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Stem cells and the vasculature

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Unraveling the contribution of stem and progenitor cells to blood vessel formation and, reciprocally, the importance of blood vessels to the production and function of stem and progenitor cells, has been a major focus of vascular research over the last decade, but has spawned many controversies. Here I review how vascular stem and progenitor cells contribute both vascular and nonvascular cells during development and in disease, and how nonvascular stem and progenitor cells might contribute to vascular lineages. I also discuss the role of the vasculature in forming stem and progenitor cell niches. Finally, I highlight the potential relevance of these relationships to disease etiology and treatment.

The concept that stem cells reside in adult tissues, where they influence normal homeostasis and disease progression, has revolutionized paradigms of physiology and pathology. This is especially true of the vascular system, which is associated with stem and progenitor cells in several ways[1,2] (see **Box 1** for terminology). Blood vessels are a source of stem and progenitor cells, which likely contribute to a variety of vascular processes and diseases. In addition, blood vessels in several organs are associated with stem cell niches that protect and regulate both vascular and nonvascular stem and progenitor cells. As with any concept that potentially changes the way we think about biology and treat disease, there is some confusion and controversy surrounding the new findings and their interpretation. Here I focus on how emerging concepts in this field could influence therapeutic approaches to diseases of blood vessels such as atherosclerosis and hemangioma. I have highlighted the functional evidence for stem and progenitor cells associated with blood vessels and their involvement in development and disease, but I have not attempted an exhaustive description of the molecular players and mechanisms or provided an extensive analysis of the markers used to identify cells because of space constraints. In each section, I cite one or more excellent reviews that provide more extensive detail.

Developmental origins and relationships of vascular cells

Blood vessels are composed of endothelial cells that provide the inner (luminal) surface of the vessel in contact with blood, and mural cells that interact with the outer (abluminal) surface of endothelial cells. Mural cells most often are pericytes, and the vessel wall of the vast majority of vessels in the body, which comprise the microcirculation, consists simply of endothelial cells surrounded by pericytes. Larger vessels, especially arterioles, arteries and veins, are invested with smooth muscle cells and elastic fibers that provide contractility, and larger arteries and veins also have an organized structure of fibroblasts that produce collagen.

The various cell types that make up the vasculature do not arise from a single embryonic source, and cells from multiple sources can ultimately acquire the same fate[3,4]. Given that blood vessels are found associated with virtually every organ and tissue in the body, it makes sense that local tissues provide precursor cells. Most of the vasculature arises from the mesoderm, although some mural cells that contribute to vessels of the great arteries and the brain come from the neural crest[5,6]. Major vessels, such as the dorsal aorta and cardinal vein, form from endothelial cell precursors called angioblasts that migrate from the lateral areas of the embryo toward the midline before coalescing into cords and forming lumens[7,8]. Mesodermal condensations called somites also produce angioblasts that migrate both medially, contributing to vessels surrounding the neural tube, and laterally, contributing to limb vessels[9,10]. Most organ rudiments produce angioblasts that vascularize the local tissue and connect to vessels that sprout from the major conduit vessels and invest the organs. However, the central nervous system (brain and spinal cord) lacks resident angioblasts. Vessels of the central nervous system are formed by angiogenic sprouting into the brain from surrounding vessels, and in some cases single angioblasts also migrate into the brain and initiate new vessel formation[11,12]. Elegant clonal analyses have shown that mural cells, including pericytes and smooth muscle cells, are predominantly recruited to vessels from local sources[13–17]. Thus, the vasculature has multiple cell types and does not arise homogeneously but is rather a mosaic of cells from different sources and locales. This diverse etiology may influence vascular function and disease propensity. For example, although susceptibility to atherosclerosis is associated with hemodynamic parameters, graft studies suggest that intrinsic differences between vessel segments may also contribute to the frequency of plaque development in arteries (ref. 4 and references therein).

Stem and progenitor cells and developing vessels

How do stem and progenitor cells contribute to vascular development? The diverse embryonic sources of both endothelial and mural cells argue against a single type of vascular stem cell, analogous to the

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BOX 1 Definitions of terms used in this review

Adventitial progenitor cells: multi-potent Sca1$^+$ progenitor cells localized to the adventitia of blood vessels.

Endothelial-to-mesenchymal transition (EndMT): a process whereby endothelial cells lose their epithelial phenotype and acquire a mesenchymal phenotype; this process may involve a multipotent progenitor cell intermediate.

Endothelial progenitor cell (EPC): a bone marrow–derived cell that may contribute structurally to neoangiogenesis. (The term EPCs has also been used to describe non-bone marrow–derived circulating cells with endothelial potential.)

Hemangioblast: a putative bipotential progenitor of endothelial cells and hematopoietic stem cells (HSCs).

Hemogenic endothelium: endothelial cells that give rise to HSCs.

High-proliferative potential endothelial colony–forming cell: a circulating endothelial cell with high proliferative capacity.

Mesenchymal stem cell: a progenitor cell that gives rise to one or more mesodermal lineages (for example, bone, muscle, fat and chrondrocytes).

Pericyte: a vascular mural cell that stabilizes endothelial cells and may have progenitor cell properties.

Stem cell niche: a localized microenvironment that supports stem and progenitor cell maintenance.

Vascular stem cell: a putative self-renewing multipotent stem cell that gives rise to vascular lineages.

hematopoietic stem cell, which would both self-renew and contribute to all vascular lineages. It can also be argued that there is no need for a vascular stem cell, as once endothelial cells differentiate they retain the capacity to proliferate and form new vessels via sprouting angiogenesis, and mesenchymal cells capable of recruitment to mural lineages reside in most local environments. However, both vascular compartments—endothelial and mural—are associated with stem cells, progenitor cells or both during development[18–20] (**Fig. 1a**). This association suggests that stem and progenitor cells could contribute to both normal and abnormal vascular development. Vascular stem or progenitor cells may also persist into adulthood and potentially contribute to disease (**Fig. 1b**). Evidence for the involvement of stem and progenitor cells in vessel development comes from studies of the hemangioblast and hemogenic endothelium, analyses of cardiac progenitor cells, and investigations of the developmental potential of pericytes, as described below, as well as from analyses of vascular tumors and malformations, as described later in the review.

Vascular and hematopoietic lineages. The hemangioblast is a putative common progenitor of hematopoietic and endothelial cells[19] (**Fig. 2a**). Although some lineage diagrams place it upstream of all hematopoietic and endothelial cells, experimental data indicate that endothelial cells are derived from hemangioblasts only infrequently; instead, most endothelial cells derive from mesoderm-derived angioblasts not directly related to the hematopoietic lineage[21,22]. The existence of the hemangioblast was first hypothesized by Florence Sabin in 1920 (ref. 23), on the basis of the close proximity of hematopoietic and endothelial cells during early development. Keller and his colleagues produced clones containing both hematopoietic and endothelial cells from partially differentiated mouse embryonic stem cells and gastrulation-stage mouse embryos, although these clones were not strictly bipotential, as they also produced smooth muscle cells[21,24]. Marker analysis of mouse embryos and transplant studies showed that the endothelial lining of the dorsal aorta was associated with labeled cells that also produced hematopoietic stem cells (HSCs), although this analysis did not rigorously establish bipotential capability[25]. The most compelling evidence for a hemangioblast progenitor cell comes from Vogeli *et al.*[22], who used laser uncaging to label single cells and track progeny in the early zebrafish embryo. This analysis showed that some cells contributed to both the hematopoietic and vascular lineages in a narrow developmental window, but it also highlighted that many cells labeled at early stages of development contributed to only a single lineage, indicating that most hematopoietic and endothelial cells do not arise from a hemangioblast progenitor.

The existence of the hemangioblast is still controversial, because current data can also be explained by the concept of a hemogenic endothelium, composed of well-differentiated endothelial cells[26], which is responsible for generating hematopoietic cells (**Fig. 2b**). Recent lineage tracing, live imaging and genetic studies in both mice and zebrafish have established that, at specific developmental stages and locales, endothelial cells give rise to hematopoietic cells, and that these cells or descendants contribute to HSCs in the adult animal[27–31]. Marker analysis and imaging indicate that the process involves transdifferentiation of endothelial cells into hematopoietic cells, although in one study endothelial cell division was closely associated with hematopoietic cell generation from hemogenic endothelium[28], suggesting that hemogenic endothelial cells sometimes undergo asymmetric division to produce an endothelial cell and a hematopoietic cell (**Fig. 2b**). At the molecular level, the transcription factor Runx1 is required for the transition but not in hematopoietic cells once they form; in differentiated endothelial cells, the transcription factor HoxA3 maintains the endothelial phenotype by downregulating Runx1 (refs. 32,33). A unifying, hybrid model has the hemangioblast producing endothelial cells that contribute to hemogenic endothelium (**Fig. 2c**). A recent study showing that mouse hemangioblast colonies produce hemogenic endothelium in culture supports this link[34]. The temporal and spatial restriction of hemogenic endothelium formation during development suggests that extrinsic factors also likely contribute to its formation.

Vascular and muscle lineages. Lineage tracing and retrospective clonal analysis reveal that single embryonic cells originating from somites contribute to both skeletal muscle and vascular endothelial cells; somitic cells that are bipotential for smooth muscle and endothelial fates have also been reported[14,15]. These *in vivo* data support an earlier *in vitro* analysis of lineage relationships in differentiating embryonic stem cells[35]. These relationships are well documented in the developing heart, which contains progenitor cells (marked by Flk-1 receptor or Isl-1 transcription factor expression in mice and Isl-1 in humans) that contribute to multiple lineages when genetically marked *in vivo*; moreover, single progenitor-cell isolates give rise to cardiomyocytes, vascular smooth muscle and endothelial cells *in vitro*[20,36–39]. Thus, data from multiple experimental approaches provide convincing evidence for the existence of bi- or multipotent progenitors in specific embryonic locations that contribute to both endothelial and muscle lineages.

Mural cells. Vascular pericytes that surround blood vessels are reported to have some properties of stem cells in both embryonic and adult tissues[18,40] (**Fig. 1**). In muscle, cells expressing pericyte markers

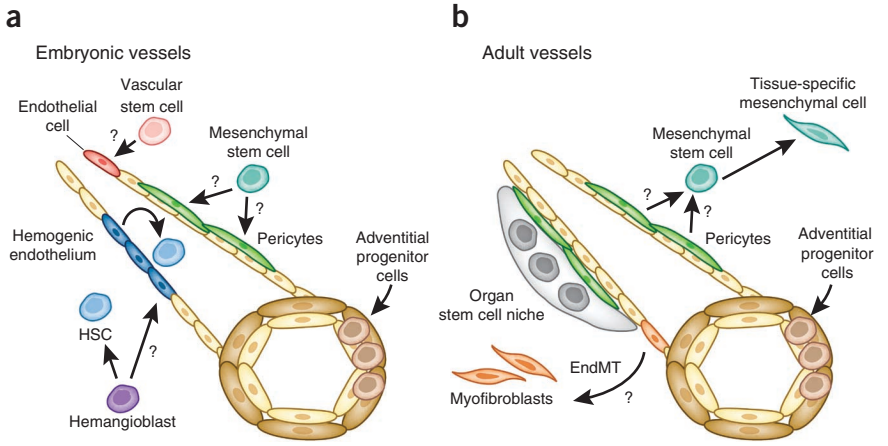

Figure 1 Proposed interactions of stem and progenitor cells with the vasculature. (**a**) In embryonic blood vessels, several types of stem and progenitor cells either reside in the nascent vessel or contribute to its formation (or both). As discussed in the text, stem and progenitor cells may contribute to vascular endothelial cells, pericytes and hemogenic endothelium. Hemogenic endothelium may in turn produce HSCs. Also indicated is an adventitial niche harboring progenitor cells. (**b**) Adult blood vessels interact with stem and progenitor cells in multiple ways. Vessels provide a niche for organ-specific stem cells, act as a reservoir of pericytes and endothelial cells that may contribute to mesenchymal lineages, and harbor adventitial progenitor cells. In both panels, interactions that are less well established are indicated with question marks.

associate with vessels and are myogenic both *in vitro* and when re-introduced *in vivo*[41]. Bianco and his colleagues[42] identified a cell type that co-expresses CD146 (MCAM) and other established pericyte markers and localizes subendothelially in bone marrow. These cells can be expanded clonally *in vitro* and re-create the hematopoietic microenvironment when injected into mice. In another study, cells expressing pericyte markers were clonally isolated from fetal and adult human muscle and other organs[43]. These cells had properties of mesenchymal stem cells in culture and contributed to skeletal muscle when re-introduced in a mouse muscle injury model. However, as no marker exists that exclusively labels pericytes, contributions from cells other than pericytes cannot be ruled out in these studies. Other groups have isolated similar progenitor cells from adipose tissue[44,45]. Taken together, these findings are consistent with a model in which mesenchymal stem cells are 'captured' by developing vessels and become pericytes but retain nascent stem cell properties; these cells may later be 'reactivated' after injury or in disease states[18] (**Fig. 1**). Recently, lineage tracing was used to mark putative pericytes expressing the marker NG2 (ref. 46). Labeled cells were found in mesenchymal cells of the tooth, suggesting that pericytes can contribute to the odontoblast lineage via a progenitor cell intermediate. However, a limitation of this study is that the lineage reporter used is not restricted to pericytes, so contributions of other cell types to marked mesenchymal cells cannot be rigorously excluded. In another study, knock-in alleles at the platelet-derived growth factor receptor-β locus that conditionally elevate signaling were shown to inhibit differentiation of pericytes or the conversion of mesenchymal cells to adipocytes, suggesting that this signaling pathway regulates the developmental potential of mural cell progenitors[47]. However, in this study as well, the genetic perturbations used were not rigorously restricted to pericytes, leaving open alternative models.

Although the existence of pericytes with mesenchymal stem cell properties has not been rigorously demonstrated, their presence in vessels, as well as that of other vascular stem or progenitor cells, could have important implications for various types of vascular disease, as described in more detail later in the review. Such diseases could perturb vascular stem and progenitor cells that reside within blood vessels; conversely, from a therapeutic perspective, stem and progenitor cells present in vessels from their earliest stages could be harvested for tissue regeneration.

Postnatal vascular stem and progenitor cells

In addition to vessel-associated stem and progenitor cells with an embryonic developmental origin, the existence and function of a

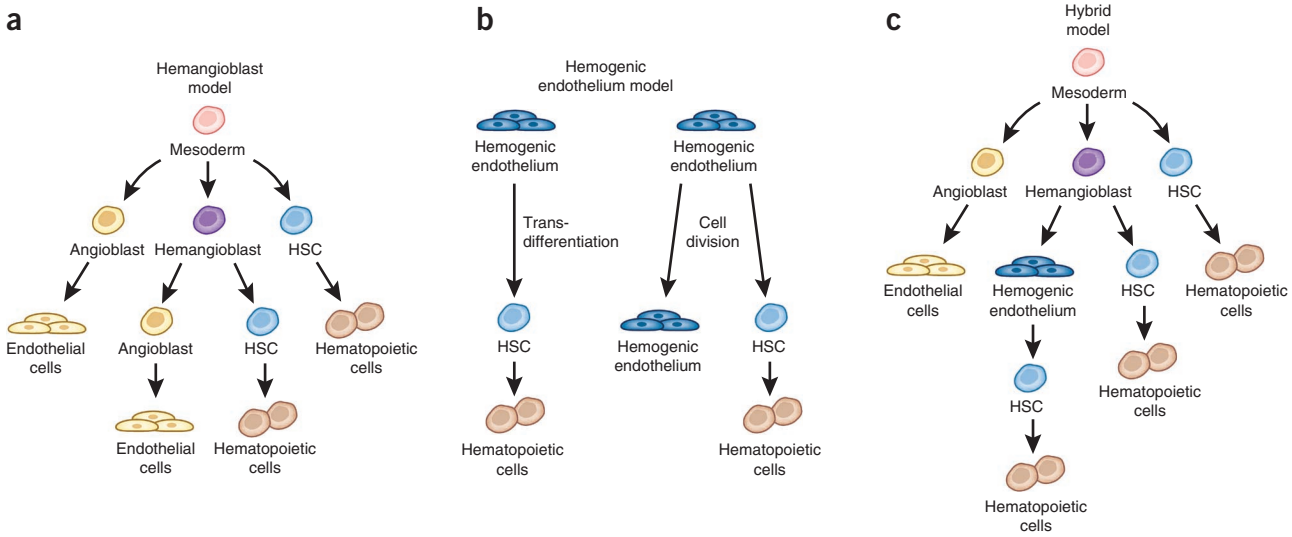

Figure 2 Potential developmental relationships of hematopoietic and endothelial cells. (**a**–**c**) Possible lineage relationships of the vascular and hematopoietic lineages. See text for details.

class of vascular progenitor cells in the adult—bone marrow–derived endothelial progenitor cells (EPCs)—has been vigorously debated. Bone marrow–derived cells contributing to neovascularization in ischemic tissues were reported over ten years ago[48]. Since then, numerous studies have described circulating bone marrow–derived EPCs that contribute to the endothelial layer of quickly forming neovessels—for example, in response to the strong hypoxia of an acute ischemic injury or of tumor microenvironments[49–52]. The existence of EPCs could have medical ramifications: if EPCs contribute significantly to neovessel formation, strategies to augment or block neovessel formation would center on the bone marrow. However, compelling studies in mice using genetically marked bone marrow transplants and parabiosis have found no evidence that bone marrow–derived cells contribute structurally to the endothelial lining of tumor neovessels in either tumor xenografts or spontaneous tumors[53–56].

Why have the findings on this topic been so disparate, and how can they be reconciled? First, substantial numbers of nonendothelial bone marrow–derived cells home to sites of neovessel formation, including monocytes and macrophages that provide paracrine cues important for tumor progression, so the finding that blockade of bone marrow–derived cells that infiltrate tumors alters tumor kinetics[55,57] does not necessarily implicate the direct contribution of these cells to the endothelium. These bone marrow–derived hematopoietic cells can express some endothelial markers and form close contacts with neovessels, which can make it difficult to determine whether such cells actually engraft in vessels. There is also a timing issue, in that some data suggest that bone marrow–derived cells transiently participate structurally in early neovessel formation but are quickly replaced by local cells[52]. However, Dudley *et al.*[56] did not find stage-specific bone marrow–derived endothelial cells in a transgenic prostate tumor model. Moreover, different cell-surface markers, marker sets and functional criteria are used to define 'EPCs' across different studies, making comparisons difficult (ref. 58 and references therein). Finally, endothelial cells do not become postmitotic; rather, they retain the capacity to proliferate and undergo sprouting angiogenesis in response to pathophysiological cues, and this response is probably sufficient for neovascularization. Yoder *et al.*[59,60] identified a subset of cultured vessel wall–derived endothelial cells with a high proliferative capacity and showed that these cells contribute to neovessel formation in a collagen implant model *in vivo*. These findings suggest that a few endothelial cells, corresponding to the high-proliferative-potential endothelial colony–forming cells identified in cell culture, are present in existing blood vessels *in situ*, and can respond to pathophysiological cues with vigorous proliferation to provide endothelial cells for neovascularization[58], although their existence in intact vessels has not been rigorously shown. Although a clear answer to the 'EPC dilemma' may not be essential for some translational aspects of neovessel formation, the most effective therapies will result from precise knowledge of the cells that respond to neoangiogenic stimuli to form new blood vessels. For example, tissue regeneration requires perfusion by blood vessels, and much effort has gone into building blood vessels outside the body to be used for tissue engineering. As *ex vivo*–expanded endothelial cells and vascular mural cells are obvious sources of cells for such vessels[61,62], it is important to have a detailed understanding of these cells' proliferative and differentiation potentials.

Endothelial cells in blood vessels form a stable epithelial tube, yet in some pathologies may release their attachment to neighboring endothelial cells and assume a mesenchymal phenotype[63,64], a so-called 'endothelial-to-mesenchymal transition' (EndMT). This transition is thought to be analogous to the process whereby endocardial cells delaminate to form the heart cushions and valves in the embryo. EndMT has been observed in the settings of cardiac fibrosis and tumor progression: single cells were found to express both endothelial and myofibroblast markers, and myofibroblast-like cells were lineage-marked for endothelial (Tie1 or Tie2) expression[65,66]. However, the possibility that nonendothelial Tie2-expressing cells, such as subsets of monocytes and macrophages, contribute to myofibroblast formation was not eliminated. EndMT may involve an intermediate, multipotent progenitor cell type induced by the transforming growth factor-β (TGF-β) and bone morphogenetic protein (BMP) receptor ALK2. A recent study showed that endothelial cells stimulated with TGF-β or BMP4 have multipotent differentiation potential in culture, and that humans with an activating mutation in ALK2 and mice overexpressing constitutively active ALK2 have cells within heterotopic ossified lesions that co-express endothelial and osteogenic or chondrogenic markers[67]. These data suggest that features of mesenchymal stem and progenitor cells may lie dormant in mature vascular endothelial cells and contribute to disease progression when reactivated.

Blood vessels and the stem cell niche

A corollary of the concept that stem and progenitor cells persist in various tissues into adulthood is the existence of specialized niches for stem and progenitor cells that provide protection and regulate their proliferation[68]. The vasculature contributes to several of these niches. The best characterized is the hematopoietic bone marrow niche for HSCs, which consists of an osteogenic niche that maintains HSC quiescence and a vascular niche that regulates progenitor cell differentiation and egress[69,70]. Recent studies make a strong case for the essential contribution of blood vessels to the stem cell niche in other organs as well. In the central nervous system, neural stem cells are found closely associated with blood vessels at two anatomical sites, the subventricular zone and the subgranular zone[71]. Coculture of endothelial cells with neural stem cells increases stem cell self renewal through Notch receptor signaling and the Notch effector Hes1 (ref. 72); interactions in this niche also involve chemokine signaling and integrins[73,74]. This neural stem cell vascular niche is likely to have a role in neurological disease. Degradation or 'exhaustion' of the vascular niche, thus reducing the number of neural progenitors available for neurogenesis, may contribute to aging and diseases characterized by memory loss, analogous to associations between reduced muscle function and aging of the skeletal muscle stem cell niche[75]. Manipulation of the vascular neural stem cell niche in neurological disease may therefore be able to bolster neural function.

Larger arteries and veins have several layers: the outermost, or adventitial, layer contains collagen and fibroblasts; the middle, or medial layer, contains smooth muscle cells; and the inner, or intimal layer, is composed of endothelial cells. These layers are important for normal vessel function, and may also form a niche that protects stem and progenitor cells during early development and into adulthood[76,77]. Hu *et al.*[78] identified $Sca1^+$ adventitial progenitor cells in atherosclerosis-prone apolipoprotein E–knockout mice that contribute to neointima formation in a vein graft model. Passman *et al.*[79] identified such $Sca1^+$ adventitial progenitor cells in late embryonic and early postnatal mice and used genetic tools to reveal a requirement for Sonic hedgehog (Shh) signaling in the perdurance of these cells. This Shh requirement is intriguing, because Shh signaling maintains stem and progenitor niches in other developmental contexts[68]. Notably, the $Sca1^+$ adventitial cells were not derived from either bone marrow or neural crest, and their origin is currently unclear[78,79]. These studies in mice are consistent with studies showing that human vessels contain adventitia-localized progenitor cells[80,81].

As discussed previously, pericytes show some properties of stem and progenitor cells when removed from the local environment of the vessel wall[18]. Thus, the vasculature may provide a niche that prevents differentiation of pericytes into mesenchymal cell derivatives. In the vessel wall, pericytes physically interact with endothelial cells to provide vessel stabilization, so pericyte-endothelial interactions may be important for maintaining pericyte properties or holding the pericyte in 'suspended animation'. Pericyte-endothelial interactions that contribute to vessel stabilization are a focus of intense study. Signaling by platelet-derived growth factor, TGF-β and Notch ligands, as well as direct cell-cell contacts mediated by N-cadherin, are involved in this crosstalk[82,83]. Further studies are needed to define the molecular pathways whereby endothelial cells, or other vascular cells, might regulate the latent progenitor cell properties of pericytes.

Stem and progenitor cells in vascular disease

Therapies targeting stem and progenitor cells might need to be distinct from those targeting differentiated cell populations. For example, many types of stem and progenitor cells express transporters that confer resistance to numerous drugs[84], so an effective therapy to block stem or progenitor cell–based neoangiogenesis might be to disable these transporters. Conversely, therapies designed to target differentiated cell types most often disrupt signaling pathways specific to the formation or maintenance of those cells, such as vascular endothelial growth factor signaling for endothelial cells or platelet-derived growth factor signaling for mural cells. The evidence for the involvement of vascular stem and progenitor cells in vascular disease is more compelling for some pathologies than for others, as discussed below.

Atherosclerosis and vessel injury. Although bone marrow–derived EPC involvement in vein graft–induced atherosclerosis has been reported[85], the Tie2 marking system used in this study was not specific to endothelial cells, and other studies found no evidence of bone marrow–derived EPC involvement in atherosclerosis in apolipoprotein E–deficient mice[86] or in an injury model of neointima formation[87]. Moreover, in the injury model, Sca1$^+$ adventitial cells increased in number and proliferative potential with neointima formation. These findings indicate that plaque development and injury repair may involve local populations of vascular progenitor cells. It will be particularly interesting to further examine the role of the adventitia-localized progenitor cells in plaque development and progression[71].

Hemangioma. Some hemangiomas are benign vascular tumors found in infants that regress or involute during childhood[88–90]. However, hemangiomas occuring on the face and neck can affect vision and breathing, and some are life threatening. Recent evidence suggests that hemangiomas can arise from one or a few stem cells, called HemSCs, whose progeny cells constitute the tumor. It was initially found that endothelial cells derived from most hemangiomas are clonal; that is, they derive from a single abnormal endothelial cell, suggesting the possibility of a stem cell origin[91,92]. Subsequently, putative HemSCs were isolated from hemangiomas that can clonally expand, differentiate into multiple lineages and form vessels after serial passage *in vivo*[93]. As hemangiomas are usually detected shortly after birth and are thought to arise during fetal development, it has been proposed that vascular stem cells, normally present during fetal development, occasionally persist and/or become dysregulated, leading to hemangioma formation[88,90]. Hemangioma involution would then be ascribed to a gradual depletion of HemSCs. Although this model is plausible, there is not yet strong independent evidence for the existence of a single type of vascular stem cell that contributes widely to blood vessels during normal development. An alternative hypothesis is that hemangiomas arise when an angioblast progenitor, present during normal development, is modified to acquire stem-like properties, a model consistent with a recent analysis of mutations associated with hemangioma-derived endothelial cells[94]. Another uncertainty surrounding the concept of a hemangioma stem cell is that the lineage potential of such a cell has not yet been clearly defined. Although it was recently reported that cultured hemangioma stem cells give rise to both pericytes and endothelial cells[95], the lack of definitive lineage tracing data in primary hemangiomas precludes a rigorous evaluation of their etiology. If further validated, the stem cell hypothesis for hemangioma would argue that treatments targeting HemSCs or their niche would have therapeutic potential.

Vascular malformations. Vascular malformations often result from one or a few somatic genetic mutations[96], so these mutations can be used to determine whether the malformations are related in individuals with multiple lesions. The most compelling evidence for the association of vascular stem or progenitor cells with vascular malformations was an analysis by Vikkula and his colleagues[97] of several individuals with multiple sporadic venous malformations. Each of two individuals had two mutations in one copy of the gene encoding Tie2 in endothelial cells isolated from multiple focal lesions at several distinct sites. The mutations were not found in blood cells, suggesting that a stem or progenitor cell sustained the two mutations and then produced progeny cells that circulated and seeded the focal malformations (although sloughing from a primary lesion could not be ruled out). If vascular malformations result from lesions in a stem or progenitor cell, systemic therapy targeting stem cells might be effective.

The vascular stem cell niche and disease. The ability of the vasculature to provide a niche for diverse stem and progenitor cells suggests that blood vessels are more widely involved in disease than is currently appreciated. For example, if vessel dysfunction disrupts an organ-specific stem and progenitor cell niche, stem cells might become prematurely exhausted and unable to maintain organ function. In a recent study, the selective deletion in osteogenic cells that contribute to the hematopoietic niche of bone marrow of either a regulator of microRNAs, Dicer, or the putative Dicer target gene *Sbds* (encoding Shwachman-Diamond-Bodian syndrome protein) led to myelodysplasia and leukemia[98]. These findings indicate that oncogenesis can result from a perturbed niche rather than solely from cell-autonomous lesions. Another study linked a premature aging syndrome to dysfunction of vascular adventitial cells[99]; although effects on adventitial progenitors were not examined, it seems plausible that disruption of an adventitial niche might contribute to this syndrome.

Stem cells and blood vessels: future directions

The case for the involvement of stem and progenitor cells in numerous aspects of blood vessel development, function and disease is strong. Moreover, stem and progenitor cells produced by blood vessels may contribute to nonvascular processes. Vessels also provide a niche for stem and progenitor cells in diverse organs. This intimate connection between blood vessels and stem and progenitor cell function adds an additional layer of complexity to the function of the vasculature in normal homeostasis and in disease.

Numerous important questions remain. First, although there is compelling evidence that some endothelial cells have hemogenic capability, whether a hemangioblast progenitor gives rise to hemogenic endothelium, and the relative role of intrinsic versus extrinsic signals in the induction of hemogenic endothelium, remain to be determined. Given that some HSCs seem to derive from hemogenic endothelium, a better understanding of this subset of endothelial cells could result in improved therapies for hematopoietic disorders. Second, many questions need to be resolved regarding the possibility that cells in the vessel wall, whether Sca 1^+ adventitial cells or pericytes, have stem or progenitor cell potential. What proportion of vascular cells have this capability, and how is it modified in different vascular beds? What molecular and cellular interactions in blood vessels keep these cells quiescent under normal circumstances and under what conditions does their stem or progenitor cell potential become activated? Can this paradigm be exploited therapeutically? Because they are defined functionally and by location rather than by unique markers, both adventitial progenitor cells and pericytes have been difficult to study rigorously. However, as more cell-type specific markers and functional assays become available, their combinatorial use should help define the potential stem and progenitor cell properties of blood vessels and the vessel wall in particular. Finally, the concept of a vascular niche for organ-specific stem and progenitor cells also raises intriguing questions. Is this function of blood vessels widespread or confined to a few organ beds? What is the crosstalk between the organ and blood vessels that sets up and maintains this niche? How does vessel dysfunction and disease affect niche properties and the organ-specific stem and progenitor cells that depend on the niche? Advances in marking and imaging vascular cells *in situ*, including live imaging, will be useful in further defining the microenvironment of the vascular niche and its role in disease.

Compared to just a few years ago, more sophisticated and rigorous cell marker panels and assays for vascular stem and progenitor cells are available, as are more precise genetic tools for lineage marking and clonal analysis *in situ*, which is the gold standard for determining the fate of putative stem and progenitor cells. These genetic tools also allow for more precise genetic manipulations *in vivo*, which will lead to less ambiguity in the interpretation of experimental results. The insights afforded by these new tools and approaches may contribute to new therapies for diverse vascular and nonvascular diseases.

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COMPETING FINANCIAL INTERESTS

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