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Resident vascular progenitor cells - diverse origins, phenotype and function

Peter J. Psaltis, M.D. Ph.D, Adriana Harbuzariu, M.D., Sinny Delacroix, M.D., Eric W. Holroyd, M.D., and Robert D. Simari, M.D.

Division of Cardiovascular Diseases, Mayo Clinic, Rochester, MN

Abstract

The fundamental contributions that blood vessels make toward organogenesis and tissue homeostasis are reflected by the considerable ramifications that loss of vascular wall integrity has on pre- and postnatal health. During both neovascularization and vessel wall remodeling after insult, the dynamic nature of vascular cell growth and replacement vitiates traditional impressions that blood vessels contain predominantly mature, terminally-differentiated cell populations. Recent discoveries have verified the presence of diverse stem/progenitor cells for both vascular and non-vascular progeny within the mural layers of the vasculature. During embryogenesis, this encompasses the emergence of definitive hematopoietic stem cells and multipotent mesoangioblasts from the developing dorsal aorta. Ancestral cells have also been identified and isolated from mature, adult blood vessels, showing variable capacity for endothelial, smooth muscle, hematopoietic and mesenchymal differentiation. At present, the characterization of these different vascular wall progenitors remains somewhat rudimentary, but there is evidence for their constitutive residence within organized compartments in the vessel wall, most compellingly in the tunica adventitia. This review overviews the spectrum of resident stem/progenitor cells that have been documented in macro- and micro-vessels during developmental and adult life and considers the implications for a local, vascular wall stem cell niche(s) in the pathogenesis and treatment of cardiovascular and other diseases.

Keywords

Angiogenesis; vascular progenitor cells; endothelial progenitor cells; hematopoietic stem cells; mesenchymal stem cells; smooth muscle progenitor cells

Introduction

The development of the vasculature and its subsequent homeostasis are integral components of embryogenesis, fetal organogenesis and the maintenance of health and normal function of adult tissues. In embryonic life, the emergence of ancestral stem/progenitor cells for both endothelial cells (ECs) and hematopoietic cells gives rise to the formation of blood vessels, which are then stabilized by the incorporation of periendothelial cells and stromal elements [1]. New blood vessel formation (neovascularization) also continues in adults and is a key contributor to both physiological and pathological processes, including tissue ischemia, repair and regeneration, atherosclerosis, tumor growth and metastasis [2].

Corresponding Author: Robert D. Simari, M.D., Division of Cardiovascular Diseases, Mayo Clinic, 200 First St SW, Rochester, MN 55905. Phone: 507-284-3727; Fax: 507-538-6418; simari.robert@mayo.edu.

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The postnatal vascular wall comprises three concentric layers consisting of a limited number of cell types. The tunica intima has an EC lining which interfaces with blood, the media contains several layers of smooth muscle cells (SMCs) and the adventitia is made up of stromal and adipose tissue, along with the vasa and nerva vasorum. Disruption of vessel wall integrity is associated with the pathogenesis of various disease processes, including atherosclerosis, aneurysm formation, vasculitis, allograft vasculopathy and post-intervention restenosis, which in turn may occlude tissue blood supply, leading to ischemia or infarction.

Up until the last decade, it was widely believed that the cellular elements of the adult vessel wall are terminally differentiated and thus relatively quiescent. Angiogenesis, defined as the formation of new blood vessels from pre-existing ECs, was considered to be the only mechanism by which neovascularization occurred after birth [2]. Similarly, paradigms of atherosclerosis emphasized the involvement of mature cell types with respect to EC turnover, inflammatory cell recruitment and migration of SMCs from the media to the neointima [3]. In recent times, these traditional views of vascular biology have been revised by the discovery of immature stem/progenitor cell populations, relevant to endothelial [4,5], smooth muscle [6-8], myeloid [9] and multipotent mesenchymal lineage [10] that have been shown to participate in postnatal vasculogenesis and vascular wall remodeling. An increasing body of evidence points to the existence of these different progenitor cell types within embryonic, fetal and adult vessel walls, where they may reside either constitutively or appear as a result of circulating migration.

This review will discuss current knowledge concerning vascular wall-resident stem/progenitor cells (VW-PCs) identified in pre- and postnatal life. Focus will be given to the identities, origins, and biological significance of these diverse cell populations, the preliminary evidence for their residence within specialized vascular wall niches, and the important implications that they may have for the pathogenesis and treatment of cardiovascular disease.

Developmental beginnings of the vessel wall as a stem cell niche

The vessel wall as a source of hematopoiesis

During embryogenesis, the earliest signs of *de novo* blood vessel formation (vasculogenesis) begin soon after gastrulation, with the migration of progenitor cells from the lateral and posterior mesoderm toward the extra-embryonic yolk sac. Here these mesodermal cells aggregate to form small clusters called “blood islands”. These blood islands are foci of bipotent cells that consist of a loose inner mass of primitive hematopoietic precursors and an outer luminal layer that gives rise to endothelial precursors (angioblasts) [1,11,12] (Fig. 1A). From the growth and patterned assembly of these angioblasts, there is coalescence and remodeling of blood islands into a functional vascular plexus that establishes the vitelline circulation.

Extraembryonic blood vessels communicate with the developing fetal circulation via the vitelline vein but do not otherwise contribute to the subsequent process of intraembryonic vasculogenesis. The latter proceeds with the establishment and migration of rudimentary angioblast strands from different regions of mesoderm, beginning with the development of the endocardium, great vessels and soon after, dorsal aorta [13]. Several signaling factors provide crucial inductive cues for hematovascular differentiation, including members of the fibroblast growth factor (FGF) and bone morphogenetic protein (BMP) families, vascular endothelial growth factor (VEGF) and its receptors VEGFR 2 (Flk1/KDR1) and VEGFR1 (Flt1) [13,14].

The primitive vascular and hematopoietic systems remain closely intertwined during intraembryonic development. Studies in zebra-fish [15,16], avian [17], amphibian [18] and mammalian species [19-22], including humans [1], reveal a conserved origin for definitive hematopoiesis within the para-aortic splanchnopleura and subsequent aorta-gonad-mesonephros (AGM) region, which comprises the dorsal aorta and surrounding mesenchyme. The ventral floor of the dorsal aorta has been specified as the primary source of hematopoietic stem cells (HSCs), although there has been contention as to whether these cells arise from the aortic endothelium or the surrounding mesenchyme [23].

Recent studies have gone a long way to resolving this ambiguity. Genetic tracing and lineage mapping, coupled with high resolution imaging, have verified that definitive HSCs directly emanate from endothelium [15,16,20,22], while the AGM mesenchyme does not seem capable of providing hematopoietic progeny [20]. The emergence of HSCs may occur through a *Runx1*-dependent process called endothelial hematopoietic transition, whereby ECs transform phenotype while undergoing abluminal bending from the aortic floor into the sub-aortic space [16] (Fig. 1B). Although the hematopoietic potential of “hemogenic endothelium” is only transient, its progeny enters the circulation and results in robust, multilineage seeding of intermediate and adult hematopoietic organs [15], in which HSCs continue to occupy perivascular niches [24].

Hemangioblasts

The intimate anatomical and temporal relationship of hematopoietic and endothelial cells during developmental life suggests that they may share a bipotent mesodermal ancestor, which has been called the hemangioblast (Fig. 2). Single cell hemangioblast potential has been demonstrated during embryogenesis in zebra-fish [25] and mice [26] and in clonal studies using murine and human embryonic stem cells (ESCs) [27,28]. Several genes and surface markers have been implicated in the regulation and differentiation of ESC-derived hemangioblasts: *Runx-1* [29], stem cell leukemia gene (*SCL*) [30], *Flk-1*, mesodermal gene *T* (brachyury) [31] and angiotensin converting enzyme (*ACE*, CD143) [32]. Although the presence of hemangioblasts remains unproven in adult life, indirect evidence can be inferred from transplantation studies showing the endothelial potential of adult HSCs (Lin⁻c-Kit⁺sca-1⁺) [33] and from the common expression of various markers and genes by endothelial and hematopoietic progenitors. Recently, hemangioblast bipotency has also been described for Flk-1⁺ progenitors derived from murine induced pluripotent stem cells [34].

Mesoangioblasts

Intriguingly, the embryonic dorsal aorta is also responsible for the emergence of a distinct population of primitive cells, called mesoangioblasts [35]. Isolated from its posterior and lateral walls (Fig. 1B), these culture-defined cells possess the capacity for self-renewal and have multipotent plasticity for both endothelial and diverse mesodermal lineages (e.g. skeletal muscle, dermis, bone, cartilage) [36]. During early and late passage culture, clonally-derived mesoangioblasts express hemangioblast markers (CD34, Flk1 and c-Kit) but unlike hemogenic ECs, do not display hematopoietic capacity. It has been hypothesized that these cells may accompany newly branching blood vessels during organogenesis, allowing them to adopt specific properties in response to the local tissue milieu. Although their presence has not been verified in adult organisms, they may be homologous with postnatal multipotent pericytes.

Smooth muscle progenitors

Historical views of SMC ontogeny focused on a part of the yolk sac mesoderm that was associated with differentiating endothelium. However, lineage mapping and specific gene targeting studies have highlighted that the embryological origins of SMCs are diverse and

anatomically distinguishable. The SMC content of vessels from the pharyngeal arch (ascending aorta, aortic arch, subclavian, common carotid and pulmonary trunk arteries) can be traced back to progenitor cells that migrate from the neural crest [37,38], coronary arterial SMCs are derived from cells in the epicardial mesenchyme (proepicardium) [39] and SMCs in the descending aorta appear to develop from segmental somites [40].

Vascular wall-resident progenitor cells in postnatal life

The development of new vessels from progenitor cells extends well beyond embryogenesis. While postnatal neovascularization plays a positive role in facilitating tissue repair in ischemic conditions, it is also centrally involved in the establishment and evolution of other diseases, underpinning the growth and spread of many malignancies and the progression and destabilization of atherosclerotic plaques [2]. Throughout adult life, blood vessels are also exposed to genetic and environmental influences that can damage their integrity and ultimately lead to different forms of vasculopathy. Thus there needs to be an ongoing state of cell turnover, growth and repair within the vascular wall.

Since the first descriptions of adult-derived “endothelial progenitor cells (EPCs)” in the late 1990s [4,41], there has been considerable energy directed to the identification and characterization of different subtypes of vascular progenitor cells. Initially, most attention was directed to the pro-vasculogenic properties of these cells and their potential regenerative/repairative value in treating ischemic conditions, such as coronary artery disease [42-44]. However, more recently an equally important emphasis has been placed on their involvement in maintaining normal vessel physiology and in contributing to pathological vascular remodeling. Although most of our understanding of vascular progenitor cells relates to those isolated from bone marrow (BM) or peripheral blood (PB), important new information is also emerging for the existence of VW-PCs in both the macro- and micro-vasculature.

Progenitor cells of endothelial lineage

The capacity for ECs to undergo replication has been known for over thirty years. Although early animal studies indicated a declining rate of EC mitosis after birth, they also showed that the rate of EC turnover can be stimulated to increase [45] and that dividing ECs are not distributed uniformly throughout the vascular system [46]. Two decades after these findings, a new era of research was born with the demonstration that progenitor cells isolated from adult PB [4] and BM [41] can differentiate into ECs and incorporate into sites of neovascularization and neoendothelialization. Many subsequent studies have verified the contribution of adult progenitor cells to endothelium in neovessels at sites of tumor formation [47], wound healing [48], peripheral [49] and myocardial ischemia [42], as well as in regions of endothelial denudation after vascular injury [50].

In the strictest sense, “EPCs” should display clonogenicity, self-renewal, high proliferative capacity and the ability to adhere to extracellular matrix molecules and to differentiate into functional, mature ECs [51]. Over the last decade, different investigators have applied a variety of isolation techniques to prepare different subsets of “EPCs” from the mononuclear cell fraction of PB and BM. These include strategies based on (1) EC outgrowth during *ex vivo* culture [52], (2) colony formation [53,54] and (3) immunoselection, typically for a panel of two or more cell surface markers, comprising CD34, VEGFR2 or CD133 [4,55], occasionally with the depletion of CD45⁺ hematopoietic cells [56]. However, this lack of methodological uniformity has resulted in some persistent controversies in this field.

The traditional surface antigens used to define “EPCs” all lack specificity, due to their shared expression by hematopoietic and endothelial cells. The initial notion that

CD34⁺VEGFR2⁺ progenitors possess pro-angiogenic, endothelial differentiation capacity [4] has since been challenged by evidence that these cells are not true EPCs but rather progenitors of hematopoietic lineage [57,58]. This ambiguity regarding the identity of “EPCs” is also emphasized by the fact that culture-based isolation results in two distinct cell populations, discernable by their temporal pattern of outgrowth [51,52].

The early colony-forming progeny of mononuclear cell culture actually consists of hematopoietic-derived cells with limited proliferative capacity and endothelial plasticity [52,59], that mediate their angiogenic actions largely by indirect, trophic mechanisms [60]. Variably referred to as endothelial colony forming units (-Hill), endothelial-like cells or circulating angiogenic cells, these cells have often been used by investigators describing the application of “EPC” number for cardiovascular risk assessment [53,61]. In contrast, late outgrowth endothelial cells (OECs), also called endothelial colony forming cells (ECFCs), do not express hematopoietic lineage markers and have more robust proliferative and vessel-forming capacity, making these cells more consistent with a *bone fide* EPC phenotype [52,58]. Therefore BM and circulating blood contain diverse subtypes of endothelial and hematopoietic cells, that have been confusingly bracketed as “EPCs”, although these cell populations may play complementary roles in neovessel formation and endothelial repair [49].

Amidst the controversy surrounding the identities of circulating “EPCs”, evidence has come to light revealing that these diverse cell populations are not restricted to a BM origin but also arise from other tissue sources. In a murine model of chimeric TIE2-LacZ BM and vessel graft atherosclerosis, Hu *et al.* reported that the reconstitution of luminal endothelium in allografts was formed from recipient progenitor cells and that a majority of these (70%) were not from BM [62]. Other groups have also described negligible or limited contributions by BM to vascular EC turnover in a mouse model of chronic endothelial dysfunction [63], a rodent study of transplant arteriopathy [64] and an endothelial-specific, inducible transgenic model of murine tumorigenesis [65]. Progenitor cells with endothelial and angiogenic potential have also been obtained from a range of non-BM adult tissues, including skeletal muscle [66], adipose [67], spleen [50], liver and intestine [68] and myocardium [69]. Although the precise niches for endothelial progenitors have not been defined for the majority of these tissues, converging lines of evidence point to their likely residence within interstitial or parenchymal blood vessels [9,67,69].

With respect to moderate and large caliber blood vessels, endothelial progenitors have been isolated from the intimal endothelial layer [5] and the inner lining of the adventitia, just external to the tunica media [9]. In a landmark study, Ingram *et al.* demonstrated that human umbilical vein endothelial cells (HUVECs) and human aortic endothelial cells (HAECs) could be passaged extensively for at least 40 population doublings, rejecting the notion that postnatal ECs are terminally differentiated [5]. Definitive single cell colony forming assays further showed that HUVECs and HAECs had comparable rates of clonogenicity when compared to umbilical cord blood-derived EPCs, and contained a mixture of low and high-proliferative potential-ECFCs, along with cells that could generate secondary EC colonies. Together, these data revealed the likely presence of a complete hierarchy of EPCs derived from postnatal vessels.

Ex vivo ring assays of human embryonic [70], fetal [71] and adult [9] arteries have also shown the sprouting of capillary-like structures, containing mature ECs, from the outer layer of the vessel wall. In one of these studies, immunostaining of internal thoracic arteries and vessels from various organs (bladder, testis, kidney, lung, heart, liver, brain) revealed the presence of CD34⁺CD31⁻ cells localized to a “vasculogenic zone” between the smooth muscle and adventitial layers [9]. In addition to forming capillary outgrowths, these

mural progenitor cells also were seen to migrate through the vessel wall, producing intraluminal vascular sprouts after prolonged culture.

Recently, clusters of c-Kit⁺/VEGFR2⁺ progenitor cells have been identified throughout all three mural layers of human epicardial coronary vessels, where they share connections via connexin-43 and N-cadherin with ECs, SMCs and fibroblasts [69]. These cells were negative for hematopoietic markers (CD45, tryptase) and displayed clonogenic multipotency towards ECs, SMCs and to a lesser extent cardiomyocytes. Transcriptional profiling demonstrated similarities in the molecular signatures of vascular progenitors and c-Kit⁺/VEGFR2⁺ myocyte progenitors, except for genes involved in EC, SMC and cardiomyocyte lineage commitment. Impressively, the functionality of these c-Kit⁺/VEGFR2⁺ cells was confirmed by showing that their transplantation resulted in *de novo* formation of arteries, arterioles and capillaries in a canine model of critical coronary stenosis.

Smooth muscle progenitor cells

The synchronized contractile activity of the medial smooth muscle layer helps to regulate normal vascular tone and blood flow, while also providing mechanical stability for newly formed adult vessels. Neointimal accumulation of synthetic SMCs is central to the pathogenesis of various types of arteriosclerosis. The traditional paradigm of neointimal formation hypothesized that in response to vascular insult, mature SMCs undergo various degrees of dedifferentiation, transforming from a quiescent, contractile state into a proliferative, synthetic phenotype and migrating from the media to the subendothelial intima [3]. In recent years this “dedifferentiation concept” has been significantly challenged by data that indicate a wide range of ancestral cell candidates for adult SMCs. These include BM and circulating smooth muscle progenitors [6,72,73], medial and adventitial VW-PCs [7,74], peri-adventitial myofibroblasts [75] and mature vascular ECs that can undergo endothelial-mesenchymal transdifferentiation [76].

Small animal studies of vascular transplant and graft arteriopathy have typically shown that the vast majority of neointimal SMCs arise from host cells [64,72,77,78]. Using various models of vascular disease in mice with chimeric GFP or LacZ-labeled BM, Sata *et al.* observed that BM-derived cells comprised a substantial proportion of SMCs (up to 82% in the neointima) in diseased arteries [72]. Concordant evidence for the existence of BM-derived smooth muscle progenitor cells (SPCs) has also been provided in human subjects, in whom neointimal SMCs of donor origin were enriched in atherosclerotic coronary arteries compared to healthy vessels, following sex-mismatched BM transplantation [79]. Recently, CX₃CR1⁺ BM SPCs have been specifically implicated in neointimal remodeling [73], while mononuclear cells with SMC plasticity have also been isolated from the peripheral circulation [6,80] and may be characterized by expression of CD14 and CD105 [81].

Despite these observations, other investigators have been unable to reproduce the finding that BM-derived SMCs participate in arteriosclerosis [64,78,82,83]. This has inspired the notion that SPCs may reside in other niches, including within the mural layers of blood vessels. Major contributions to our current knowledge of vascular-resident SPCs have been provided by Xu and colleagues, who first identified the abundant expression of various progenitor markers (Sca-1 ~21%, c-Kit ~9%, CD34 ~15%) in the adventitia of the proximal aorta in *ApoE*^{-/-} mice [7]. Using lineage tracing, these investigators showed that Sca-1⁺ adventitial cells were not of hematopoietic origin and therefore were likely to be constitutively resident within the vessel wall. Sca-1⁺ cells could be differentiated into SMCs and ECs *in vitro* and after external application to irradiated vein grafts were shown to migrate into the neointima, where they made up 30% of cells after 4 weeks and acquired expression of the SMC marker, SM22. Furthermore, the migratory capacity of these progenitor cells was much greater than that of Sca-1⁻ adventitial fibroblasts, whose

contribution to neointimal growth has been under some conjecture [75,84]. Other groups have confirmed the presence of progenitor cell markers within the inner adventitial lining of human arteries [9,85,86] and these appear to be enriched in the setting of atherosclerosis [87].

In another insightful study, Passman *et al.* reported that a sonic hedgehog (Shh) signaling domain is restricted to the adventitial layer of murine arteries during embryonal and postnatal life, where it supports the maintenance of resident Sca-1⁺ cells that have SMC, EC and osteogenic plasticity [8]. These vascular Sca-1⁺ progenitors first appeared in the perivascular space between the ascending aorta and pulmonary trunk during prenatal development, after the complete development of the tunica media, and were shown not to originate from neural crest-derived SMCs.

A distinct population of mural progenitor cells has also been isolated from the medial layer of healthy arteries in adult mice [74]. Selected by their ability to extrude Hoechst-dye, these “side population” cells displayed a Sca-1⁺c-Kit^{-/lo}lin⁻CD34^{-/lo} profile and comprised 6-15% of cells from the tunica media but were not isolated from the adventitia. Although they possessed no capacity for hematopoiesis, these cells were bipotent for EC and SMC lineages and formed vascular-like cords containing both cell types, when cultured on matrigel.

Collectively, these data build a convincing case for the different progenitor origins of SMCs in adult life and the existence of ancestral SPC populations within the vascular wall. The diverse sources of SMCs both pre- and postnatally may account for the phenotypic and functional heterogeneity that they display in healthy and diseased blood vessels [88]. As is the case for endothelial progenitors, substantial work remains to determine the extent to which vessel-resident SPCs participate in local vascular remodeling and contribute to a systemic, circulating progenitor population.

Pericytes and Mesenchymal stromal/stem cells

At a strictly etymologic level, the term “pericyte” refers to the ubiquitous periendothelial cells that surround capillaries and microvessels. These contractile cells are closely juxtaposed to their overlying ECs, with which they share a basement membrane and are connected by elongated processes and intercellular junctions [89]. They play crucial roles during angiogenesis and in the established microvasculature, helping to pattern vascular networks, modulate EC growth and differentiation, regulate vessel tone, caliber and permeability and provide mechanical stability through their physical interactions with ECs and by synthesizing basement membrane proteins. Despite having some consistent characteristics, pericytes are morphologically and functionally heterogeneous and have been traced to diverse origins including both neuroectodermal and mesodermal tissue [89].

A solid body of evidence has now highlighted convincing similarities between pericytes and multipotent MSCs. These non-hematopoietic cells have been shown to occupy niches predominantly localized to perivascular and sinusoidal sites in a wide range of postnatal tissues, most notably in BM, but also in adipose, skeletal muscle, heart, lung, dental pulp, periodontal ligament, placenta and umbilical cord [90]. *In situ* pericytes and MSCs both express common cell markers in their native state, such as CD44, CD73, CD90, CD105, CD146, stromal precursor antigen-1 (STRO-1), platelet-derived growth factor receptor β (PDGF-R β), the neural glial antigen NG2, alkaline phosphatase and alpha-smooth muscle actin (α -SMA), at the exclusion of hematopoietic, endothelial and myogenic markers [91-96].

During *ex vivo* culture, pericytes from different fetal and adult sources also display an MSC-like phenotype, with respect to morphology, surface antigen profile, proliferative kinetics

and clonal capacity for self-renewal and trilineage differentiation for bone, fat and cartilage [95]. They may also possess striking plasticity and regenerative properties specific to their host tissue type, as demonstrated elegantly for skeletal muscle [92,97] and nervous tissue [98]. Finally, both pericytes and MSCs share a common propensity to provide mechanical and paracrine support of other cell types [86,99,100], underpinning their pro-angiogenic potential and their respective importance in maintaining endothelial and hematopoietic homeostasis.

Overall, the data compellingly point to a common ancestor of MSCs that is natively associated with the vascular wall and more specifically belongs to a subset of perivascular cells [95,101]. Although it remains unclear what proportion of pericytes actually contains progenitor cell potential, the omnipresence of these cells may explain the pan-organ distribution of multilineage progenitor cells. Subtle variations in tissue-specific plasticity of MSCs may reflect a degree of lineage allegiance that ancestral perivascular cells have acquired to their host tissue. Although unproven, this may be an extension into adult life of the emergence of mesoangioblasts from vascular sites into developing organs during embryogenesis.

In addition to their microvascular niches, pericyte-like cells have also been identified within each of the mural layers of large, medium and small arteries and veins, including in the adventitia where they encircle the vasa vasorum [102]. It is therefore not surprising that MSC-like cells have been isolated from postnatal rat aorta [103], the tunica media of bovine arteries [10] and the tunica adventitia of human saphenous veins [86,104], pulmonary [105] and thoracic arteries [85,106], although in some cases these cells have displayed an atypical antigenic profile (CD34⁺CD31⁻CD146⁻) [85,86]. Vessel-resident MSCs have been passaged to high yield and display similar characteristics with MSCs from more conventional sources. These include the potential for vascular and non-vascular cell differentiation [85,104], the capacity to support hematopoietic colonies [10] and the trophic mediation of angiogenesis [86].

These cells appear well-placed to serve as another progenitor source of vascular ECs and SMCs and to participate in local vasculogenesis, such as in the emergence and expansion of the vasa vasorum and the neovascularization of atheroma. It is also speculated that in response to various stimuli (e.g. mechanical stress, glucose, reactive oxygen species, TNF- α) and BMP2-Wnt signalling, adventitial and perivascular MSCs may undergo osteogenic, chondrogenic or adipogenic transformation. This may account for the ancestry of macrovascular myofibroblasts and calcifying vascular cells (CVCs), which have been charged with the pathogenesis of calcific vascular and valvular disease [107].

Hematopoietic progenitor/stem cells

The striking connections established between vascular and hematopoietic systems *in utero* pose the possibility that the vascular wall may also contain hematopoietic progenitors after birth. Cells of hematopoietic origin participate in both ischemic and tumor neovascularization and during the pathogenesis of vascular disease. Macrophages are involved in the initial proliferative phases of angiogenesis [108] and during the development of atherosclerosis, with the accumulation of foam cells in atheromatous plaque. Lymphocytes, mast cells, platelets and polymorphonuclear leukocytes are also heavily implicated in the inflammatory cascades which lead to atheroma, plaque destabilization and rupture [109].

Traditional paradigms have focused on the recruitment of these cells from the peripheral circulation to blood vessels [110], however, occasional studies have also pointed to the participation of local, rather than BM-derived, hematopoietic precursors in vascular

remodeling. In rodent studies of hindlimb ischemia, depletion of circulating monocyte numbers by cyclophosphamide has not been found to affect the accumulation of macrophages in collateralizing vessels, indicating the activation of tissue resident macrophages or their vessel-resident precursors [9,111]. The latter cells have also been inferred from *ex vivo* ring assays of human arterial specimens, in which the content of CD68⁺ macrophages increases markedly during culture, starting in the “vasculogenic zone” of the adventitia before extending to the entire adventitial layer and newly formed vascular sprouts [9]. Furthermore, CD45⁺ cells within the vessel wall have consistently been localized to the inner adventitia, sharing this region with other cells that express hematopoietic progenitor markers (c-Kit, CD34, Sca-1) [7,9,85].

These observations prompt consideration of a new hypothesis for inflammation in vascular disease proposing a role for local hematopoietic stem/progenitor cells, possibly resident within a vascular adventitial niche. The existence of such progenitors could explain the local emergence of adventitial macrophages and mast cells, both of which are implicated in the progression of vascular disease [112,113] and the intriguing phenomenon of extramedullary hematopoiesis, whereby BM elements form within advanced vascular lesions (Fig. 3). However, it must be emphasized that current evidence for the existence of vascular wall HSCs is largely circumstantial and more rudimentary than for the other VW-PC subtypes described above. Future studies are challenged to confirm the multilineage hematopoietic potential of VW-PCs by performing definitive transplantation experiments, while lineage tracing strategies should also help to clarify whether these cells are constitutively resident in the vascular wall or the by-product of trafficking from BM [114].

Vessel Wall as a Stem Cell Niche

The existence of stem cells within compartmentalized, highly organized microenvironments has been best illustrated for HSCs within their endosteal and vascular niches in BM [115]. Fundamentally, niches are anatomically well-defined and consist of an ancestral hierarchy of stem, progenitor and precursor cells residing in a milieu that is enriched with supporting cells, specific extracellular matrix components and growth modulating signals that can maintain stem cell survival and regulate their self-renewal and differentiation [116]. Additional molecular processes within these microenvironments modulate the crucial balance of stem cell quiescence/proliferation, adhesion/deadhesion, chemoattraction/chemoretenion and mobilization during the course of steady-state or injury-induced turnover of mature daughter cells.

The perivascular localization of HSCs in BM and MSCs throughout a range of tissues illustrates that the microvasculature is an indispensable component of these respective niches. Moreover, there is now cumulative evidence for the presence of diverse, but interrelated, progenitor cells within the mural layers of macrovessels, providing rationale that specialized niches may also exist within the vascular wall that help maintain vascular integrity and homeostasis.

Although vessel-resident progenitors have been identified in a variety of vascular territories, they do not appear to be uniformly distributed. This is suggested by the clustering of focal areas of rapid EC growth in different regions of the aorta [46], the predilection for adventitial Sca-1⁺ expression to the aortic root [7] and the discrepancy in the frequency of medial side-population cells between carotid arteries and the aorta [74]. Such anatomical heterogeneity may bear relevance to the variable susceptibilities of different vascular segments to atherosclerosis and inflammatory disease that are not solely dependent on environmental factors, such as hemodynamic flow patterns or shear stress [117].

There is also non-uniformity in progenitor cell distribution between the different vessel wall layers (Fig. 4). The greatest body of data currently points to a niche for progenitor/stem cells within the tunica adventitia, specifically along its border with the tunica media. This region is strategically located, especially as its proximity to the vasa vasorum provides an interface with the peripheral circulation, whereby bidirectional signals interconnect the vessel wall with remote stem cell niches, including BM. As corroborated by various groups, the inner adventitial zone is enriched with progenitor markers, and cells with a high level of proliferation and diverse differentiation potential. On the evidence at hand, there is apparent symbiosis between multipotent MSCs, myofibroblasts, and progenitors with endothelial, smooth muscle and hematopoietic potential [7,9,85] and preliminary data indicates that some of these populations constitutively reside in the adventitia, having become established there during prenatal developmental [8].

To date, the immunophenotypic characterization of adventitial progenitor cells has been rather non-specific (Sca-1⁺ [7,8], CD34⁺CD31⁻ [9,85]), making it possible that these are overlapping populations, whose broad spectrum of plasticity may be due to a heterogeneous mixture of lineage-specific precursor cells and genuine multipotent progenitors. The recent isolation of c-Kit⁺/VEGFR2⁺ cells in the human coronary vasculature may have narrowed down a more specific population of clonogenic VW-PCs with bipotent capacity for EC and SMC differentiation [69]. However, it is still undetermined whether adult blood vessels actually contain common ancestral stem cells for both vascular and non-vascular progeny, that would represent a postnatal extension of embryonic hemangioblasts and mesoangioblasts. Further research is also required to delineate hierarchical organizations for the different subpopulations of VW-PCs, as has been so precisely documented for the HSC niche in BM [118].

The mobilization of BM-derived vascular progenitor cells and HSCs into the peripheral circulation and their subsequent homing to different tissues has been extensively studied, with a long list of regulatory cytokines, growth factors and pathophysiological stimuli now identified [119,120]. By comparison, little is known about the local and systemic release of VW-PCs and further characterization of the vascular wall as a stem cell niche will necessitate a more complete understanding of the molecular mechanisms responsible for their activation, differentiation and migration. The CXC chemokine CXCL12 (SDF-1 α) and its receptor CXCR4, are known to be instrumental during embryogenic vascularization and in the postnatal mobilization of HSCs and endothelial progenitors from BM. It is therefore not surprising that CXCL12 expression by cells in the tunica media and adventitia has been strongly implicated in the recruitment of BM-derived SPCs during neointimal hyperplasia [121] and in the perivascular retention of circulating leukocytes [122]. Pending future studies with direct relevance to VW-PCs, it seems very likely that the regulation of these cells will also be under the influence of the CXCL12/CXCR4 axis, along with a host of other chemokine and signaling pathways.

Implications for VW-PCs in Disease Pathogenesis and Prognosis

Although the exact involvement of VW-PCs in vascular wall remodeling and neovascularization is not yet fully understood, useful inferences can be drawn from the vast literature that relates to progenitor cells isolated from BM and PB, especially “EPCs”. Over the past ten years, a wealth of information has become available concerning the physiological and pathophysiological cues responsible for the peripheral mobilization of “EPCs” [123]. Attenuation of circulating levels of “EPCs” and their biological function (e.g. survival, proliferation, migration, pro-angiogenesis) has been ascribed to various cardiovascular risk factors (advanced age, cigarette smoking, diabetes mellitus, hypercholesterolemia) and to chronic disease states, including advanced heart failure, renal

failure, established coronary atherosclerosis (particularly multivessel disease) and endothelial dysfunction [119,123]. Conversely, augmentation of peripheral “EPC” number has been shown to occur after acute myocardial infarction and unstable angina and to accompany various pharmacological (e.g. ACE inhibitors, statins, PPAR γ agonists) and non-pharmacological (exercise, coronary revascularization) interventions [119,123-127]. In some of these situations, nitric oxide (NO) has been implicated as a specific mediator of “EPC” release from BM [128] and this important vaso-active molecule also looms as a likely candidate for the mobilization of VW-PCs into the peripheral circulation.

Several studies have indicated a favorable role for “EPCs” in cardiovascular disease by showing an inverse relationship between circulating “EPC” levels and adverse outcome in patients with stable coronary atherosclerosis [126,129], myocardial infarction [130] and congestive heart failure [131]. In the context of coronary angioplasty and stenting, the early mobilization of angiogenic monocytes and lymphocytes provides paracrine impetus for the proliferation of resident endothelial cells and the recruitment of definitive BM-derived and local endothelial progenitors [132-134]. The robust and rapid involvement of these cells is thought to accelerate intimal re-endothelialization and limit the inflammatory responses, extracellular matrix deposition and neointimal hyperplasia that result in the development of in-stent restenosis. Preclinical studies have confirmed the endothelial benefits of administering angiogenic BM cells and cultured EPCs after vascular insult [50,135]. In the clinical setting this has formed the basis for the development of bio-engineered stents coated with monoclonal antibodies to attract “EPCs” and facilitate endothelial recovery after percutaneous coronary intervention [136].

The influence of progenitor cells on vascular health may vary depending on disease context, such that it cannot be easily categorized as adaptive or maladaptive. In one cautionary study, “EPC” infusion in *ApoE*^{-/-} mice accentuated spontaneous atherosclerotic burden, resulting in plaques with increased lipid cores, thinner fibrous caps and greater inflammatory infiltrates, probably mediated by their pro-inflammatory, proteolytic and pro-angiogenic effects [137]. Smooth muscle progenitors have also displayed paradoxical actions on vessel remodeling. On the one hand they may promote neointimal growth after vascular injury, intervention or grafting [121] and on the other they may help to stabilize plaque in chronic atherosclerotic disease [138].

As described earlier, experimental studies of atherosclerosis, restenosis and transplant arteriopathy have provided conflicting results as to the origins of cells involved in endothelial repair or SMC accumulation in the neointima, especially with respect to the participation of BM-derived cells [62,72,139]. This was elegantly highlighted by a series of murine experiments performed by Tanaka *et al.* in which three different models of vessel injury (wire injury, perivascular cuff and arterial ligation) were accompanied by substantially different contributions of GFP-labeled BM cells to their respective lesions [140]. Although the specific involvement of progenitor cells is more difficult to assess in human patients, it is also likely that progenitor cell compositions differ between clinical atherosclerosis, post-angioplasty restenosis and vein graft intimal hyperplasia [141].

Based on the evidence available for BM-derived and circulating progenitors, VW-PCs can also be expected to have a dual role in the evolution of vascular disease. Beneficial actions of VW-PCs after vascular injury may broadly include physical reconstruction of tissue, paracrine support for growth of endogenous cells and a limiting effect on inflammation. Recent proteomic and metabolomic analysis of adventitial Sca-1⁺ cells has revealed their similarities with mature SMCs [142], hinting that these cells are intrinsically primed to provide direct, rapid replacement of SMCs, as would be required after massive, injury-induced medial apoptosis [143]. Similarly, intimal EPCs appear to be ideal candidates to

initiate re-endothelialization after endothelial denudation [5]. Conversely, detrimental effects of VW-PCs in arteriosclerosis may include neointimal hyperplasia (SPCs) [7], ectopic calcification (MSCs) [10], adventitial inflammation (HPCs) and expansion of the vasa vasorum (EPCs) [9], which itself contributes to plaque vascularization and neointimal hyperplasia [144,145].

There are also contrasting data with respect to the involvement of circulating and BM-derived endothelial cells in tumor vascularization [47,65]. Angiogenic progenitor cells, located within the inner adventitia, have shown the capacity to mobilize preferentially in an abluminal direction following exposure to peri-adventitial cues, including those elicited by cancer cells [9]. This may enable them to be recruited into their surrounding interstitium more rapidly than circulating progenitors, which need to migrate through a luminal EC and basement membrane barrier. In this way, adventitial EPCs could provide an early impetus for neovascularization at sites of ischemia or tumor growth, where their incorporation into newly formed vessels may be supported by mechanical and paracrine interactions with adjacent inflammatory cells and MSCs.

Therapeutic Implications for VW-PCs

As more information becomes available on the physiologic and pathogenic roles of VW-PCs in health and disease, focus is likely to shift toward the possible therapeutic applications of these cells. The cardiovascular reparative properties of MSCs continue to undergo exhaustive investigation [90] and studies have also directly demonstrated the broad potential of tissue-derived pericytes for both mesenchymal [92] and vascular regeneration [146]. These cells are particularly attractive for cellular therapy, given their pan-tissue accessibility, proclivity for culture-based expansion and potential for allogeneic use. Recently, high yields of MSCs with angiogenic potential have been isolated from the mononuclear cell fraction of fresh human thoracic aortas (5cm segments) harvested from multi-organ donors [85]. With respect to endothelial and smooth muscle progenitors, practical difficulties have related to the need for autologous supply and the fact that *ex vivo* expansion requires relatively high volumes of PB or BM, especially if yield is compromised by increased age, chronic disease or cardiovascular illness. An important objective of future studies will be to determine whether these cells can be prepared more efficiently from vascular sources.

By virtue of their location, it is conceivable that VW-PCs are relatively protected from direct exposure to peripheral blood, which may have implications for their responsiveness to pharmacological substances (e.g. anti-angiogenic chemotherapy, statins, ACE inhibitors) and their susceptibility to systemic factors (e.g. reactive oxygen species, advanced glycosylation end-products). Future strategies to treat atherosclerosis, restenosis, allograft arteriopathy and tumor angiogenesis may therefore be advised to target VW-PCs more specifically. An example of this is the developing field of perivascular gene transfer, which aims to direct treatment preferentially to the adventitia as both a reservoir for progenitor cells and an important nidus for neovascularization and inflammation [147].

Conclusion

An impressive array of direct and indirect evidence has converged to uncover the dynamic nature of the vasculature as a source of progenitor cells throughout pre- and postnatal life. Undeniably, the vascular wall plays an integral role during embryogenesis and fetal organogenesis, highlighted by the emergence of definitive HSCs and multipotent mesoangioblasts from the embryonic dorsal aorta. There is also a considerable case to argue for the persistence of endothelial, smooth muscle, hematopoietic and mesenchymal

progenitors in the mural layers of blood vessels after birth and to postulate the involvement of these cells in normal vessel homeostasis, endogenous tissue repair and vascular disease throughout adult life. In particular, a local stem/progenitor cell hypothesis has major implications for the pathogenesis and treatment of both atherosclerosis and solid malignancies, the disease processes that are by far most responsible for morbidity and mortality in Western societies. Future studies are now pressed with many searching questions to further our understanding of the identities and relative importance of the diverse VW-PC populations and to determine whether they actually exist in constitutive niches, analogous to the highly orchestrated microenvironments for HSCs and MSCs in BM. Ultimately it is hoped that the answers to such questions will lay the foundation for the successful manipulation of these vascular resident cells in clinical therapeutics.

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Fig 1. Vascular origins of stem cells during embryogenesis

The formation of blood islands from mesodermal cells in the yolk sac ultimately gives rise to both extraembryonic vasculogenesis and primitive hematopoiesis (a). Within the developing embryo, the first intraembryonic site to exhibit hematopoietic activity is the para-aortic splanchnopleura and subsequent aorta-gonad-mesonephros (AGM) region (days 9.5 to 12.5 postcoitum in mice), which comprises the dorsal aorta and its surrounding splanchnic mesoderm (b). Recent consensus indicates that definitive hematopoietic stem cells (HSCs) emerge from hemogenic endothelium in the aorta's ventral floor. This process may involve budding of HSCs into the aortic lumen (1) or the abluminal bending of endothelial cells into HSCs (endothelial hematopoietic transition), so that they appear in the surrounding mesenchyme (2) [16]. Circulation of the HSC progeny results in robust engraftment in subsequent sites of hematopoiesis (3). In addition, multipotent mesoangioblasts, devoid of hematopoietic potential, reside in the roof and lateral walls of the dorsal embryonic aorta. Although their developmental role is unclear, these cells may accompany newly branching blood vessels into other tissues (4).

Fig. 2. Hemangioblast ancestry of endothelial and hematopoietic lineages

Schematic diagram showing the purported derivation of endothelial and hematopoietic cells from a common bipotent, hemangioblast ancestor.

Fig. 3. Extramedullary hematopoiesis in advanced carotid plaque

Carotid plaque excised surgically at the time of carotid endarterectomy, showing dense calcification, hemorrhage and the presence of bone marrow elements (inset box).

Hematoxylin and Eosin. ×10 (a), ×100 (b). Images were kindly provided by Dr Dylan Miller, Mayo Clinic, Rochester, MN.

Fig. 4. Mural distribution of VW-PCs

Different populations of vascular wall-resident progenitor cells (VW-PCs) have been identified within the discrete layers of the vessel wall. Progenitor cells with endothelial potential (EPCs) occupy both the subendothelial space in the intima and the inner region of the tunica adventitia ("vasculogenic zone") which also has a rich supply of smooth muscle progenitors (SPCs) and multipotent mesenchymal stromal/stem cells (MSCs) or pericytes. Immunophenotypic profiles used to isolate and study these cells include Sca-1^+ , $\text{c-Kit}^+\text{VEGFR2}^+$ and $\text{CD34}^+\text{CD31}^-$. In addition, pericytes/MSCs have been demonstrated in the other mural layers and distinct side population cells with bipotent smooth muscle and endothelial plasticity have been localized to the tunica media. The local activation and migration of VW-PCs is believed to contribute to cell turnover during physiologic and pathogenic vascular remodeling and neovascularization, while there may also be dynamic, bidirectional exchange of progenitor cells between the vessel wall and peripheral circulation throughout adult life.