: Table summarising cellular analysis technique, phenotype markers, preferred nomenclature and function of cells often termed as EPC in current literature.

ability have been found to been enhanced by laminar shear stress [110-113] suggesting that they may contribute to autologous vascular repair. This is an important finding raising the possibility of using these cells as viable treatment option for cardiovascular patients [90]. However any future use in as a treatment option would require an ex-vivo production due to the low concentrations of LO-EPC in-vivo [87,114].

The use of ECFCs and MACs are preferentially used terms as this definition accurately describes the phenotype and function of these cell-types [102,115]. Figure 1 adapted from Medina et al. [29] summarizes cellular analysis technique, phenotype markers, preferred nomenclature and function of cells often termed as EPC in current literature.

Conclusion

Since the first reported reports of bone marrow derived circulating cells differentiating to endothelial cells some there have been a number of cells termed EPCs despite being unable to differentiate into functional endothelial cells. However currently there remains a lack of consensus on phenotypic and functional definition of endothelial precursor cells. Intense research is being undertaken into elucidating a consensus on classification and identification of EPCs. This will allow a better understanding of the function of EPCs in both health and disease but may also path the way for use of EPC as a viable treatment modality.

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