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Endothelial Progenitors: A Consensus Statement on Nomenclature

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SUMMARY

Endothelial progenitor cell (EPC) nomenclature remains ambiguous and there is a general lack of concordance in the stem cell field with many distinct cell subtypes continually grouped under the term "EPC." It would be highly advantageous to agree on standards to confirm an endothelial progenitor phenotype and this should include detailed immunophenotyping, potency assays, and clear separation from hematopoietic angiogenic cells which are not endothelial progenitors. In this review, we seek to discourage the indiscriminate use of "EPCs," and instead propose precise terminology based on defining cellular phenotype and function. Endothelial colony forming cells and myeloid angiogenic cells are examples of two distinct and well-defined cell types that have been considered EPCs because they both promote vascular repair, albeit by completely different mechanisms of action. It is acknowledged that scientific nomenclature should be a dynamic process driven by technological and conceptual advances; ergo the ongoing "EPC" nomenclature ought not to be permanent and should become more precise in the light of strong scientific evidence. This is especially important as these cells become recognized for their role in vascular repair in health and disease and, in some cases, progress toward use in cell therapy. *STEM CELLS TRANSLATIONAL MEDICINE 2017;6:1316–1320*

SIGNIFICANCE STATEMENT

There is need for a cytotherapy to facilitate new blood vessel formation in damaged organs, which is highly relevant for ischemic diseases and three-dimensional tissue engineering. Therefore, there has been increased interest in identifying endothelial progenitors as the "building blocks" of these vascular units. Unfortunately, as the research field has expanded, nomenclature relating to these cells has become increasingly complex and does not always align with the latest scientific evidence. Ensuing confusion around endothelial progenitor cell identity and function has sometimes diminished confidence in the field and the intent of this article is to raise concerns on current standard practices and propose alternative, more accurate, terminology.

AMBIGUITY IN CURRENT DEFINITION FOR ENDOTHELIAL PROGENITORS

Endothelial progenitor cells (EPCs) have been typically defined as cells that are able to differentiate into endothelial cells and contribute to the formation of new blood vessels. While this theoretical definition remains broadly correct, it fails to align precisely with the current scientific evidence and this has allowed a wide

variety of different cell types to be named and used as EPCs [1]. Some researchers have come to consider EPC as a highly heterogeneous population and because published studies cannot be easily compared, this has significantly hampered scientific advances in the field and clinical translation. Moreover, this has resulted in conflicting results reporting both incorporation [2] and lack of incorporation [3] into host vasculature, and created confusion about the role of these cells in health and disease [4].

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Working definitions for EPCs have also been criticized for lack of specificity. For example, the EPC phenotype in culture is frequently defined by the combination of CD31 (PECAM1) expression, AcLDL uptake, and lectin (UEA) binding. However, it has been demonstrated that cultured hematopoietic cells can effectively acquire an EPC phenotype by passive transfer of platelet microparticles containing CD31 [5]. In addition, markers classically associated with endothelium such as CD31 and vascular endothelial growth factor receptor 2 (VEGFR2) are also expressed in some monocyte subpopulations [6]. AcLDL uptake and lectin binding are not specific to endothelial progenitors as they are conventionally used for characterization of both macrophages and mature endothelial cells. These deficiencies in current practices highlight a major issue: expression of CD31, uptake of acLDL, and lectin binding are insufficient to define an endothelial progenitor in vitro. This confusion arose because initially “putative” endothelial progenitors were isolated from circulating blood mononuclear cells expressing CD34 and Flk-1, and identified in culture by acLDL-Dil uptake and CD31 expression [7]. Advances in knowledge and technology currently enable a more detailed and accurate definition.

CONFUSING NOMENCLATURE

Since Asahara et al. coined the terminology “putative EPCs” in 1997 [7], various different names to describe endothelial progenitors have entered the scientific literature causing considerable confusion in the field. To facilitate understanding, it is important to recognize that there are two distinct approaches used for studying endothelial progenitors: (a) Flow cytometry-based assays in blood samples; and (b) In vitro cell culture isolation methodologies [8] (Fig. 1).

Using flow cytometry, circulating EPCs are frequently quantified as the percentage of mononuclear cells expressing CD34 and VEGFR2. Because CD34+ VEGFR2+ cells may also identify circulating mature endothelial cells sloughed from vasculature, some research groups have included CD133 as an additional progenitor marker [9]. While enumeration of CD34+ VEGFR2+ cells appears to be a useful biomarker for cardiovascular risk [10], the use of CD133 as an additional progenitor marker remains controversial. There is evidence to demonstrate that CD34+ CD133+ cells give rise to endothelial cells [11]; however, there is also evidence to the contrary, demonstrating that CD34+ CD133+ VEGFR2+ cells do not normally yield endothelium but remain hematopoietic [12].

For EPCs isolated using cell culture methodologies, there is now agreement that two different populations can be distinguished. Originally, these were known as early and late EPCs in relation to their time of appearance in culture [13], although the phenotype of these two cell populations is very different; one being hematopoietic, and the other endothelial, respectively [14]. This led to a proposal to rename these cells as “hematopoietic EPCs” and “nonhematopoietic EPCs” [15], based on the hypothesis that hematopoietic EPCs will give rise to nonhematopoietic EPCs and ultimately endothelial cells. Since the existence of an “adult hemangioblast” has not been convincingly demonstrated, we should avoid considering adult hematopoietic cells as endothelial progenitors. Moreover, since the term hematopoietic EPCs is based on unproven assumptions and creates confusion, it is our recommendation that researchers in the field avoid using this term.

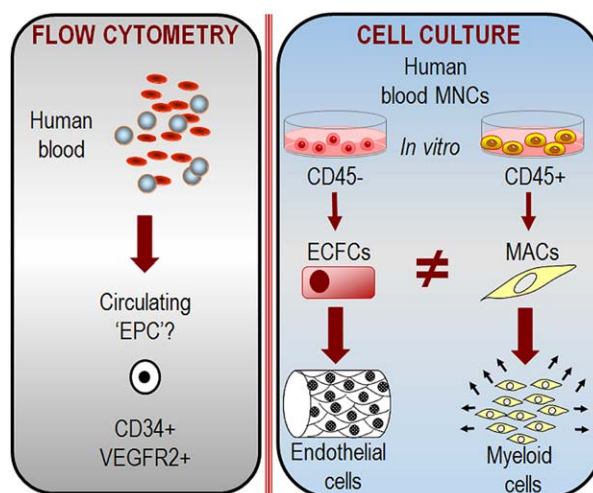


Figure 1. Methodology used to study human endothelial progenitors. Enumeration of EPCs circulating in blood is performed using flow cytometry. Cell culture technology consistently allows the isolation of well-defined cell populations with vasoreparative properties such as ECFCs and MACs. ECFCs are fully committed to the endothelial lineage while MACs exhibit a phenotype similar to M2 macrophages. \neq indicates that ECFCs and MACs represent highly distinct cell populations evidenced by their immunophenotype and pro-angiogenic mechanism of action. Abbreviations: ECFCs, endothelial colony forming cells; EPC, endothelial progenitor cell; MACs, myeloid angiogenic cells; VEGFR2, vascular endothelial growth factor receptor 2.

The name “circulating angiogenic cells” (CACs) is frequently used to identify early EPCs [16]. This nomenclature assumes that these cells circulate in blood, and while this is likely, currently there is insufficient proof to confirm such “circulating” status in vivo. In fact, it has been suggested that CACs might be generated “in vitro” due to cell culture conditions that do not exist “in vivo.” Therefore, we recommend referring to these cells as myeloid angiogenic cells (MACs) to clarify both their lineage and function. MACs are defined as cultured cells derived from peripheral blood mononuclear cells grown under endothelial cell culture conditions, which are characterized by an immunophenotype depicted as positive for CD45, CD14, CD31, and negative for CD146, CD133, and Tie2 [17, 18]. MACs do not have capacity to become endothelial cells, but promote angiogenesis through a paracrine mechanism [19].

In culture, a second cell population can also be obtained and the name “endothelial outgrowth cells” was used to identify these late EPCs [20]. These cells are now more commonly known as endothelial colony forming cells (ECFCs) and represent an endothelial cell type with potent intrinsic angiogenic capacity, capable of contributing to vascular repair of injured endothelium as well as de novo blood vessel formation [21]. This vasculogenic property is further enhanced by their role as trophic mediators through release of paracrine factors [22].

There have been major controversies when trying to define “bona fide EPCs” and disparate results are difficult to reconcile, due to differences in compositions of cell populations used [23]. Most reports have tried to demonstrate the superiority of one cell type versus another. We need to recognize that cell composition, purity, and mechanisms of action differ greatly, which makes meaningful comparisons difficult. In addition, different cell types

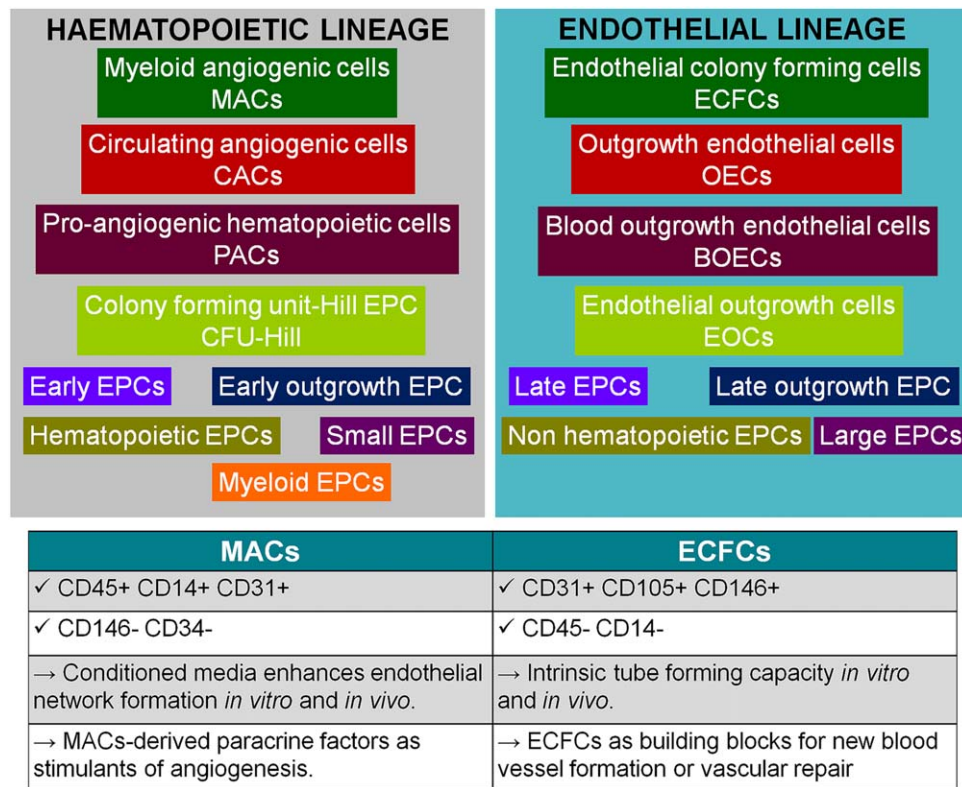


Figure 2. Cell types studied for their pro-angiogenic properties. Complex nomenclature can be divided into two distinct groups according to phenotypic lineage: hematopoietic and endothelial. Table provides minimal criteria to define MACs and ECFCs based on immunophenotype, including negative markers for purity; and function, assessed as a potency assay. Abbreviations: BOECs, blood outgrowth endothelial cells; CACs, circulating angiogenic cells; CFU, colony forming unit; ECFCs, endothelial colony forming cells; OECs, endothelial outgrowth cells; EPC, endothelial progenitor cell; MACs, myeloid angiogenic cells; PACs, pro-angiogenic hematopoietic cells.

may play dissimilar roles and there is evidence to suggest a synergistic effect when distinct EPCs are used together [13]. To guide scientists new to the complex field of cells studied for vascular regeneration, we have grouped the different names into two main categories according to their hematopoietic (myeloid) or endothelial lineage (Fig. 2).

REVISED TERMINOLOGY

It is widely appreciated that the current EPC nomenclature is inaccurate and current standards were suggested over a decade ago [24]. While this was valuable in providing a starting framework for understanding EPC biology, we feel it is now time to use the most recent molecular and functional data to update terminology with obvious benefits for research continuity, clinical associations and enhance progress to mainstream cell therapy.

In order for cell nomenclature to be informative, accurate and biologically meaningful, we propose that it should describe two interrelated characteristics: a specific phenotype and a biological function. On these lines, for the two distinct EPC subtypes isolated in culture, we support the terminology ECFCs [25] and MACs [19] because they accurately describe the phenotype and function of these cell-types. We do not support the use of the term EPCs because of its intrinsic ambiguity. In particular, EPCs should not be used to name cells such as MACs/CACs because these cells are not endothelial nor progenitor cells [23], but myeloid cells, albeit with potent pro-angiogenic, vasoreparative functionality [26–28],

through a paracrine mechanism [29]. It is important to highlight that MACs do not give rise to endothelial cells, but remain true to their hematopoietic nature [17, 19]. Ideally the term endothelial progenitor, if ever used to describe a population of cells, should be strictly reserved for cells with an endothelial phenotype, self-renewal potential, and capacity for de novo, *in vivo* blood vessel formation.

While discrepancies in the field may be inevitable, standardized tests such as potency assays are required to harmonize standards and improve accuracy. In this context, endothelial behavior such as the capacity to form a vascular network *in vitro* and *in vivo*, coupled with a detailed identity immunophenotype, should be used as routine standards during cell characterization. In addition, clonogenicity and proliferative capacity should become standard criteria to distinguish true progenitors from mature endothelial cells. Based on the collective experience in the authors' laboratories, the minimum requirements for an ECFC are: unequivocal endothelial cell phenotype, significant proliferative potential, and capacity to self-assemble into functional blood vessels *in vivo*. We operationally defined ECFCs as cultured cells derived from umbilical cord blood or peripheral blood mononuclear cells, grown under endothelial cell culture conditions, which are characterized by an immunophenotype depicted as positive for CD31, VE-Cadherin, von Willebrand factor, CD146, VEGFR2, and importantly negative for CD45 and CD14 [30–32]. These cells also express CD34, although expression level of this antigen may decline during *in vitro* expansion. Functionally, ECFC must exhibit

significant proliferative capacity and possess vascular network forming potential *in vitro* and *in vivo*.

NEXT GENERATION ENDOTHELIAL PROGENITORS

Thanks to advances in cell characterization methodologies and technologies, we are starting to recover and identify cell subpopulations within homogenous cell populations such as ECFCs. For example, well-defined hierarchies based on ECFC proliferative potential and *in vivo* vasculogenic activity have been described [30, 33]. Similarly, it has been reported that ECFCs can be classified according to aldehyde dehydrogenase (Alde) activity [34] with low-Alde levels being associated with higher reparative capacities in ischemic tissues than high-Alde ECFCs. Such studies provide enhanced precision in the field, and further advancements in technology such as flow cytometry, cell sorting, and single cell genomics will facilitate ever-improving understanding of endothelial progenitor biology. As an example, using induced pluripotent stem cell (iPSC) technology, protocols have been designed for differentiation of human iPSCs into ECFCs [35], which may allow detailed investigation into molecular mechanisms driving endothelial differentiation. Characterization of ECFC pro-angiogenic activity has significant translational potential because such functionality can be used as a surrogate marker for personalized medicine [36, 37].

While bone marrow is considered the classical source for endothelial progenitors, emerging evidence supports the existence of vascular progenitor cells which are resident in various organs [38] and carry significant vascular homeostatic and regeneration capacity [39]. For example, it has been suggested that the endothelial lining of blood vessels is a major source of ECFCs, and a complete hierarchy of ECFCs based on single cell clonogenic capacity has been identified in endothelial cultures from umbilical vein and aorta [40]. ECFCs have also been isolated from human placenta [41] and human white adipose tissue [42]. In addition, a “vasculogenic zone” within the vascular wall has also been reported to contain endothelial precursors and multipotent mesodermal stem cells [43]. Future research into defining the precise tissue niche of true endothelial progenitors *in vivo* is warranted [44]. Likewise, it is important to determine if there is organ-specificity for progenitors as this has been demonstrated for microvascular endothelial cells [45]. Determining the niche where progenitors reside will provide further important information to guide nomenclature.

Future research optimizing and improving methodology for ECFC isolation and expansion *in vitro* coupled with transferring academic laboratory protocols to GMP grade standard operating procedures will facilitate translation of preclinical research into

clinical trials. Examples of these recent advances are the development of a microfluidic system to capture ECFCs from human adult peripheral blood [46]; and the replacement of FBS with human platelet lysate for ECFC culture [47, 48]. Strategies to enhance ECFC vasculogenic potential when delivered into ischemic tissues are also being investigated [49, 50].

CONCLUSION

Accurate cell definitions represent a critical barrier for translation of cell therapies into the clinic. Indeed, identity and purity are essential requirements to define any cell therapy product. The working definition for EPCs, as cells from circulating blood that promote new blood vessel formation is not sufficiently accurate in the era of precision medicine. This is especially true as our field progresses toward clinical use of efficacious cell therapy products [51], which require a detailed phenotypic identity, a measurement of purity, and consistent functional readouts as minimal essential release criteria. We believe that biologists working in the endothelial progenitor field should seek to align with these basic requirements, not just to aid future clinical application, but to help advance our basic scientific knowledge of these important cells. Therefore, we endorse the terms ECFCs and MACs as well-defined cell populations isolated in culture with potential for therapeutic angiogenesis.

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AUTHORS CONTRIBUTIONS

R.J.M., C.L.B., F.S., F.D.-G., J.M.M.-M., K.K., O.O., A.M.R., J.K.Y.C., T.Y., V.W.M.V.H., M.C.Y., and A.W.S.: conception and design, manuscript writing, final approval of manuscript; R.J.M. and T.Y.: figures design.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

K.K. is an inventor on a patent licenced to AngioStem. The other authors indicated no potential conflicts of interest.

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