



Endothelial Progenitors: A Consensus Statement on Nomenclature

Reinhold J Medina, Chad L Barber, Florence Sabatier, Francoise Dignat-George, Juan M Melero-Martin, Kiarash Khosrotehrani, Osamu Ohneda, Anna Randi, Jerry K.Y. Chan, Teruhide Yamaguchi, et al.

► To cite this version:

Reinhold J Medina, Chad L Barber, Florence Sabatier, Francoise Dignat-George, Juan M Melero-Martin, et al.. Endothelial Progenitors: A Consensus Statement on Nomenclature. Stem Cells Translational Medicine, 2017, 6 (5), pp.1316-1320. 10.1002/sctm.16-0360 . hal-01777824

HAL Id: hal-01777824

<https://amu.hal.science/hal-01777824>

Submitted on 25 Apr 2018

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Endothelial Progenitors: A Consensus Statement on Nomenclature

REINHOLD J. MEDINA,^a CHAD L. BARBER,^b FLORENCE SABATIER,^c FRANCOISE DIGNAT-GEORGE,^c JUAN M. MELERO-MARTIN,^{d,e,f} KIARASH KHOSROTEHRANI,^{g,h} OSAMU OHNEDA,ⁱ ANNA M. RANDI,^j JERRY K.Y. CHAN,^k TERUhide YAMAGUCHI,^l VICTOR W.M. VAN HINSBERGH,^m MERVIN C. YODER,ⁿ ALAN W. STITT^a

^aCentre for Experimental Medicine, Queen's University Belfast, Belfast, United Kingdom; ^bDepartment of Biology, California Lutheran University, Thousand Oaks, California, USA; ^cVascular Research Centre Marseille, INSERM, Aix Marseille Université, Marseille, France; ^dDepartment of Cardiac Surgery, Boston Children's Hospital, Massachusetts, USA; ^eDepartment of Surgery, Harvard Medical School, Boston, Massachusetts, USA; ^fHarvard Stem Cell Institute, Cambridge, Massachusetts, USA; ^gUniversity of Queensland Centre for Clinical Research, Herston, Queensland, Australia; ^hUniversity of Queensland Diamantina Institute, Translational Research Institute, Woolloongabba, Queensland, Australia; ⁱLab of Regenerative Medicine and Stem Cell Biology, University of Tsukuba, Tsukuba, Japan; ^jNational Heart and Lung Institute (NHLI) Vascular Sciences, Imperial College London, London, United Kingdom; ^kDepartment of Reproductive Medicine, KK Women's and Children's Hospital, Singapore; ^lUniversity of Kanazawa Technical Institute, Ishikawa, Japan; ^mDepartment of Physiology, Institute for Cardiovascular Research, VU University Medical Center, Amsterdam, The Netherlands; ⁿDepartment of Pediatrics, Indiana University School of Medicine, Indianapolis, Indiana, USA

Key Words. Angiogenesis • Cellular therapy • Endothelial cell • Progenitor cells

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].

Correspondence: Reinhold J. Medina, M.D., Ph.D., Centre for Experimental Medicine, Queen's University Belfast, Belfast, United Kingdom BT9 7BL. Telephone: 028 9097 6477; e-mail: r.medina@qub.ac.uk

Received August 1, 2016; accepted for publication December 5, 2016; published online First on 10 March 2017. ©AlphaMed Press 1066-5099/2016/\$20.00/0; <http://dx.doi.org/10.1002/sctm.16-0360>

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

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.

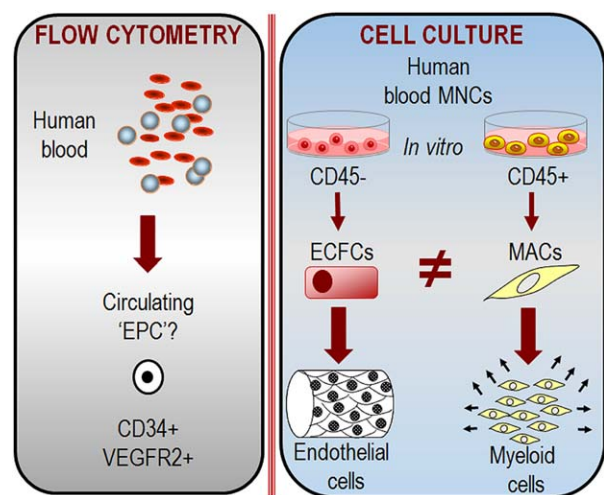


Figure 1. Methodology used to study human endothelial progenitors. Enumeration of EPCs circulating in blood is performed using flow cytometry. Cell culture technology consistently allows the isolation of well-defined cell populations with vasoreparative properties such as ECFCs and MACs. ECFCs are fully committed to the endothelial lineage while MACs exhibit a phenotype similar to M2 macrophages. ≠ 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

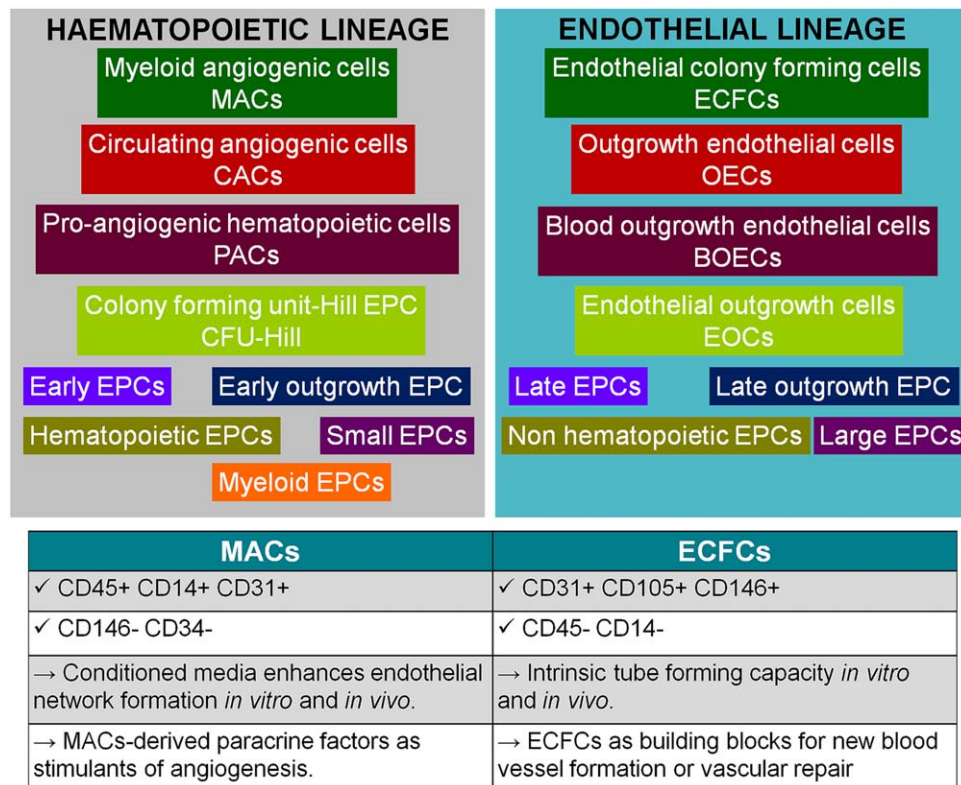


Figure 2. Cell types studied for their pro-angiogenic properties. Complex nomenclature can be divided into two distinct groups according to phenotypic lineage: hematopoietic and endothelial. Table provides minimal criteria to define MACs and ECFCs based on immunophenotype, including negative markers 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.

ACKNOWLEDGMENTS

R.J.M. was supported by the Juvenile Diabetes Research Foundation, Fight for Sight, the Leverhulme Trust, and the National Eye Research Centre. K.K. was supported by the Australian National Health and Medical Research Council (NHMRC). M.C.Y. was partially supported by the National Institutes of Health Grant DK106846 and the Riley Children's Foundation. A.W.S. was supported by the Sir Jules Thorn Trust, Fight for Sight, and Invest Northern Ireland.

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.

REFERENCES

- Basile DP, Yoder MC. Circulating and tissue resident endothelial progenitor cells. *J Cell Physiol* 2014;229:10–16.
- Yoder MC, Mead LE, Prater D et al. Redefining endothelial progenitor cells via clonal analysis and hematopoietic stem/progenitor cell principals. *Blood* 2007;109:1801–1809.
- Purhonen S, Palm J, Rossi D et al. Bone marrow-derived circulating endothelial precursors do not contribute to vascular endothelium and are not needed for tumor growth. *Proc Natl Acad Sci USA* 2008;105:6620–6625.
- Barber CL, Iruela-Arispe ML. The elusive endothelial progenitor cell: Identities, functions and clinical implications. *Pediatr Res* 2006;59:26R–32R.
- Prokopi M, Pula G, Mayr U et al. Proteomic analysis reveals presence of platelet microparticles in endothelial progenitor cell cultures. *Blood* 2009;114:723–732.
- Kim SJ, Kim JS, Papadopoulos J et al. Circulating monocytes expressing CD31: Implications for acute and chronic angiogenesis. *Am J Pathol* 2009;174:1972–1980.
- Asahara T, Murohara T, Sullivan A et al. Isolation of putative progenitor endothelial cells for angiogenesis. *Science* 1997;275:964–967.
- Medina RJ, O'Neill CL, O'Doherty TM et al. Endothelial progenitors as tools to study vascular disease. *Stem Cells Int* 2012;2012:346735.
- Peichev M, Naiyer AJ, Pereira D et al. Expression of VEGFR-2 and AC133 by circulating human CD34(+) cells identifies a population of functional endothelial precursors. *Blood* 2000;95:952–958.
- Werner N, Kosiol S, Schiegl T et al. Circulating endothelial progenitor cells and cardiovascular outcomes. *N Engl J Med* 2005; 353: 999–1007.

- 11 Wu X, Lensch MW, Wylie-Sears J et al. Hemogenic endothelial progenitor cells isolated from human umbilical cord blood. *STEM CELLS* 2007;25:2770–2776.
- 12 Case J, Mead LE, Bessler WK et al. Human CD34+AC133+VEGFR-2+ cells are not endothelial progenitor cells but distinct, primitive hematopoietic progenitors. *Exp Hematol* 2007;35:1109–1118.
- 13 Yoon CH, Hur J, Park KW et al. Synergistic neovascularization by mixed transplantation of early endothelial progenitor cells and late outgrowth endothelial cells: The role of angiogenic cytokines and matrix metalloproteinases. *Circulation* 2005;112:1618–1627.
- 14 Medina RJ, O'Neill CL, Sweeney M et al. Molecular analysis of endothelial progenitor cell (EPC) subtypes reveals two distinct cell populations with different identities. *BMC Med Genomics* 2010;3:18.
- 15 Asahara T, Kawamoto A, Masuda H. Concise review: Circulating endothelial progenitor cells for vascular medicine. *STEM CELLS* 2011;29:1650–1655.
- 16 Vaughan EE, O'Brien T. Isolation of circulating angiogenic cells. *Methods Mol Biol* 2012;916:351–356.
- 17 O'Neill CL, Guduric-Fuchs J, Chambers SE et al. Endothelial cell-derived Pentraxin 3 limits the vasoreparative therapeutic potential of circulating angiogenic cells. *Cardiovasc Res* 2016;112:677–688.
- 18 Stitt AW, O'Neill CL, O'Doherty MT et al. Vascular stem cells and ischaemic retinopathies. *Prog Retin Eye Res* 2011;30:149–166.
- 19 Medina RJ, O'Neill CL, O'Doherty TM et al. Myeloid angiogenic cells act as alternative M2 macrophages and modulate angiogenesis through interleukin-8. *Mol Med* 2011;17:1045–1055.
- 20 Lin Y, Weisdorf DJ, Solovey A et al. Origins of circulating endothelial cells and endothelial outgrowth from blood. *J Clin Invest* 2000;105:71–77.
- 21 Tasev D, Koolwijk P, van Hinsbergh VW. Therapeutic potential of human-derived endothelial colony-forming cells in animal models. *Tissue Eng Part B Rev* 2016;22:371–382.
- 22 Lin RZ, Moreno-Luna R, Li D et al. Human endothelial colony-forming cells serve as trophic mediators for mesenchymal stem cell engraftment via paracrine signaling. *Proc Natl Acad Sci USA* 2014;111:10137–10142.
- 23 Pearson JD. Endothelial progenitor cells—an evolving story. *Microvasc Res* 2010;79:162–168.
- 24 Murasawa S, Asahara T. Endothelial progenitor cells for vasculogenesis. *Physiology (Bethesda)* 2005;20:36–42.
- 25 Prasain N, Meador JL, Yoder MC. Phenotypic and functional characterization of endothelial colony forming cells derived from human umbilical cord blood. *J Vis Exp* 2012;62:3872.
- 26 Chambers SE, O'Neill CL, O'Doherty TM et al. The role of immune-related myeloid cells in angiogenesis. *Immunobiology* 2013;218:1370–1375.
- 27 Kanayasu-Toyoda T, Tanaka T, Kikuchi Y et al. Cell-surface MMP-9 protein is a novel functional marker to identify and separate proangiogenic cells from early endothelial progenitor cells derived from CD133(+) cells. *STEM CELLS* 2016;34:1251–1262.
- 28 Urbich C, Heeschen C, Aicher A et al. Relevance of monocytic features for neovascularization capacity of circulating endothelial progenitor cells. *Circulation* 2003;108:2511–2516.
- 29 Urbich C, Aicher A, Heeschen C et al. Soluble factors released by endothelial progenitor cells promote migration of endothelial cells and cardiac resident progenitor cells. *J Mol Cell Cardiol* 2005;39:733–742.
- 30 Ingram DA, Mead LE, Tanaka H et al. Identification of a novel hierarchy of endothelial progenitor cells using human peripheral and umbilical cord blood. *Blood* 2004;104:2752–2760.
- 31 Medina RJ, O'Neill CL, O'Doherty TM et al. Ex vivo expansion of human outgrowth endothelial cells leads to IL-8-mediated replicative senescence and impaired vasoreparative function. *STEM CELLS* 2013;31:1657–1668.
- 32 Poitevin S, Cussac D, Leroyer AS et al. Sphingosine kinase 1 expressed by endothelial colony-forming cells has a critical role in their revascularization activity. *Cardiovasc Res* 2014;103:121–130.
- 33 Patel J, Wong HY, Wang W et al. Self-renewal and high proliferative colony forming capacity of late-outgrowth endothelial progenitors is regulated by cyclin-dependent kinase inhibitors driven by notch signaling. *STEM CELLS* 2016;34:902–912.
- 34 Nagano M, Yamashita T, Hamada H et al. Identification of functional endothelial progenitor cells suitable for the treatment of ischemic tissue using human umbilical cord blood. *Blood* 2007;110:151–160.
- 35 Prasain N, Lee MR, Vemula S et al. Differentiation of human pluripotent stem cells to cells similar to cord-blood endothelial colony-forming cells. *Nat Biotechnol* 2014;32:1151–1157.
- 36 Reed DM, Paschalaki KE, Starke RD et al. An autologous endothelial cell: Peripheral blood mononuclear cell assay that detects cytokine storm responses to biologics. *FASEB J* 2015;29:2595–2602.
- 37 Paschalaki KE, Starke RD, Hu Y et al. Dysfunction of endothelial progenitor cells from smokers and chronic obstructive pulmonary disease patients due to increased DNA damage and senescence. *STEM CELLS* 2013;31:2813–2826.
- 38 Torsney E, Xu Q. Resident vascular progenitor cells. *J Mol Cell Cardiol* 2011;50:304–311.
- 39 Khmelevski E, Becker A, Meinertz T et al. Tissue resident cells play a dominant role in arteriogenesis and concomitant macrophage accumulation. *Circ Res* 2004;95:E56–64.
- 40 Ingram DA, Mead LE, Moore DB et al. Vessel wall-derived endothelial cells rapidly proliferate because they contain a complete hierarchy of endothelial progenitor cells. *Blood* 2005;105:2783–2786.
- 41 Patel J, Seppanen E, Chong MS et al. Prospective surface marker-based isolation and expansion of fetal endothelial colony-forming cells from human term placenta. *STEM CELLS TRANSL MED* 2013;2:839–847.
- 42 Lin RZ, Moreno-Luna R, Munoz-Hernandez R et al. Human white adipose tissue vasculature contains endothelial colony-forming cells with robust in vivo vasculogenic potential. *Angiogenesis* 2013;16:735–744.
- 43 Zengin E, Chalajour F, Gehling UM et al. Vascular wall resident progenitor cells: A source for postnatal vasculogenesis. *Development* 2006;133:1543–1551.
- 44 Goligorsky MS, Salven P. Concise review: Endothelial stem and progenitor cells and their habitats. *STEM CELLS TRANSL MED* 2013;2:499–504.
- 45 Nolan DJ, Ginsberg M, Israely E et al. Molecular signatures of tissue-specific microvascular endothelial cell heterogeneity in organ maintenance and regeneration. *Dev Cell* 2013;26:204–219.
- 46 Lin RZ, Hatch A, Antontsev VG et al. Microfluidic capture of endothelial colony-forming cells from human adult peripheral blood: Phenotypic and functional validation in vivo. *Tissue Eng Part C Methods* 2015;21:274–283.
- 47 Kim H, Prasain N, Vemula S et al. Human platelet lysate improves human cord blood derived ECFC survival and vasculogenesis in three dimensional (3D) collagen matrices. *Microvasc Res* 2015;101:72–81.
- 48 Tasev D, van Wijhe MH, Weijers EM et al. Long-term expansion in platelet lysate increases growth of peripheral blood-derived endothelial-colony forming cells and their growth factor-induced sprouting capacity. *PLoS One* 2015;10:e0129935.
- 49 Stalin J, Harhour K, Hubert L et al. Soluble CD146 boosts therapeutic effect of endothelial progenitors through proteolytic processing of short CD146 isoform. *Cardiovasc Res* 2016;111:240–251.
- 50 Goto K, Takemura G, Takahashi T et al. Intravenous administration of endothelial colony-forming cells overexpressing integrin beta1 augments angiogenesis in ischemic legs. *STEM CELLS TRANSL MED* 2016;5:218–226.
- 51 Chong MS, Ng WK, Chan JK. Concise review: Endothelial progenitor cells in regenerative medicine: Applications and challenges. *STEM CELLS TRANSL MED* 2016;5:530–538.