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Hemato-vascular Origins of Endothelial Progenitor Cells?

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Abstract

Numerous studies have suggested the presence of precursor cells in various tissues and organs with potential to differentiate into endothelial and mural cells, and contribute to blood vessel formation in different physiological and pathological circumstances. Although there is still a lack of consensus in the field regarding the origin, and phenotypic and functional characteristics of putative vascular progenitor cell populations, all agree that further studies are needed to fully explore and exploit their great potential as cell therapy for vascular diseases, as modulators of postnatal blood vessel formation, and as disease biomarkers. Herein, we will review the phenotypic and functional characteristics of endothelial progenitor/precursor cell types thought to be derived from the hematopoietic and vascular systems and contribute to postnatal blood vessel formation, and discuss their potential lineage relationships.

Keywords

endothelial progenitor cells (EPC); endothelial cell; vasculogenesis; angiogenesis; hematopoietic stem cell

Introduction

Blood vessel formation plays an essential role in many physiological and pathological processes, including normal tissue growth and healing, as well as progression of tumorigenesis and retinopathy. The process by which blood vessels are formed de novo from undifferentiated cell types (i.e. multi-lineage progenitors and committed precursors) is termed vasculogenesis (Risau W. et al., 1995; Flamme, I., et al. 1997; Goldie L.C. et al., 2009); whereas, the expansion and remodeling of existing blood vessel networks is referred to as angiogenesis (Risau, W., 1997). Both vasculogenesis and angiogenesis occur during embryonic development, but for many years, angiogenesis was believed to be the sole mechanism responsible for postnatal blood vessel formation, maintenance and repair.

This paradigm was questioned when “endothelial progenitor cells” (EPC) were isolated from adult human and mouse peripheral blood, and shown to contribute to endothelial cell

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formation in vitro and postnatal neovascularization in vivo (Asahara, T. et al., 1997). Since these observations, various other studies have identified progenitor/precursor cells with vascular potential from different tissue sources based on cell surface protein expression and/or cell culture methods. However, it is still not clear how individually identified populations of putative vascular progenitor/precursor cells arise and how they are interrelated.

Without consensus about the origin, identity and functional characteristics of such cells, it is difficult to compare data generated from different laboratories investigating distinct cell populations. However, the existence of progenitor/precursor cell types with vascular potential within postnatal tissues cannot be denied. Confusion regarding the terminology and biology of such cells reflects our current limited understanding of the diversity, interrelationship and endogenous functions of various populations of cells that exhibit vascular potential. Thus, properly naming such cell populations and defining their distinct and/or similar properties is a challenge. Nonetheless, it is imperative that we continue to investigate, and discuss within our field, the phenotypes, origin(s) and interrelationship(s) of progenitor/precursor cell types, as well as their relative contributions to blood vessel formation. Only in so doing, will we be able to fully exploit and optimize their use for vascular therapies, for tissue regeneration, and as biomarkers of vascular diseases. In this review, we will provide a brief history of the identification of endothelial progenitor/precursor cells and discuss their potential origins, with specific focus on the hematopoietic and vascular systems.

Historical Review

Although adult vascular progenitor cells were not widely studied prior to the late 1990s, the presence of blood vessel-forming cells within circulating blood was actually reported decades earlier. In 1932, capillary-like structures were found to appear in cultures of blood-derived leukocytes (Hueper, W.C., et al., 1932), and organized vessels forming in cultures of chicken blood cells were reported in 1933 (Parker, R.C., 1933). Similarly, in 1951, adult chicken bone marrow cells were found to give rise to vessel structures in vitro (White, J.F., et al., 1951), and in 1987, Leu et al. (Leu, H.J., et al. 1988) found that circulating mononuclear cells give rise to pre-endothelial cells that then mature into cells with endothelial-specific organelles (i.e. Weibel-Palade bodies). In addition, endothelialization of implanted vascular prostheses by cells from systemic blood circulation has long been suggested, (Parker, R.C., 1933; Stump, M., et al. 1963; Mackenzie, J.R., et al., 1968; Shi, Q., et al. 1994; Scott, S.M., et al. 1994). Although these studies suggested the presence of endothelial cells or precursors thereof, in circulating blood, they did not define their phenotype.

In 1997, Asahara and colleagues isolated a subpopulation of circulating cells that appeared to possess in vitro and in vivo endothelial cell potential (Asahara, T., et al., 1997). Human CD34+ and mouse Flk+ peripheral blood mononuclear cells (PBMC), respectively, were shown to become endothelial-like cells in vitro and contribute to neovascularization in response to hindlimb ischemia in vivo, suggesting that these subpopulations of circulating blood cells, presumed to be derived from bone marrow, represented or contained vascular progenitor cells. It still not entirely clear to what extent these cells are related to circulating endothelial cells (CEC), which are thought to be differentiated endothelial cells shed from existing vessel lumens in response to vascular injury or in association with tumor angiogenesis (Martin, C.F., et al. 1896), myocardial infarction (Mutin, M., et al., 1999), lupus (Wellicome, S. M., et al., 1993), sickle cell anemia (Solovey, A., et al., 1997), septic shock, etc. (George, F., et al. 1992; Mutin, M., et al., 1999; Solovey, A., et al. 1997; Mutuuga, M., et al., 2001; Clancy, R., et al., 2001). Nonetheless, our review will focus on

cell types identified as non-endothelial progenitors/precursors that can give rise to endothelial cells in vitro and in vivo.

Definition of Endothelial Progenitor Cells

The term “progenitor” is widely used in the stem cell field to describe primitive cells with limited clonal expansion, and from which more than one type of lineage-related cells can be derived (Bryder, D., et al., 2006). Thus, the term progenitor may not be appropriate to describe all cells that give rise to endothelial cells. “Endothelial precursor cells” may be a more reasonable term to describe cells that are endothelial lineage-restricted progenitors and/or cells that can give rise to endothelial cells through another mechanism such as transdifferentiation. However, currently within the field, “endothelial progenitor cells” (EPC) is broadly defined as non-endothelial cells that can give rise to endothelial cells in vitro and/or in vivo; therefore, there are multiple types of EPC identified, which may have distinct origins, characteristics, and functions.

Proposed Origins of Endothelial Progenitor Cells

The hematopoietic and vascular systems develop in parallel, in an interdependent manner, during embryogenesis. Multi-lineage hematopoietic progenitors are derived from the endothelium within the yolk sac (Goldie L.C. et al., 2009) and embryo proper (Zovein, A.C. et al. 2008). Whether the same is true in adults has not been ruled out. In fact, there is close physical association between endothelial and hematopoietic stem cells (HSC) in postnatal bone marrow (Morrison, S.J., et al., 2008), and vascular endothelial and hematopoietic cell types share many cell surface markers (Jackson, K. A. et al., 2001). Thus, it is not surprising that several identified EPC populations exhibit hematopoietic characteristics, although nonhematopoietic sources of EPC have also been identified. Herein, we will briefly review proposed hematopoietic and vascular origins of endothelial progenitor cells; importantly, it is not known to what extent different populations are biologically related vs. develop independently.

Blood

Both peripheral blood and umbilical cord blood appear to contain progenitors/precursors with endothelial potential. The first EPC were isolated from human peripheral blood based on expression of CD34 (Asahara, T. et al., 1997), which is historically a marker of hematopoietic stem and progenitor cells (Shizuru J. A., et. al., 2005). Since then, the heterogeneous population of CD34-expressing blood cells has been further fractionated in attempts to isolate the specific subpopulation(s) of cells with endothelial cell potential. Thus, putative EPC have been identified in blood using a variety of hematopoietic and/or endothelial lineage markers, or combination(s) thereof, including CD34, VEGFR2 (KDR, Flk1), CD133 and CD14 (Nieda, M., et al. 1997; Shi. Q., et al. 1998; Lin. Y., et al. 2000; Gulati, R., et. al. 2003; Loges, S., et al. 2004; Stauffer, B.L., et al. 2008; Harraz, M., et al., 2001; Schmeisser, A., et. al. 2001; Raemer, P.C., et al., 2009; Kim, S. J., et al. 2009). Although similar strategies have been applied to the isolation of cells from both peripheral and cord blood, there are differences in the proportions of cell types within each. For example, cord blood contains a higher proportion of CD34⁺ and CD133⁺ cells compared to adult peripheral blood, and cord blood cells exhibit a higher proliferation capacity and express telomerase (Ingram, D. A., et al., 2004). Thus, although similar cell populations can be derived from both blood sources, they are likely to exhibit distinct phenotypic and functional characteristics.

Bone Marrow-derived Cells

Bone marrow contains many cell types including HSC and hematopoietic progenitor cells, mesenchymal stem cells (MSC) and stromal cell types, including vascular cells; all of which have been proposed as sources of EPC.

HSC—Soon after the existence of peripheral blood EPC was reported, the presence of similar cells within bone marrow was suggested. Transplantation of Flk1- or Tie-2-LacZ bone marrow cells into lethally irradiated, genetically matched (wild type) recipient mice resulted in donor-derived LacZ-positive vascular endothelial cells (Asahara, T., et al., 1999). Subsequent experiments demonstrated that highly enriched, genetically tagged hematopoietic stem and progenitor populations contributed to various extents to neovascularization after transplantation into lethally irradiated recipient mice (Crosby, J.R. et al., 2000; Jackson, K.A., et al., 2001; Llevadot, J., et al., 2001; Lyden, D., et al., 2001; Murayama, T., et al., 2002; Grant, M.B., et al., 2002; Garcia-Barros, M., et al., 2003). Single HSC transplantation has also revealed that they can give rise to both blood cells and vascular endothelial cells (Bailey, A. S. et al., 2004; Grant, M. B. et al., 2002; Pelosi, E. et al., 2002). However, other studies suggested purified HSC, isolated from Flk1-LacZ or Tie1-LacZ mice and transplanted into lethally irradiated wild type mice, do not result in donor-derived endothelial cells in the recipients (Purhonen, S., et al., 2008). Therefore, whether HSC, per se, give rise to endothelial progenitor/precursor cells, and mature endothelial cells, remains controversial.

Myeloid Progenitor Cells—Whether specific hematopoietic progenitors can give rise to endothelial cells, or progenitors thereof, is also being investigated. Monocytes are thought to exhibit plasticity and have the potential to differentiate into specialized cell types including macrophages and dendritic cells (Giavazzi, R. et al., 1993; Shima, M., et al. 1995; Austin, J. M., et al., 1998). Freshly isolated CD14⁺ myeloid cells share common markers with human umbilical vein and microvascular endothelial cells, such as CD36 (Trezzi, C. et al., 1990), CD68 (Strobl, H. et al., 1995), CD54 (Icam-1) (Ocklind, G. et al., 1992) and CD31 (Watt, S. M. et al., 1995); expression of CD45 is used to distinguish monocytes (CD45⁺) from endothelial cell types (CD45[−]). However, in the presence of angiogenic growth factors, human CD14⁺ mononuclear cells have been shown to exhibit endothelial-like properties, including expression of vWF, VE-cadherin, CD105, CD36 (thrombospondin receptor), FLT-1 (VEGF receptor-1), and to a lesser extent, KDR (VEGF receptor-2) (Fernandez, P. B. et al., 2000). In addition, CD14⁺/CD34[−] monocytes have been shown to integrate into the endothelium in vivo (Schmeisser, A. et al., 2001; Harraz, M. et al., 2001; Krenning, G., et al., 2007). Ex vivo expanded CD14⁺ cells also incorporate into newly formed blood vessels in vivo and appear to improve neovascularization of injured tissues (Urbich, C., et al., 2003). These data suggest that bone marrow- and blood-derived CD14⁺ cells have properties shared by, or can give rise to, endothelial cells; however, CD14 is not uniformly expressed on monocytes. A subset of monocytes dimly expresses CD14, but highly express CD16 (Clanchy, F. I. et al., 2006); thus, additional experiments are necessary to determine whether any subset(s) of CD14⁺ cells exhibit, or can be induced to take on, endothelial cell characteristics, and to what extent they function as endothelial cells within vessel lumens in vivo.

In other studies to investigate the vascular potential of HSC-derived progenitor populations, common myeloid progenitors (CMP) and granulocyte/macrophage progenitors (GMP) were transplanted into recipient mice (Bailey, A. S. et al., 2006). The progenitors were found to localize within the endothelium and express CD31, von Willebrand factor, and Tie2, but not the hematopoietic markers CD45 and F4/80 or the mural cell markers desmin and smooth muscle alpha actin. Since myeloid cells can function as fusion partners in hepatocyte repair

(Vassilopoulos, G. et al., 2003; Willenbring, H. et al., 2004), lineage-tracing analysis was performed to rule out that these cells fuse with endothelial cells. However, other in vivo studies have suggested that monocyte-like cells secrete cytokines that promote vessel formation (Rehman, J. et al., 2003; Grunewald, M., et al., 2006). Whether there are multiple mechanisms by which myeloid lineage cells can contribute to neovascularization is still unclear, as is whether the observed low levels of their incorporation into blood vessels (1.3% donor-derived CMP and 0.8% donor-derived GMP) is physiologically significant.

Mesenchymal Stem Cells—Mesenchymal stem cells (MSC) are multi-potent cells present in bone marrow, as well as other adult somatic tissues, and have been shown to differentiate in vitro and in vivo into multiple cell lineages including bone, cartilage, adipose, tendon, ligament or even muscle (Caplan, A.I. 1991; García-Castro, J., et al., 2008; De Matteis, R., et al., 2009; Gimble, J. M., et al. 2009; Lu, F., et al. 2009; Quirici, N., et al., 2009; Jukes, J. M., et al., 2009; Fan, J., et al., 2009). Bone marrow MSC are usually isolated by adherent-selection from mononuclear cells; non-adherent cells are discarded and adherent cells are cultured as MSC. MSC, in general, are known to express CD105, CD73, CD90, CD166 and CD44, but do not express endothelial-specific markers. However, in VEGF-containing medium, cells from within this heterogeneous adherent fraction have been shown to exhibit endothelial-like properties in vitro and improve neovascularization in vivo (Khaldi, A., et al., 2003; Oswald, J., et al., 2004). Similarly, adherent bone marrow mononuclear cells depleted of CD45+ and glycophorin A+ blood cells, termed multipotent adult progenitor cells (MAPC) are also reported to exhibit endothelial properties in vitro and contribute to vessel formation in vivo during tumor angiogenesis and wound healing (Reyes, M., et al. 2002). The marrow-derived MAPC have been further defined as CD34−, CD44−, CD45−, ckit−, MHC classI−, MHC classII−, Flk1-low, Sca1-low, CD13+ and SSEA1+ cells (Jiang, Y., et al. 2002; Check, E., 2007); however, all MSC populations appear to be heterogeneous and lack unique surface antigens that could be used for positive selection. Therefore, the exact phenotype of vascular progenitor/precursor cells within these adherent and expanded fractions of bone marrow MSC are not clearly defined.

Blood Vessels

The existence of tissue-resident vascular progenitor/precursor cells has been reported within different adult organs, as well as within blood vessels, specifically, which are common components of almost all tissues and organs (Aarum, J., et al. 2003; Conboy, I. M., et al., 2003). Using multi-channel laser scanning confocal microscopy of whole-mounted tissues to study angiogenesis in chimeric mice, it was revealed that genetically marked syngeneic bone marrow-derived endothelial cells do not significantly contribute to tumor- or cytokine-induced neovascularization. Instead, peri-endothelial cells are consistently detected at sites of tumor- or vascular endothelial growth factor-induced angiogenesis (Rajantie, I., et al., 2004). Single cell clonogenic assays have also revealed that cells derived from the vessel wall can integrate into human endothelial cell monolayers in vitro, can be passaged for many population doublings in vitro, and contain higher telomerase levels compared to mature vascular cell types (Ingram, D.A., et al. 2005). Therefore, progenitor cells that contribute to postnatal neovascularization might reside within the vascular wall.

In fact, tissue resident cells, rather than blood borne cells, have been proposed to be the major source of vascular progenitors during the initial phases of new vessel formation (Khmelevski, E., et al. 2004), although in these studies, surface markers were not used to identify or isolate vessel-derived progenitor cells. However, Zengin and coworkers (Zengin, E., et al. 2006) subsequently suggested that CD34+Flk1+Tie2+CD31− cells reside within a 'vasculogenic zone' of the walls of adult human blood vessels. CD34+ cells can also be

isolated from the walls of human internal thoracic arteries, and form capillary sprouts *ex vivo*, and contribute to tumor vascularization.

Other recent studies showed that cells isolated from human saphenous vein and mammary arteries could be expanded *in vitro*, express high levels of CD133 and CD144, and could be induced to exhibit endothelial cell properties (Ranjan, A.K., et al., 2009). However, the procedure used to isolate these cells was a common protocol for endothelial cell isolation and propagation, and markers such as CD133 and CD144 can be expressed by both endothelial cells and progenitors thereof. Therefore the phenotype of cells within blood vessels that can, indeed, function as progenitors needs to be more carefully characterized.

Adipose Stroma-vascular Fraction

Human adipose tissue is also reported to contain multi-potent cells that can differentiate to various lineages, including vascular cell types (Zuk, P.A., et al. 2001). However, the stroma-vascular fraction (SVF) of adipose that is proposed to contain such potential is heterogeneous, containing adipocytes, as well as microvascular endothelial cells and pericytes (Hutley, L.J., et al. 2002; Frye, C.A., et al., 2002). Attempts have been made to characterize SVF cells, and they have been shown to express markers such as CD34, CD13, CD45, CD14 and CD144, form vascular-like structures in Matrigel, and enhance neovascularization in ischemic tissues (Lin. Y., et al. 2000); however, the phenotype of the cells that contribute to vessel formation, specifically, was not defined. Other groups have shown that it is the CD34+ fraction of SVF cells that can incorporate into the vasculature and improve blood flow in the hindlimb ischemia model (Miranville, A., et al., 2004). However, CD34 is also expressed by many cell types within the hematopoietic lineage (Cortés, F., et al., 1999); moreover, the presence of HSC in adipose SVF has been suggested (Cousin, B., et al., 2003). Therefore, whether the cells within adipose SVF are hematopoietic or vascular in nature remains to be determined.

Hematopoietic vs. Vascular Origin of EPC: A Circular Argument?

Although we have discussed vascular and hematopoietic sources of EPC as separate entities, based on existing literature, it is entirely possible that some, or all, of the identified cell populations are interrelated in a continuing cycle of: circulation, tissue deposition, mobilization, etc. If so, then the somatic tissues and blood vessel structures from which distinct populations have been derived, including bone marrow, may merely be a stop along the way. Cells can be deposited within tissues and blood vessels via systemic circulation, and their phenotype slightly adjusted to meet the demands/needs of the specific microenvironment in which they currently reside.

This theory, although not proven, is supported by previous studies conducted to understand the relationship between bone marrow- and muscle-derived side population (SP) cells (Majka, S.M et al., JCI 2003). In these studies, bone marrow SP cells from genetically marked (LacZ+) donor mice were transplanted into lethally irradiated, genetically matched wild type recipients. Muscle-derived SP cells were then isolated from the transplanted mice over a time course and found to be LacZ+, and the proportion of which was donor-derived increased over the life of the recipients. Furthermore, the phenotype of the SP cells within muscle was slightly different from the transplanted bone marrow SP cells from which they were derived and, in response to injury, contributed to muscle neovascularization. These data suggested that vascular progenitor cells residing within muscle tissue are derived from bone marrow cells via blood circulation, and this cellular compartment is continuously turned over throughout postnatal life. Similar paradigms have been suggested for marrow-derived mesenchymal progenitors (i.e. fibrocytes) that express both blood cell and fibroblast markers, and are thought to traffic in the body, serving as a ready source of fibroblasts and

myofibroblasts for physiologic and pathologic tissue remodeling and repair (Strieter et al., 2009). HSC are also thought to traffic through the body, as such, and reside in extramedullary niches (Schulz, C. et al., 2009).

Perhaps the same is true for vascular progenitor populations, in general. Perhaps such cells circulate all over the body through peripheral blood, take up residence within somatic tissues, or blood vessels therein, and are either stored and turned over, or mobilized in response to injury to function as vascular progenitor/precursor cells. It is equally plausible that distinct cell populations, with various degrees of vascular potential, arise independently within different tissue sites and are not biologically related. Neither theory has yet to be definitely proven.

Summary

In summary, EPC were identified more than a decade ago, and although there has been a huge amount of research in this field, basic questions still remain regarding the precise phenotypic and functional characterization of these cells, as well as the interrelationships among various identified populations. Evidence suggested that EPC may have multiple origins; however, lack of specific cell surface markers or cellular “signatures” has made it difficult to carefully track their origin(s) and fate(s). Thus, we need further define the phenotype of cells within adult tissues, including blood and blood vessels, and develop the appropriate in vivo tools for lineage tracking and functional characterization. Addressing such basic questions would enable us to better elucidate the underlying mechanism(s) of their mobilization, differentiation, and contribution to neovascularization in health and disease. These insights could then be applied to the optimization of their clinical utilization.

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