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Circulating and tissue resident endothelial progenitor cells

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Abstract

Progenitor cells for the endothelial lineage have been widely investigated for more than a decade, but continue to be controversial since no unique identifying marker has yet been identified. This review will begin with a discussion of the basic tenets originally proposed for proof that a cell displays properties of an endothelial progenitor cell. We then provide an overview of the methods for putative endothelial progenitor cell derivation, expansion, and enumeration. This discussion includes consideration of cells that are present in the circulation as well as cells resident in the vascular endothelial intima. Finally, we provide some suggested changes in nomenclature that would greatly clarify and demystify the cellular elements involved in vascular repair.

Introduction

If one had to point to a single paper that established the field of endothelial progenitor cell (EPC) biology, the 1997 paper by Asahara et al. (6) would have to be the sentinel choice. In this work, the authors reasoned that since angioblasts and hematopoietic cells emerged near simultaneously in extra-embryonic blood islands during mouse development and shared expression of many cell surface antigens, these lineages may have been derived from a common precursor. They also inferred that the ability of circulating hematopoietic stem cells (HSC) to reconstitute the hematopoietic system of recipient mice as evidence that some circulating stem cells were present in the systemic bloodstream. Thus, the authors sought to determine whether circulating adult human peripheral blood cells could differentiate into endothelial cells. Magnetic beads were used to isolate cells expressing CD34 and/or Flk-1 (vascular endothelial growth factor receptor-2) and purity of the isolated fractions were noted to be 15.7% and 20%, respectively. Overall, a limited number of CD34+ cells attached, became spindle shaped, and proliferated over 4 weeks of in vitro culture. When the CD34+ cells were labeled with a fluorescent dye (DiI) and then co-cultured with CD34− peripheral blood cells, a 10-fold increase in cell proliferation was observed compared to the plated CD34+ cells alone. Furthermore, the CD34+ cells in co-culture with CD34− cells for only 12 hours formed numerous cell clusters. After 5 days of co-culture the CD34+ cells began to ingest acetylated low density lipoprotein (ac-LDL) and emerged as spindle shaped cells from the base of the cell clusters (whereas the round cells atop the clusters failed to

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ingest ac-LDL). Since the spindle shaped CD34+ derived cells also displayed less CD45, but greater amounts of CD31, CD34, Flk-1, Tie-2, and E-selectin than the freshly isolated CD34+ cells and were stimulated to express nitric oxide in response to acetylcholine and vascular endothelial growth factor (VEGF) administration, the authors postulated that the peripheral blood cells had become endothelial cell-like during the culture. When 500,000 freshly isolated human peripheral blood CD34+ cells (co-labeled with DiI) were injected intravenously into athymic mice that had undergone unilateral femoral artery excision, numerous DiI-labeled cells were detected 1–6 weeks in the injured limb but not the contralateral healthy limb. DiI-labeled cells were detected in 13.7% of the host capillaries in the injured limb and were determined to co-express CD31, Tie-2, and the receptor for *Ulex europaeus agglutinin-1* lectin (UEA-1). As a secondary proof of concept, Flk-1+ cells were isolated from the whole blood of 10 donor transgenic mice expressing β-galactosidase and injected into host syngeneic mice following unilateral femoral artery excision. Some β-galactosidase expressing cells were identified in the ischemic limbs post-injection in close apposition with host capillaries and small arteries that co-stained with CD31 and *Bandeiraea simplicifolia-1* lectin (BS-1). These results were interpreted as evidence that the circulating human CD34+ cells and murine Flk-1+ cells were capable of contributing to vascular structures in ischemic limbs of host mice in vivo. In sum, this seminal paper proposed that some circulating cells are capable of serving as progenitors of the endothelial lineage both in vitro and in vivo. The fact that the circulating cells could contribute to vascular regeneration in vivo suggested these cells were undergoing postnatal vasculogenic responses.

Key elements selected from this seminal paper (6) that subsequently became foundational and/or controversial concepts of features that define a circulating EPC included: 1) human and murine blood contains circulating EPC, 2) human and murine putative EPC displayed different markers; human EPC were CD34+, Tie-2+, CD31+, UEA-1+, ac-LDL+, and some expressed CD45 while murine EPC were Flk-1+, BS-1+, and CD31+, 3) circulating putative EPC integrated into regenerating host vessels in an area of injury (but not in uninjured sites), 4) putative human EPC displayed low proliferative potential that was augmented by other non-EPC peripheral blood cells, 5) putative human EPC attached to non-EPC to form cord-like structures in vitro, 6) putative EPC formed clusters when co-cultured in vitro with non-EPC, and 7) circulating putative EPC contributed via postnatal vasculogenesis to vascular repair and tissue regeneration. Given this introduction to some of these foundational concepts, we will examine the methods that are currently used to identify and define putative EPC.

Methods to define human EPCs

Putative human EPCs have been identified using several approaches. Human peripheral blood cell mononuclear cells can be plated into culture plates coated with fibronectin and are commonly grown in a commercial medium (Endothelial growth medium 2 [EGM2], Lonza) with addition of varying growth factors that include vascular endothelial growth factor (VEGF), fibroblast growth factor 2 (FGF2), insulin-like growth factor 1 (IGF1), and epidermal growth factor (EGF) (34, 42). The adherent cells that persist for 5–7 days and display the capacity to take up ac-LDL and bind UEA-1 have been defined as EPCs, based in large part on the original observations of Asahara et al. (6). However, circulating platelets commonly contaminate the plated mononuclear cells and may release membrane particles that bind to the adherent putative EPC (67). Since the platelet membranes and vascular endothelial cells share expression of many cell surface proteins, it is not uncommon for putative EPC that are not actively transcribing these proteins to display the platelet-endothelial protein markers on their cell surface. Thus, this assay for adherent putative EPCs cannot be considered valid (too many false positive cells) unless contaminating platelets can be completely eradicated from the starting cell material (67). Furthermore, peripheral blood

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monocytes are known to be isolated using attachment to fibronectin coated dishes and may therefore also contaminate the putative EPC subset (32). Since monocytes cultured with VEGF express numerous endothelial-like cell surface proteins (VEGF receptors, CD144, CD31, and von Willebrand factor(vWF), upregulate endothelial nitric oxide synthase (eNOS), and take up ac-LDL, these cells display all of the in vitro features of the putative EPC (74, 75). Indeed, EPCs isolated by the methods described above have been reported to more closely resemble VEGF treated monocytes than cultured endothelial cells when examined at a whole transcriptome level (1, 22, 59). Nonetheless, the cultured adherent cells isolated by this method demonstrate a variety of angiogenic promoting properties in vitro and in vivo and thus, may be more accurately defined as circulating angiogenic cells (CACs) (69, 72). Despite these clear published limitations and deficiencies, this now “unreliable” assay continues to be used to “identify” putative EPC (Fig. 1).

A second method used to define putative EPCs relies upon fluorochrome-labeled antibodies and fluorescence activated cell (FACS) analysis to discriminate peripheral blood subsets. Human peripheral blood cells expressing CD34, AC133, and KDR have been proposed as a fundamental EPC phenotype (6, 66), but, none of these proteins is specifically restricted in expression to the EPCs and all are expressed by hematopoietic stem and progenitor cells (2, 13). Other antigens (CD105, CD144, CD106, CD117, and CD45) and some enzymatic activities (aldehyde dehydrogenase) have also been proposed as EPC markers (Fig. 1), but none discriminate the EPCs from circulating blood cells (27, 35). Thus, in the absence of unique antigens to identify the EPC, one must consider that cells previously identified as EPC actually represent circulating hematopoietic cells. Since a variety of hematopoietic stem and progenitor cell subsets have recently been reported to display pro-angiogenic activities (89) it is quite possible these cells play the role of the circulating cell subsets with vascular reparative activities previously attributed to EPC (27, 51, 64, 84).

Finally, two colony forming assays have been used to identify putative EPCs. Peripheral blood cells are plated on fibronectin-coated dishes and 1–2 days later, the cells that fail to attach are removed and then replated on dishes coated with fibronectin. Using features originally described in the Asahara et al. paper (1), clusters of cells emerging in 4–9 days that display spindle-shaped cells emerging from the base are referred to as colony forming unit-Hill (CFU-Hill) EPCs (Fig. 1) (34). The CFU-Hill frequency in human peripheral blood highly predicts adverse cardiovascular risk in human subjects with cardiovascular disease (34). Both myeloid progenitor and lymphoid cells participate in the formation of these CFU-Hill clusters (70, 71). Rohde et al. (70) have suggested that lymphoid cell derived chemokines stimulate the myeloid progenitor cells comprising the clustered cells that mature into the spindle-shaped macrophages that emerge from the clusters. The pattern of gene expression in the CFU-Hill clusters is indistinguishable from CAC (above) or cultured monocytes, but distinctly different from cultured endothelial cells (1, 22, 59). CFU-Hill derived cells, like CAC cells, do not display replating potential and display low proliferative potential. Thus, this colony assay does not identify a cell that serves as a progenitor of an endothelial cell, but rather measures a mixture of peripheral blood hematopoietic subsets that can be informative as a biomarker of cardiovascular risk status.

The second colony forming assay identifies circulating endothelial cells that possess proliferative potential. Adult peripheral blood cells are placed in culture wells coated with type 1 collagen and culture medium is added (41). After 2–3 weeks in culture, adherent endothelial cell colonies become visible. The emergent colonies can be removed and plated to expand the cells or analyzed for endothelial characteristics. The progeny of the isolated colonies display typical endothelial antigens similar to vascular endothelial cells, form capillary-like structures when plated on Matrigel, but also form human blood vessels when suspended in collagen or Matrigel scaffolds and implanted in immunodeficient mice (16, 48,

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60, 61, 95). Analysis of the individual cells comprising the colonies indicates that some of the cells display heterogeneous clonal proliferative potential (41, 95). Some of the single cultured cells do not divide over a two week culture period while other clones form small clusters of progeny (2–50 cells) and some of the clones display high proliferative potential (>2000 progeny per colony). Endothelial colony forming cells (ECFCs) with high proliferative potential (Fig. 1) display robust replating potential and high telomerase activity and appear to generate more human vessels when implanted into immunodeficient mice than ECFC with lower proliferative potential (95). Infusion of ECFC into pre-clinical animal models of hindlimb ischemia (96), myocardial infarction (23), retinal ischemia (59), and islet transplantation (45) promote enhanced vascular recovery with evidence of ECFC formed vessels in most models. Thus, ECFCs appear to display many features of circulating cells that are consistent with the original criteria for an EPC (Fig. 1); circulating cells that give rise in vitro to colonies of endothelial cells with inherent heterogeneous proliferative potential, high replating activity, high telomerase activity, endothelial cell surface phenotype, in vivo vessel forming ability, and demonstrated ability to play a role in vascular repair or regeneration in pre-clinical animal models of human vascular disorders.

Methods to identify rodent EPCs

The approaches to identify putative murine EPC are similar in concept but deviate considerably in detail from the methods employed in human subjects. The circulating murine EPC is thought to be derived from the bone marrow compartment. The cell surface markers used to identify putative EPC are highly variable and have included different combinations of stem cell antigen-1 (Sca-1), c-Kit (CD117), Flk-1, vascular endothelial growth factor receptor-1 (Flt-1), CD31, Tie-2, CXCR4, CD133, or CD144 expression (7, 19, 30, 31, 33, 43, 46, 49, 56, 62, 65, 81, 88, 90, 91). While some of these cell surface markers are similar to those utilized originally used to identify human EPC, many are unique to the murine system. An alternative approach currently employed is to plate the mouse peripheral blood or bone marrow cells onto fibronectin-coated culture wells and to identify the adherent cells that persist in the culture at 4–7 days that display uptake of ac-LDL and the plant lectin BS-1 (14, 15) similar to the approach used in the human CAC assay (above). Like the human CAC assay, it is now well recognized that myeloid cells are the predominant cells isolated in the fibronectin-coated dishes and Tie-2 monocytes are the predominant pro-angiogenic cells comprising these cultures (18, 21, 36). While the proliferative ECFC from human adult peripheral blood are rare, murine circulating blood cells do not appear to possess any ECFC at an individual animal level. Specifically, whole blood cells pooled from 4–6 mice >8 weeks of age resulted in successful outgrowth of a single ECFC in 28% of attempts; a frequency far below a single event per animal (79). Thus, mice like human subjects display circulating hematopoietic cells with pro-angiogenic activity that participate in vascular repair, but differ from human subjects in failing to exhibit circulation of proliferative endothelial cells under homeostatic conditions. Selection of blood samples from younger mice or mice infused with VEGF does significantly augment the capacity to identify circulating ECFC (79). Understanding the origin of the circulating endothelial cells with proliferative potential would facilitate better understanding of the regulation of this process in both mouse and man.

Identification of tissue resident ECFCs

The identification of ECFCs can be placed within the context of established data regarding known endothelial turnover rates based on studies using whole animal tritiated thymidine labeling (77, 78). Mapping of proliferating cells in rat aorta reveals that they are not localized diffusely and heterogeneously, but rather are found in discrete foci in the endothelial intima (77). Such observations are consistent with the presence of a hierarchy of

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ECFC which may be distributed throughout the tissue and activated upon demand to meet the reparative needs of the endothelium (93). While normal steady-state endothelial turnover is slow, endothelial proliferation rates are increased in response to injury and reparative potential may be viewed as a function of cells within the tissue wall (12, 29, 80). For example, in disease prone states, where reduced endothelial repair may represent a component of the pathophysiology (as may occur for example in atherosclerosis), one would predict a reduction in the percentage of tissue resident ECFC and diminished capacity for endothelial proliferation (12, 29, 80, 93). Likewise, such situations may be associated with diminished recovery of circulating endothelial cells from the bloodstream (11).

As described above, while pro-angiogenic hematopoietic cells (PACs) do not represent actual endothelial progenitors, they are recruited to sites of active angiogenesis following injury. Adoptive transfer of these PACs facilitates vascular repair without stable integration into the remodeled endothelium (5). A model of cooperative interaction between PAC and ECFC during injury and/or physiological vascular remodeling has been proposed (Fig. 2) (40, 94). In the proposed model, hematopoietic PAC rapidly home to sites vascular injury to create a proangiogenic environment (39). The secretion of paracrine factors by PACs recruits ECFCs from either the circulation or the local vascular wall. Migration and proliferation of ECFCs are guided by PAC activity leading to vascular repair and/or angiogenesis (40). In support of this hypothesis, co-adoptive transfer of both cell types results in superior in vivo neo-angiogenesis and restoration of blood flow when compared to single cell transfers alone (96). When viewed from this perspective, the degree of vascular remodeling stimulated by PAC is dependent, in part, on the resident proliferative potential of ECFC within the tissue or the amount of circulating ECFC that can be recruited into the tissue (Fig. 2). If this is true, one would predict a correlation between the tissue ECFC content and the angiogenic or vascular proliferative capacity of a specific tissue bed. Different vascular beds have different angiogenic and repair capacities, reflecting their unique physiology and/or susceptibility to disease (93). A greater understanding of the cellular basis for such heterogeneity has been proposed to result from regulation of the local growth and angiogenic cues contained within the perivascular environment (68). However, the induction of local paracrine factors alone may not be sufficient to initiate or sustain angiogenesis since the complement of ECFC with proliferative potential may be limiting.

The lung microvasculature is well-known to actively engage in physiological and pathological angiogenic remodeling, for example, following acute lung injury or secondary to pulmonary hypertension or asthma (85). In addition to the proliferation of new cells, pulmonary microvascular cells may become apoptosis resistant in pulmonary hypertension, leading to excessive endothelial proliferation and angioproliferative occlusion (85). Rodent models of pulmonary ischemia (87) or inflammation (58) can recapitulate the robust angiogenesis of the lung microvasculature, suggesting that an understanding of rodent lung angiogenesis has relevance to human disorders. Pulmonary microvascular endothelial cells are a frequently used model system for understanding endothelial cell growth in vitro because they are relatively easy to establish in culture (57). Recently, Alvarez et al. (3), compared the growth potential of EC derived from the rat pulmonary microvasculature (RPMVEC) with that of endothelial cells derived from the rat pulmonary artery (RPAEC). Endothelial cells from both sources maintained cobblestone morphology, incorporated ac-LDL, and expressed CD31, and VEGFR-2, but not CD45 (4). In culture, these cells maintained a high degree of electrical resistance consistent with establishment of barrier function and were capable of forming blood-perfused vessels when suspended in collagen gels and implanted in vivo. When subjected to single-cell colony forming assays, the majority (~60%) of RPAEC remained as non-dividing cells and only ~ 15% of the RPAECs were capable of forming large colonies indicative of emergence from single cells with high proliferative potential (>2000 cells/colony). In contrast, only a small percentage of

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RPMVEC were non-dividing cells, while greater than 50% of RPMVEC formed large colonies (>2000 cells/colony). Thus, one explanation for the overall differences in growth potential of the RPMVEC versus the RPAEC resides in the increased content of HPP-ECFC in the RPMVEC (4).

Other investigators have also identified robust colony forming capacity in endothelial cells isolated from mouse lung. For example, Schneidermann et al (76), isolated CD31+ mouse lung endothelial cells and discovered a small subpopulation capable of forming large endothelial colonies in vitro. The mouse lung endothelial cells were also capable of forming blood-perfused vessels when suspended in Matrigel and implanted in vivo (76). The molecular pathways that permitted these rare lung ECFC to retain proliferative potential, while the vast majority of the isolated lung endothelial cells displayed minimal proliferative potential, have not been identified. However, alterations in specific pro-growth or progenitor regulatory proteins in some lung ECFC have been described. For example, the regulatory protein nucleosome associated protein-1 (NAP-1) was shown to be preferentially more expressed in rat lung HPP-ECFC and RPMVEC compared to the level of expression in RPAEC (17). In addition, Fang et al., suggested that lung derived ECFC activity co-segregated with a CD117/c-Kit+ subpopulation of pulmonary vasculature (28).

Despite the fact that the pulmonary microvasculature appears enriched with a complement of HPP-ECFC activity, further exploration of the hypothesis that angiogenic/remodeling potential is a function of local tissue resident ECFC content requires analysis of ECFCs from a variety of tissue vascular sources. One would predict that tissues capable of undergoing physiological angiogenesis such as brain, corpus luteum, placenta, or skeletal muscle would be enriched in HPP-ECFCs. While previous investigators in many cases propagated EC cultures from these various sources demonstrating robust growth properties (37, 44, 63, 86), a systematic evaluation to identify resident ECFC populations should be conducted in these tissues.

On the opposite end of the proposed spectrum, one would predict that a tissue with poor vascular reparative function would be depleted of HPP-ECFC. Such is the case with the renal vasculature. Renal capillary rarefaction promotes renal hypoxia and fuels progression of renal fibrosis in acute kidney injury (AKI) (8). Rarefaction is commonly observed in all models of chronic kidney disease as well as human patients with CKD (8). Capillary rarefaction is also observed in patients following AKI (50). In rats following experimental AKI, parenchymal cells undergo repair, but capillaries remain rarefied and show no evidence of vascular repair (8). The reason for impaired capillary regeneration is unclear. Several studies have been carried out to determine if hematopoietic pro-angiogenic cells may participate and facilitate vascular repair following AKI. Bone marrow (BM)-derived cells expressing endothelial cell markers (likely corresponding to PAC) infiltrate into the renal vasculature following acute injury due to ischemia reperfusion, Adriamycin, or administration of the anti Thy1.1 antibody (24, 25, 38, 54, 55). In these studies, evidence of revascularization by BM-derived cells was assessed by colocalization of the donor cells with a vascular marker such as CD31 or vWF. However, it is well known that the expression of surface endothelial markers in bone marrow cells is not sufficient to define an endothelial cell (92). Moreover, in a study by Li et al (53), BM-derived cells expressing endothelial cell markers peak within 14 days and decline by 28 days in kidney following adriamycin injury. Such a result is consistent with the transient homing of PACs to support local vascular remodeling, rather than stable incorporation and differentiation into more long-lived vascular endothelial cells.

Adoptive transfer studies of cells likely corresponding to PAC have been shown to preserve renal function in response to various acute injuries (52, 65, 83). These responses appear

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largely protective of the initiating insult rather than sustaining a growth regenerating response since there is no evidence that adoptively transferred cells stably integrate into vessels (83). Moreover, there are no studies in rodent models in which PACs have been shown to stimulate vascular regeneration following an established injury. In summary, despite significant interest in the trafficking of endogenous or exogenous PACs to injured kidney, such activity seems largely ineffective since vascular rarefaction remains the predominant feature of these models.

We have suggested that the lack of vascular repair in kidney may be reflective of low resident ECFC proliferative capacity. In contrast to the lung microvascular cells or HUVECs, which are widely utilized because of they are relatively easy to establish and maintain in culture, kidney endothelial cells (KEC) are notorious for being difficult to establish and maintain in long term culture conditions (82). Until recently, the only reports of long-term cultures of rodent kidney endothelial cells described cell lines derived secondary to transformation (73). After optimization, our laboratory was recently able to generate and maintain primary rat KEC in long term cultures (10). Multiple KEC were obtained but all were characterized by exceptionally slow growth rates relative to RPAEC, RPMVEC and HUVECs. Colony forming assay demonstrated that ~90% of cells remained as single/non-dividing colonies, while only a small percentage of cells were capable of forming moderately sized colonies, and there was no evidence of any population that would be defined as an HPP-ECFC (ie., colony > 2000 cells). The nature of the impaired growth of KEC is not clear, but it does not appear to be due to senescence or a reduction in the VEGFR, since VEGF-R expression is significantly greater in KEC vs PMVEC (10). The lack of ECFC proliferative capability is consistent with the relatively low proliferation rates observed in the kidney in vivo. In recent studies, using repetitive BrdU administration to rats, the identification of proliferating KEC was shown to be exceedingly rare and was not affected by kidney injury or that exogenous administration of VEGF (9). Although other studies have also identified some proliferating KEC using PCNA immunohistochemistry, these structures are also typically few in number (47). Therefore, in the setting of acute renal injury, persistent vascular loss occurs not because of a lack of bone marrow derived PACs, but rather that the resident ECFC population is either inherently low in number or actively under some form of proliferative repression that is dominant over PAC paracrine effects.

Summary

Our understanding of the circulating cells in the bloodstream that contribute to vascular repair has increased a great deal over the past 15 years. In many ways, the reported identification of an EPC has spurred this interest in the field. However, we have now accumulated sufficient evidence to demonstrate that the only circulating cell that displays all the features of the originally defined EPC are circulating ECFC. While numerous hematopoietic cells play key roles as pro-angiogenic paracrine activators of vascular repair and regeneration, they do not become specified to an endothelial state or fate in vivo and thus, are not EPC. Going forward the field should more fully analyze the specific molecules secreted by the proangiogenic hematopoietic cell subsets to design potential molecules that may be used to augment vascular repair. In addition, the field must learn how to interrogate the small pool of ECFC residing in the vascular endothelium to understand how these progenitors protect the vasculature from injury, senescence, and disease.

Reviewing the foundational and/or controversial concepts of features that were originally penned to define a circulating EPC we can now state: 1) human blood contains circulating ECFC and human and murine blood contains circulating PACs, 2) human and murine PACs display different markers; human PAC are CD34+CD133+CD45+CD31+CD14-CD235a- but also express other markers (20, 26, 27) while murine PACs are not strictly defined but

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generally display a Sca-1+Flk-1+CD31+ phenotype (above), 3) circulating PACs may lodge, emigrate, and accumulate in a peri-endothelial location at sites of vascular injury but do not become integrated as long-lived endothelial cells (ECFC have not yet been shown to integrate into vessels following prospective isolation without culture), 4) human PACs display low proliferative potential (ECFC display a hierarchy of proliferative potentials), 5) human PACs attach to HUVEC- or ECFC-derived capillary-like structures in vitro (cultured ECFC form vessels when injected in vivo), 6) human PACs form clusters in vitro in the presence of lymphoid cells or certain cytokines, and 7) circulating human and murine PACs promote vascular repair and regeneration via paracrine secretion of molecules that affect resident endothelial cells with residual ECFC activity. Given the status of the field, we are better served to use the above terminology or define a specific cellular subset by phenotype and function, rather than use the less precise term EPC.

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	CFU-Hill	CAC	ECFC
Clonal proliferative status	-	-	+
Replating ability	-	-	+
In vitro tube formation	+/-	+/-	+
In vivo de novo vessel formation	-	-	+
Homing to ischemic sites in vivo	+	+	+
Paracrine augmentation of angiogenesis	+	+	+
Phenotypic appearance	$CD34^{+/-}$	$CD34^{+/-}$	$CD34^{+/-}$
	$CD133^{+}$	$CD133^{+}$	$CD133^{-}$
	$VEGFR2^{+}$	$VEGFR2^{+}$	$VEGFR2^{+}$
	$CD45^{+/-}$	$CD45^{+/-}$	$CD45^{-}$
	$CD146^{+/-}$	$CD146^{+/-}$	$CD146^{+}$
	$CD115^{+}$	$CD115^{+}$	$CD115^{-}$
	$CD31^{+}$	$CD31^{+}$	$CD31^{+}$
	$ALDH^{bright}$	$ALDH^{bright}$	$ALDH^{bright}$
	acLDL uptake	acLDL uptake	acLDL uptake

Figure 1. Comparison of the phenotypic and functional characteristics of putative EPC determined by different assays

The cells isolated by the CFU-Hill and CAC assays identify essentially similar proangiogenic hematopoietic subsets. In contrast the cells isolated in the ECFC assay differ from the proangiogenic cells by displaying clonal proliferative potential, replating ability, and in vivo vessel forming ability. In addition the phenotype of the ECFC can be distinguished from the proangiogenic cells by the lack of CD133, CD45, and CD115 expression by the ECFC. Abbreviations: ALDH: aldehyde dehydrogenase, acLDL: acetylated low density lipoprotein, VEGFR2: vascular endothelial growth factor receptor 2.

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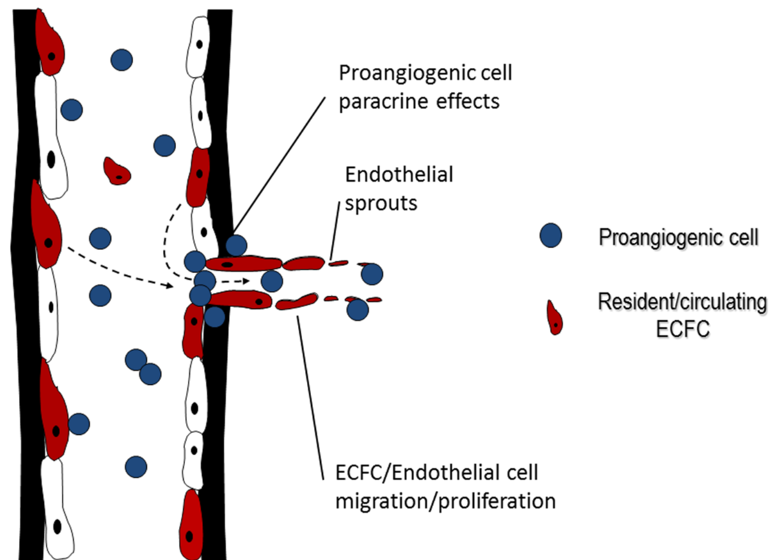

Figure 2. Collaborative interaction of proangiogenic hematopoietic cells and ECFC in the formation of new blood vessels

Bone marrow-derived proangiogenic cells are recruited to sites of tissue ischemia or damaged endothelium and secrete paracrine molecules to recruit circulating and tissue resident ECFC to participate in new blood vessel formation.

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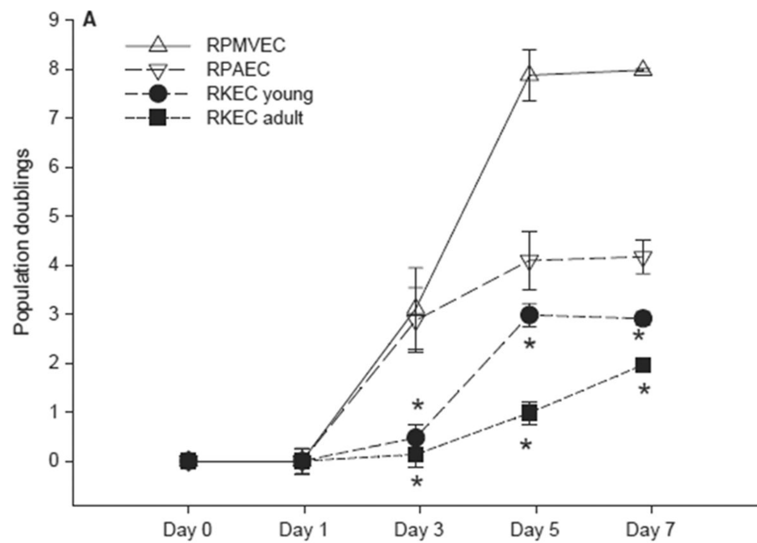

Figure 3. Tissue specific and age-related differences in proliferative potential of endothelial cells
Rat pulmonary microvascular endothelial cells (PMVEC) and pulmonary arterial endothelial cells (PAEC) proliferate more rapidly than endothelial cells isolated from 9–11 day old rat kidney (RKEC young) and 8–10 week old adult rat kidney (RKEC adult). Modified from, Basile DP, et al. Microcirculation 19:598–609, 2012.