REVIEW

Resident vascular progenitor cells: An emerging role for non-terminally differentiated vessel-resident cells in vascular biology

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Abstract Throughout development and adult life the vasculature exhibits a remarkably dynamic capacity for growth and repair. The vasculature also plays a pivotal role in the execution of other diverse biologic processes, such as the provisioning of early hematopoietic stem cells during embryonic development or the regulation of vascular tone and blood pressure. Adding to this importance, from an anatomical perspective, the vasculature is clearly an omnipresent organ, with few areas of the body that it does not penetrate. Given these impressive characteristics, it is perhaps to be expected that the vasculature should require, or at least be associated with, a ready supply of stem and progenitor cells. However, somewhat surprisingly, it is only now just beginning to be broadly appreciated that the vasculature plays host to a range of vessel-resident stem and progenitor cells. The possibility that these vessel-resident cells are implicated in processes as diverse as tumor vascularization and adaptive vascular remodeling appears likely, and several exciting avenues for clinical translation are already under investigation. This review explores the various stem and progenitor cell populations that are resident in the microvasculature, endothelium, and vessel walls and vessel-resident cells capable of phenotypic transformation.

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Introduction

The significant recent research efforts directed toward stem and progenitor cells as they relate to cardiovascular biology have led to many unexpected discoveries of profound importance. These have included the description, although controversial, of both resident cardiac progenitor cells (Beltrami et al., 2003) and endothelial progenitor cells (Asahara et al., 1997). As a result, several long-held paradigms are now being challenged, and clinicians and basic scientists alike are tantalized by the prospect that these insights may be directed toward clinical therapeutic applications (Kovacic et al., 2005).

A surprising new paradigm also to arise from this work, and the focus of this review, is the possibility that stem or progenitor cells may reside within the vessel wall (Fig. 1). Even more striking, it appears likely that several distinct stem/progenitor populations may reside in this location during development or adult life. Currently our understanding of these mural stem/progenitor populations is rudimentary, their possible biologic function is unclear, and the prospect that any of these populations are associated with a stem cell niche is yet to be rigorously investigated. Nevertheless, potential biologic roles for vessel-resident stem/progenitor cells are easily envisaged, such as homeostatic replacement of aged and apoptotic cells and in the response to vascular injury.

The core focus of this review is vessel-resident stem/progenitor cells. Therefore, we have excluded stem/progenitor populations that are considered to reside outside the vasculature, but which may nevertheless be potent for the formation of vascular structures. As an example, both embryonic stem cells and certain cardiac progenitor cells appear able to give rise to vascular cells (endothelial cells, smooth muscle cells, etc.). However, as neither embryonic stem cells nor cardiac progenitor cells appear to reside within the vessel wall, they are not formally considered in this review. Interested readers are referred to the following excellent review articles on these populations: for cardiac progenitor cells, Anversa et al. (2006) and Wu et al. (2008); for embryonic stem cells, Murry and Keller (2008).

Vasculogenesis and Early Vessel Formation

Vasculogenesis, defined as the de novo formation of a primitive vascular plexus from mesodermal progenitor cells (Kovacic et al., 2008), and the subsequent formation of an embryonic circulation are of critical importance to the developing embryo. Our knowledge of these developmental programs is based on a large body of research dating back to the late 1800s and early 1900s (Sabin, 1917)—a fact that undoubtedly contributes to the cornerstone position vasculogenesis holds in our current-day framework for understanding vessel formation.

Vasculogenesis is initiated soon after gastrulation by the aggregation of mesodermal progenitor cells into small clusters known as blood islands—the earliest discernable vascular structures (Sabin, 1917; Gonzalez-Crussi, 1971). In response to a series of cues, including fibroblast growth factor and vascular endothelial growth factor (VEGF), the cells of these blood islands undergo partial lineage commitment such that the outermost cells become endothelial precursors (termed angioblasts), while the central cells become hematopoietic precursor cells (Risau and Flamme, 1995). The observation that hematopoietic cells and angioblasts arise in close proximity has long fueled speculation that a bipotent cell (Sabin, 1917), the hemangioblast (Murray, 1932), is the common blood island progenitor. Recently, numerous sources have provided strong evidence to support the existence of such bipotent hemangioblast-like cells, including clonal in vitro embryonic stem cell data (Choi et al., 1998) and in vivo research performed in both zebrafish (Vogeli et al., 2006) and mice (Huber et al., 2004). However, well-conducted studies have also emerged arguing against the possibility that individual (clonal) hemangioblast progenitors give rise to all endothelial and hematopoietic cells within blood islands (Ueno and Weissman, 2006).

Regardless of this controversy, it is established that a critical player during vasculogenesis is VEGF receptor-2 (VEGFR-2), with homozygous VEGF-2 knockout mice dying in utero due to a severe reduction in hematopoietic progenitors and an absence of intra-and extraembryonic vessel formation (Shalaby et al., 1995). Correspondingly, the common blood island progenitor.

Figure 1  The anatomy of a vessel. (A) Four-color confocal microscopic image of mouse femoral artery acquired using a Zeiss LSM 510 UV laser scanning confocal microscope system (Carl Zeiss GmbH, Germany). Staining was with 4′,6-diamidino-2-phenylindole shown in blue, rabbit anti-SM22a (Abcam ab14106, Cambridge, MA, USA) in red, and rat anti-CD31 (eBioscience 14-0311, San Diego, CA, USA) in white, in conjunction with appropriate anti-rabbit and anti-rat secondary antibodies (Invitrogen, Carlsbad, CA, USA). The internal and external elastic laminae (shown in green) were not stained, but were imaged using autofluorescence emitted when excited with a 488-nm laser line. (B) Schematic representation of adjacent confocal image identifying vascular anatomic structures/layers.
VEGFR-2 is an important progenitor cell marker during development, potentially identifying not only hematopoietic blasts (Choi et al., 1998), but also progenitors with broad mesodermal potential (Ema et al., 2006), including cells fated to give rise to cardiomyocyte and smooth muscle cell lineages (Kattman et al., 2006).

Blood islands soon coalesce and fuse, concurrently laying down a vascular extracellular matrix and developing a central lumen. While remaining incompletely understood, distinct differences appear to exist between vasculogenesis in the emerging extra- and intraembryonic vascular systems and the respective roles played by hematopoietic precursors in each (Risau and Flamme, 1995). In particular, the central core of primitive hematopoietic precursors appears far more prominent in the emerging extraembryonic system. On the other hand, intraembryonic vasculogenesis proceeds with rudimentary vascular/angioblast strands first forming along the lateral edges of somites. The dorsal aorta thus emerges as a continuous cord at the ventrolateral somite edges, continuing into the head to fuse with the ventral aorta, and forming the first aortic arch by the six-somite stage (Coffin and Poole, 1988). The generation of hematopoietic stem cells (HSCs) is also an integral aspect of this program; however, unlike the extraembryonic system, intraembryonic HSCs appear to emanate from a discrete region of the wall of the dorsal aorta and other major embryonic vessels.

The Vessel Wall during Development—the Emergence of Hematopoietic Stem Cells and Mesoangioblasts

As alluded to above, the origins of hematopoietic cells and HSCs have been shrouded in controversy for decades. Difficulty characterizing these cells arises because they appear during a very brief window of time in embryonic development, with various cell types (primitive erythroblasts, erythromyeloid progenitors, “definitive” HSCs) appearing in rapid succession. The delineation of the origins of these cells is further confounded by the fact that they are actively redistributed to different extra- and intraembryonic host tissues via the primitive circulation soon after their emergence. Thus, one of the most controversial aspects of this field is the contribution made by extraembryonic sites (placenta, yolk sac) to definitive intraembryonic HSCs with complete lineage competency, with various publications attesting that extraembryonic contributions are either unlikely (Cumano et al., 1996, 2001) or highly probable (Ghiaur et al., 2008; Lux et al., 2008; Rhodes et al., 2008).

Nevertheless, consensus exists that in mice and humans the first intraembryonic site to exhibit hematopoietic activity is the para-aortic splanchnopleura, which comprises the dorsal aorta and the surrounding splanchnic mesoderm (8.5–10 days postcoitum in mice) (Godin et al., 1995, 1999). By days 10–12 postcoitum (mice), the aorta, gonads, and mesonephros have arisen from within the para-aortic splanchnopleura, and this structure is then called the AGM (aorta-gonads-mesonephros). From approximately day 9.5 to 12.5 postcoitum (mice), the ventral domain of the dorsal aortic floor is a particularly rich source of HSCs, constituting 1 in 12 of all cells in this region (Fig. 2) (Godin et al., 1995, 1999; Taoudi and Medvinsky, 2007). Although the umbilical and vitelline arteries may also generate HSCs (de Bruijn et al., 2000), there is evidence to suggest that in mammals a significant proportion of the initial wave of intraembryonic HSCs is derived from aortic endothelial cells (de Bruijn et al., 2002). These “hemogenic” aortic endothelial cells appear to undergo a phenotypic switch and establish specialized intra-aortic HSC-bearing “clusters” that are in continuity with the luminal surface of the dorsal aortic floor and which are marked by Runx1 expression (Jaffredo et al., 1998; North et al., 2002; Dieterlen-Lievre et al., 2006). Human data, although limited, also suggest that intraembryonic HSCs may arise from aortic endothelial cells (Tavian et al., 2005). However, we note several contradictory reports, including the suggestion that in zebrafish, aortic endothelial cells may not directly give rise to definitive HSCs, but rather, HSCs may emerge from the subaortic mesenchyme (Kissa et al., 2008). Furthermore, alternative explanations as to the origins of AGM-derived HSCs in mammals have been proposed. These include the possibility that HSCs are generated in “subaortic patches” and then migrate toward the aortic lumen, merely protruding through (rather than arising from) the endothelium (Bertrand et al., 2005). Strong evidence is also mounting to suggest that, rather than directly giving rise (de novo) to HSCs, the ventral domain of the dorsal aortic floor may act to provide local inductive cues that convert yolk sac-derived (extraembryonic) hemogenic cells into definitive HSCs (Samokhvalov et al., 2007; Metcalf, 2008).

Regardless of these controversies, aorta-derived HSCs are released into the circulation and, at least in mammals, appear to circulate to other organs such as the fetal liver and spleen, where hematopoiesis is promptly established (Godin et al., 1999). These secondary hematopoietic sites rapidly become the predominant site for HSC residence and expansion, with aortic HSC generation extinguished by embryonic day 13 postcoitum (mice) (Godin et al., 1999).

Figure 2 Histologic analysis of mouse aorta at embryonic day 10.5, with the dotted square delineating the area of higher magnification shown in the upper-left corner. Arrow indicates hematopoietic cells on the ventral wall of the dorsal aorta. This image was originally published in Ghiaur et al. (2008) and is reproduced with permission of the publisher; copyright by The American Society of Hematology.
In turn, HSCs from these secondary hematopoietic sites, possibly including subsequent waves of HSCs that emerge separate from those originally from the embryonic aorta (Emambokus and Frampton, 2003), circulate and establish definitive hematopoiesis in the bone marrow (BM). These BM HSC niches, well known to be the sites of adult mammalian hematopoiesis, have been referred to as “vascular” and “perivascular” niches. However, within the adult BM, HSCs appear merely to reside in close association with the BM vasculature (and also the endostium), rather than being actually vessel-resident or vessel-derived per se (Kiel et al., 2005; Sugiyama et al., 2006). Therefore, although these so-called adult BM HSC vascular niches clearly add to the overall importance of the vasculature with respect to stem cell biology, they fall outside the scope of this review and will not be formally considered.

It is also likely that a second population of stem/progenitor cells, known as mesoangioblasts, arises during embryogenesis in parallel with aorta-derived HSCs, but from the roof and lateral walls of the dorsal aorta (Cosso and Bianco, 2003). Mesoangioblasts are putative multipotent progenitors able to give rise to endothelium and that have been shown to differentiate in vivo into skeletal muscle and multiple other mesoderm-derived connective tissues (bone, cartilage, dermis, etc.) (Minasi et al., 2002). While the possibility that mesoangioblasts and HSCs may arise from a common ancestor has been raised, this remains speculative at the current time (Minasi et al., 2002). In culture, freshly established mesoangioblast clones consistently express markers of early endothelial lineage commitment: CD34, VEGFR-2, vascular-endothelial (VE)-cadherin, and stem cell antigen-1 (Sca-1), while mature endothelial markers are not expressed (Minasi et al., 2002; Cosso and Bianco, 2003). Other investigators have also confirmed the existence of endothelial progenitor cell (EPC)-like CD34+CD31+ cells in the outer stromal layer of the human embryonic aorta (Alessandri et al., 2001). Of note, unlike HSCs that arise from the dorsal aortic floor, mesoangioblasts appear devoid of hematopoietic activity (Cosso and Bianco, 2003).

The developmental role of mesoangioblasts is unclear, as the bulk of postnatal mesoderm-derived tissues are established via other canonical embryonic sources such as somites. Furthermore, mesoangioblasts may not be “fated” to give rise to skeletal muscle, but may do so only if exposed to a “myogenic field,” such as during muscle development or in response to specific injuries (Cosso and Biressi, 2005). Recent indirect evidence suggests that embryonic mesoangioblasts may give rise to a population of interstitial pericytes that populate skeletal muscle in adult humans and that are able to generate skeletal muscle in vitro and in vivo (Dellavalle et al., 2007). Indeed, mesoangioblasts share several common features with pericytes (see below), most notably a broad mesenchymal differentiation potential. However, the spatial and hierarchical relationships of embryonic mesoangioblasts and adult pericytes remain to be clearly defined, and it is also possible that a subset of postnatal skeletal muscle satellite progenitor cells, which differ from skeletal muscle pericyte progenitors, may also arise from the dorsal aorta or other regions of the vascular system (De Angelis et al., 1999).

Taken as a whole, it is clear that embryonic mesoangioblasts possess a level of developmental complexity that is yet to be fully understood. While these and many other questions relating to these cells remain to be addressed, both HSCs and mesoangioblasts serve to highlight what we perceive to be an early and conserved biological precedent for stem and progenitor cells to reside within the vasculature.

**Intimal Endothelial Progenitor Cells**

Arguably, the modern era of endothelial research began in the early 1970s when studies using tritiated thymidine uptake first documented the ability of endothelial cells to divide and proliferate (Schwartz and Benditt, 1973). It was soon appreciated that the daily rate of aortic endothelial replication in rodents was 13% at birth, but this declined to only 0.1–0.3% at ~6 months of age. However, the rate of aortic endothelial cell replication was observed to increase significantly in response to an appropriate stimulus or injury (Schwartz and Benditt, 1977). These studies also documented that replicating endothelial cells were not randomly distributed, but rather appeared as clusters within the vasculature that were interpreted to be “growth centers” or “high-turnover regions” (Schwartz and Benditt, 1976).

Another milestone in endothelial biology occurred in 1997 when Asahara et al. (1997) first identified circulating EPCs that could be isolated from adult humans and that contribute to capillogenesis (capillogenesis being a novel term describing the universal process of new capillary formation; Kovacic et al., 2008). However, despite over a decade of further research, currently the precise nature and origin of EPCs remain enigmatic. Perhaps because EPCs can be derived from the blood and BM, and as early reports suggested their surface antigen expression was somewhat similar to HSCs, the adult BM has been widely assumed to be the likely reservoir for EPCs. Incorrectly reinforcing this assumption, EPC status was erroneously ascribed to colonies of blood-derived cells (referred to as early outgrowth colonies or CFU-EC) that are now understood to be closely related to macrophages/monocytes and/or T cells (Gulati et al., 2003; Rohde et al., 2007; Yoder et al., 2007). Adding further to this confusion, while some groups have recently recapitulated the potential HSC origins of EPCs/endothelium (Bailey et al., 2006; Rota et al., 2007), others have suggested that HSCs do not give rise to EPCs or to progeny with an endothelial phenotype (Case et al., 2007; Timmermans et al., 2007; Yoder et al., 2007). Somewhat unexpectedly, studies have now also suggested that circulating non-BM-derived progenitor cells may account for a large proportion of adult neovascularization (Aicher et al., 2007) and endothelial repair (Xu et al., 2003). Needless to say, at the current time the precise origin(s) of circulating EPCs remains a matter of intense debate (Kovacic et al., 2007).

In the midst of this controversy, an interesting possibility to emerge is that some circulating EPCs may be derived from the intimal vascular endothelial layer. This revelation has arisen from the fact that (vessel-derived) endothelial cells such as human umbilical vein endothelial cells (HUVECs) and human aortic endothelial cells (HAECs) can be passaged in vitro for ~40 population doublings—a fact that stands in contrast to the widely held belief that these are terminally differentiated, mature endothelial cells. Thus, in a succinct but important study, Ingram et al. (2005) recently undertook an in vitro analysis of HUVECs and HAECs and addressed the
issue of whether these populations contain EPCs. These authors used a novel single-cell deposition assay that discriminates EPCs on the basis of their proliferative and clonogenic potential. In brief, HUVECs, HAECs, and human umbilical cord blood-derived EPCs were compared, and it was found that single cells from these populations were able to undergo at least one division with a frequency of 52, 53, and 55%, respectively. Single endothelial cells derived from cord blood EPCs displayed greater capacity to form large colonies (47%) than HUVECs (28%) and HAECs (27%); however, these vessel-derived populations clearly exhibited significant capacity in this regard. Together, these findings suggest that certain endothelial cells derived from the human aorta or umbilical vein possess progenitor cell potency that is comparable to that of cord blood-derived EPCs.

Although yet to be replicated, this study has significant implications with respect to our understanding of capillogenesis and vascular biology. For example, if EPCs reside among other terminally differentiated and relatively nonproliferative endothelial cells, we must at least consider the possibility that an EPC niche exists in the intimal lining of arteries and/or veins. An intimal EPC niche may be of clinical importance in various scenarios such as reendothelialization after vascular stenting. However, whether this niche exists, and what its characteristics may be, remains speculative at the current time. Also of importance, the distinctions that were previously drawn between vasculogenesis and angiogenesis may now require careful reconsideration. Formerly, these processes were at least partially distinguished on the basis that only vasculogenesis was thought to involve progenitor cell proliferation. The fact that the vessel wall itself may harbor EPCs now suggests that angiogenesis, like vasculogenesis, may be a progenitor cell-mediated phenomenon (Kovacic et al., 2008).

Pericytes were first described by Eberth (1871) and Rouget (1879) over 100 years ago and are ubiquitous subendothelial cells that reside in vessels ranging in size from the microvasculature to the aorta (Fig. 3). While their developmental origins are complex and yet to be fully understood, in formed vessels pericytes are elongated cells embedded within and sharing a common basement membrane with overlying endothelial cells. Pericytes and endothelial cells are intimately connected by tight and gap junctions, and each pericyte may be in contact with several endothelial cells via elongated processes wrapping around and along the blood vessel (Gerhardt and Betsholtz, 2003; Collett and Canfield, 2005). In larger vessels, pericytes may be observed not only as subendothelial cells in the inner intimal layer, but also in the media and adventitia associated with the vasa vasorum (Andreeva et al., 1998). Arguably, however, the best characterization of these cells has been in the microvasculature, where pericytes closely encircle endothelial cells, adding anatomical and functional stability to capillary networks (Benjamin et al., 1998, 1999). The ratio of pericytes to endothelial cells varies widely across differing microvascular beds, from approximately 1:1 in retinal vessels to 1:10 in the lung and 1:100 in striated muscle (Shepro and Morel, 1993). However, the pericyte coverage of any given endothelial cell is typically only partial, with pericyte-free areas thought to be of importance for transvascular transport of gases and nutrients (Gerhardt and Betsholtz, 2003).

Pericytes play an important role during capillogenesis, in which, under the control of platelet-derived growth factor B, they invest budding endothelial sprouts and control the proliferation and differentiation of endothelial cells (Betsholtz et al., 2005). Putative mechanisms whereby pericytes may modulate these processes include initial patterning of vascular networks, regulation of EPC/endothelial cell proliferation and differentiation, regulation of capillary size, and synthesis of extracellular matrix proteins. Studies of genetic knockout mice have demonstrated that capillaries and microvessels devoid of pericytes develop a series of abnormal features, including endothelial hyperplasia, tortuosity, abnormal endothelial junctions, and increased leakage of plasma and erythrocytes (Hellstrom et al., 2001; Betsholtz et al., 2005; Collett and Canfield, 2005). A further important feature of pericytes is that they possess smooth muscle cell-like contractile properties and are implicated in the regulation of microvasculature tone (Shepro and Morel, 1993).

Of relevance to this review, significant evidence also points to the existence of perivascular stem/progenitor cells and a potential perivascular stem cell niche. This concept is arguably traceable to the 1980s, when it was described that pericytes are able to differentiate into osteoprogenitor cells and participate in cranial bone formation (Sato and Urist, 1985). Several years later it was noted that pericyte-like cells, derived from various sites including the retinal microvasculature (Schörl et al., 1990) and the medial aortic layer (Bostrom et al., 1993), were capable of osteoblastic differentiation and were likely responsible for arterial calcification during the atherosclerotic process (Bostrom et al., 1993).
Subsequent studies have clarified that pericytes express α-smooth muscle actin and various characteristic marker proteins or surface antigens, including alkaline phosphatase (ALP), CD146, and NG2 (Dellavalle et al., 2007; Peault et al., 2007). In contrast, pericytes do not express myogenic (CD56) or common endothelial antigens (VE-cadherin, von Willebrand factor, or CD31). A precise phenotypic characterization at the cellular level of what proportion of pericytes are multipotent progenitor cells remains to be performed. However, current evidence suggests that a significant proportion may be competent for the generation of a wide variety of mesenchymal tissues, including skeletal muscle (Dellavalle et al., 2007; Peault et al., 2007), osteoblasts, cartilage (Doherty et al., 1998), and adipose tissue (Farrington-Rock et al., 2004).

Recently, numerous lines of evidence have converged to suggest that several “tissue-specific” populations of oligopotent mesenchymal progenitors are actually pericytes. This has implications for our understanding of progenitor cell biology, as it suggests that the notion of tissue-specific progenitor populations, at least for mesenchymal tissues, may have been overplayed. Rather, ubiquitous pericytes, with a pan-organ distribution but perhaps with a predilection to form the principal cells of their host tissue (adipocytes, skeletal myocytes, etc.), may be at the core of many of these “tissue-specific” populations (Andreva et al., 1998; Peault et al., 2007). For example, it has been previously demonstrated that the “stromal vascular fraction” obtained after the enzymatic digestion and culture of adipose tissue has a broad mesenchymal differentiation potential (Zuk et al., 2002). Recently, independent groups have provided strong evidence to suggest that adipose-derived pericytes are the principal progenitors within the “stromal vascular fraction” responsible for this mesenchymal differentiation potency (Traktuev et al., 2008; Zannettino et al., 2008). As a further example, the sorting of cells from human skeletal muscle as being ALP−CD56− (Dellavalle et al., 2007) or CD146−CD34−CD45−CD56− (Peault et al., 2007) has been proposed to identify a population of purified pericytes that are potent for skeletal muscle regeneration. In follow-on experiments, the use of pericytes for the generation of normal skeletal muscle is now under investigation for the treatment of myopathic disorders such as Duchenne muscular dystrophy (Dellavalle et al., 2007). With respect to teeth, Shi and Gronthos (2003) have localized and identified human dental pulp progenitor cells as being pericytes, while others have shown that dental pulp-derived progenitors are potent for the generation of odontoblast-like cells (Alliot-Licht et al., 2005). Finally, Dore-Duffy et al. (2006) have reported that, at least in vitro, central nervous system pericytes are able to differentiate into cells characteristic of neurons and glial cells.

Interestingly, pericytes and BM-derived mesenchymal stromal cells (MSCs) share several properties, including the repertoire of mesenchymal tissues they are able to give rise to and the expression of certain surface antigens, including STRO-1 (Doherty et al., 1998; Shi and Gronthos, 2003; Alliot-Licht et al., 2005). Provocatively, studies aimed at identifying non-BM MSCs have shown that, in fact, MSCs reside in virtually all postnatal organs and tissues in a subendothelial perivascular location. Furthermore, like pericytes, MSCs frequently express α-smooth muscle actin (da Silva Meirelles et al., 2006). Thus, taken as a whole, evidence is accumulating to suggest a close relationship between MSCs and pericytes. Conceivably, given that MSCs (and pericytes) isolated from disparate tissues exhibit subtle variability in differentiation capability, it is possible that MSCs are ubiquitous perivascular progenitor cells distributed throughout the body, but with a degree of lineage allegiance to their host tissue. This hypothesis would also stand for BM-derived MSCs, of which, residing within a perivascular niche within postnatal BM (Shi and Gronthos, 2003), one of their tissue-specific roles is the support and maintenance of the HSC niche. Consistent with this, it has been shown that non-BM-derived MSC-like cells from the artery wall are able to support hematopoietic colonies (Tintut et al., 2003). However, as mentioned above, it is possible that pericytes may also be competent for the generation of nonmesenchymal lineages such as neural cells (usually considered to be ectoderm-derived). Evidently, our understanding of these mysterious but omnipresent vessel-resident progenitor cells is far from complete.

Medial and Adventitial Progenitor Cells

Inspired by subtle signals indirectly suggesting the existence of mural vascular progenitor cells, Hu et al. (2004) are to be credited with providing the first systematic evaluation of the possibility that progenitor cells reside in the media or adventitia of mature vessels. Using immunohistochemical staining of vessels from Apo-E-deficient mice, they found that the adventitia of the aortic root harbors large numbers of cells bearing typical stem/progenitor cell markers, including Sca-1 (21%), c-kit (9%), and CD34 (15%). Only very rare cells with these surface antigenic characteristics were identified in either the media or the intimal vascular layers—an important negative finding, as Sca-1 is also expressed by apparently mature (intimal) endothelial cells (van de Rijn et al., 1989). Turning their attention to the adventitial Sca-1−fraction, Hu et al. (2004) determined that these cells could differentiate into smooth muscle cells both in vitro and in vivo. Thus, adventitial Sca-1−progenitor cells gave rise to the majority of smooth muscle cells that formed the proliferative neointimal component in a vein graft transplantation model in Apo-E knockout mice. Importantly, it was also shown that adventitial progenitor cells were not derived from circulating BM cells, but rather, these appeared to be bona fide resident vascular progenitor cells (Hu et al., 2004).

Grenier et al. (2007) also provided support for the existence of a vessel-resident Sca-1−progenitor. These authors described a population of Sca-1−CD31−Lin− cells that could be purified by the enzymatic digestion of “small” murine vessels obtained from dermis, adipose tissue, and skeletal muscle. While these Sca-1−CD31−Lin− progenitor cells did not display robust in vitro adipogenic, neurogenic, or myogenic potential, they were able to generate high numbers of apparently mature endothelial cells. Further, Sca-1−CD31−Lin− progenitor cells, or their progeny, were able to form cord-like vascular structures in vitro and participated in capillogenesis in a rodent ischemic hind-limb model (Grenier et al., 2007). However, while Hu et al. (2004) (see above) carefully described that their Sca-1−progenitor cell is resident within the adventitia, a major limitation of the Grenier et al. (2007) study is that a precise anatomical localization of their EPC-like Sca-1−cells was not performed.
<table>
<thead>
<tr>
<th>Stem/progenitor cell (or process)</th>
<th>Localization of stem/progenitor cell (or process)</th>
<th>Major daughter cells and/or progeny arising from this cell/location</th>
<th>Important features of these vessel-resident cells</th>
<th>Other comments</th>
</tr>
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<tbody>
<tr>
<td>Embryonic hemogenic cells and hematopoietic stem cells</td>
<td></td>
<td>HSCs, which are released from the embryonic aortic wall into the circulation and which then circulate and establish secondary hematopoiesis in distal sites (liver, spleen, etc.)</td>
<td>HSCs emanate from the ventral domain of the dorsal embryonic aorta and play an important role in establishing definitive hematopoiesis in the embryo</td>
<td>HSCs are generated in embryonic aorta only for a brief period during development; the exact nature of this process is controversial</td>
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<tr>
<td>Embryonic mesoangioblasts</td>
<td></td>
<td>Endothelium, skeletal muscle, and various other mesoderm-derived tissues (bone, cartilage, dermis, etc.)</td>
<td>Reside in the roof and lateral walls of the dorsal embryonic aorta; devoid of hematopoietic capacity</td>
<td>Developmental role is unclear—the bulk of postnatal mesoderm-derived tissues are established via other canonical embryonic sources such as somites</td>
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<td>Intimal endothelial progenitor cells</td>
<td></td>
<td>Endothelial cells</td>
<td>Capacity of intimal EPCs to divide and proliferate is comparable to that of umbilical cord blood-derived EPCs</td>
<td>Recognized only recently; biologic role remains to be determined</td>
</tr>
<tr>
<td><strong>Pericytes</strong></td>
<td>Broad mesenchymal differentiation potential, often with predilection to form the principal cell of their host tissue (adipose, skeletal muscle, etc.)</td>
<td>Ubiquitous subendothelial cells that reside in vessels ranging in size from the microvasculature to the aorta</td>
<td>Pericytes show contractile ability, but also significant phenotypic and functional overlap with MSCs</td>
<td></td>
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<tr>
<td><strong>Medial and adventitial progenitor cells</strong></td>
<td>Different medial and adventitial progenitor populations are able to give rise to endothelial and/or smooth muscle cells</td>
<td>Several populations described in both the medial and the adventitial layers, including SP, CD34⁺, and Sca-1⁺ cells</td>
<td>The interrelationships of these medial and adventitial progenitor populations are unclear; significant overlap may exist, including also with pericyte</td>
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<td><strong>Endothelial to mesenchymal transition</strong></td>
<td>Smooth muscle cells, fibroblasts, and other mesenchymal cells</td>
<td>Certain endothelial cells exhibit capacity for phenotypic modulation to other mesenchymal cell phenotypes</td>
<td>An important developmental program; importance in adult pathologic processes and malignancy is increasingly recognized; not considered a “stem cell phenomenon”</td>
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Therefore, it is possible that these Sca-1+CD31−Lin− cells were merely peripherally associated with “small vessels,” rather than being actually resident within the wall. Nonetheless, these EPC-like Sca-1+ cells seem unlikely to have been pericytes, as they expressed a broad range of endothelial antigens and were unable to give rise to the classic pericyte repertoire of mesenchymal tissues.

In contrast to the findings of Hu et al. (2004), it has now been described that progenitor cells also reside in the medial layer of healthy arteries of adult mice (Sainz et al., 2006). Isolated based on their ability to extrude Hoechst-dye, these cells were described by Sainz et al. (2006) as so-called “side population” (SP) cells, which also display a Sca-1+CD31−Lin−CD34+/low profile and represent 6% of all cells in the media of the adult murine aorta but were not present within the adventitia. Medial SP cells were unable to give rise to hematopoietic colonies in vitro, but were competent for the generation of both endothelial and smooth muscle cell progeny and able to form arborescent vessel-like structures when cultured on Matrigel (Sainz et al., 2006).

Remarkably, a third population of progenitor cells has been reported to reside between the medial and the adventitial layers of a wide range of large and medium-sized human arteries and veins (Zengin et al., 2006) and the thoracic aorta (Pasquinelli et al., 2007). In the first of these studies, using predominantly human internal thoracic artery samples, Zengin et al. (2006) identified CD34+CD31− cells at the inner border of the adventitial layer, in close proximity to the external elastic membrane and lying immediately outside the medial smooth muscle layer. Unlike the populations described by Hu et al. (2004) or Sainz et al. (2006), but similar to those described by Greinier et al. (2007), these CD34+CD31− cells were an EPC-like population, providing a significant endothelial contribution to newly formed capillary-like tubes ex vivo. Although robust lineage-tracking systems were not used, the ability of these CD34+ progenitors to give rise to smooth muscle cells was thought to be low, leading the authors to adopt the term “vascular wall-resident CD34+/low endothelial precursor cells.” The existence of CD34+CD31− cells at the medial-adventitial border zone was also confirmed in vessels from a wide range of human organs, including bladder, kidney, heart, brain, lung, and liver (Zengin et al., 2006). Subsequently, Pasquinelli et al. (2007) corroborated and extended this work by reporting that CD34+ cells are also resident between the medial and the adventitial layers in the human thoracic aorta.

In the same publication but with no clear relationship to the above-mentioned CD34+ population, Pasquinelli et al. (2007) also performed ex vivo culture of digested whole thoracic aortic segments. This gave rise to cells that exhibited the surface antigenic characteristics of MSCs (CD44+CD90+CD105+) and that were able to form capillary-like structures (Pasquinelli et al., 2007). Others have similarly described the isolation of progenitor cells with broad mesenchymal differentiation potential after ex vivo culture of digested vessel segments (Tintut et al., 2003). While it is possible that MSC-like populations occupy a specific (and hitherto undescribed) niche within the vascular wall, to the casual observer it would perhaps appear more likely that these investigators had merely isolated and expanded pericytes that were resident within the vascular wall, perhaps from the vasa vasorum. This example emphasizes the possibility, or perhaps the likelihood, that there may be significant overlap between pericytes and the above-mentioned medial and adventitial progenitor populations. Moreover, the apparently contradictory findings of several of these publications relating to the presence of progenitor cells in the medial versus the adventitial layers, or their profile of surface antigen expression, highlight the need for further research to characterize and delineate these populations properly.

**Endothelial-to-Mesenchymal Transition**

In addition to potentially harboring EPCs, a further fascinating property of the endothelial intimal layer is endothelial-to-mesenchymal transition or transformation (EndMT). EndMT is a specific example of the broader and well-described biologic process known as epithelial-to-mesenchymal transition or transformation (EMT) (E.M. Zeisberg et al., 2007a). While purists might reasonably argue that this is not a stem or progenitor cell-mediated phenomenon, we believe that EndMT exhibits sufficient “progenitor-like” features to warrant further exploration in the final sections of this review.

During development, as the heart tube begins to loop, a subset of endothelial cells that form the atrioventricular and outflow-tract endocardium undergo EndMT. This involves the loss of intercellular adhesions and detachment (“delamination”) of endocardial endothelial cells from neighboring cells, dramatic changes in cell morphology and cytoskeletal structure, and degradation of the basal lamina. These transformed (now mesenchymal) cells migrate into the adjacent cardiac jelly (an underlying expanded layer of extracellular matrix) resulting in the formation of the cushion mesenchyme and, ultimately, the cardiac valves and part of the septa (reviewed in Mjaatvedt et al., 1999). Endocardial EndMT occurs in response to regionalized myocardial signals, including the transforming growth factor (TGFB)-β (Brown et al., 1996; Ransdell and Markwald, 1997; Nakajima et al., 1998), bone morphogenic protein (BMP) (Mjaatvedt et al., 1999), Wnt/β-catenin (Liebner et al., 2004), and Notch (Timmerman et al., 2004) signaling pathways. This developmental program is likely to be under strict spatiotemporal control involving the action of several or all of these pathways in their correct sequence, with clear evidence that the isolated application of even the predominant pathway (TGFB-β) induces only incomplete transition to a smooth muscle cell phenotype (Hautmann et al., 1999).

Apart from cardiac valve formation, EndMT/EMT may be involved in the development of aortic smooth muscle cells (DeRuitter et al., 1997; Arciniegas et al., 2000), the outermost (epicardial) cardiac layer (reviewed in Wessels and Perez-Pomares, 2004), and certain other organs (Thiery, 2002; Nawshad et al., 2004). EMT has also been implicated in several pathological states, including oncogenesis and tumor metastasis (Thiery, 2002; Timmerman et al., 2004) and chronic fibrosing-type injuries in the liver (M. Zeisberg et al., 2007b), kidney (Zeisberg et al., 2003), and other organs (Kalluri and Neilson, 2003). A likely mechanism whereby EMT may contribute to chronic fibrosis is the formation of fibroblasts from resident epithelial cells, for example, from hepatocytes (M. Zeisberg et al., 2007b) or renal epithelia (Iwano et al., 2002). Similar to EMT during development, in fibrosing conditions EMT has been predominantly described as being guided by the TGF-β and BMP signaling pathways.
However, an important contribution to our molecular understanding of this process was the discovery that a fundamental aspect of tumorigenesis arising from EMT is the repression of E-cadherin transcription and that the transcription factor Snail is a “master gene,” which governs E-cadherin down-regulation (Cano et al., 2000). That E-cadherin repression is involved in EMT has biologic plausibility, as E-cadherin is fundamental for cell-cell adhesion, and its loss would therefore facilitate epithelial delamination. Adding clinical relevance, an E-cadherin germ-line mutation has been associated with early-onset high-grade gastric tumors in a large kindred from New Zealand (Guilford et al., 1998).

Multiple publications have also demonstrated that endothelial cells, including those derived from the adult, can be induced to undergo EndMT in vitro (Arciniegas et al., 1992; Paranya et al., 2001; Frid et al., 2002; Ishisaki et al., 2003). These studies have included proofs that clonally derived populations may give rise to both endothelial and smooth muscle cells, prompting some authors to adopt terms such as “plasticity” and “transdifferentiation” and to suggest that EndMT may arise from progenitor cells (Paruchuri et al., 2006). Others have also suggested that inflammatory stimuli may direct microvascular endothelial cells to transition into mesenchymal-like cells (Romero et al., 1997) and that pulmonary arteriolar endothelial cells may transdifferentiate into smooth muscle-like cells under the influence of myocardin (Zhu et al., 2006).

Of great relevance, albeit as somewhat of a surprising paradigm shift, endothelial cells were very recently described as participating in cardiac fibrosis via EndMT. Using murine models of cardiac fibrosis/heart failure in association with a Cre-lox genetic system to mark permanently cells that have ever expressed Tie1 (an endothelial marker), E.M. Zeisberg et al. (2007a) reported that cardiac fibrosis is associated with the emergence of fibroblasts derived from endothelial cells. In addition, like during cardiac valve formation, TGF-β1 was found to induce cultured endothelial cells to undergo EndMT, whereas BMP-7 preserved the endothelial phenotype. Of potential clinical relevance, the systemic administration of recombinant human BMP-7 significantly inhibited EndMT and the progression of cardiac fibrosis (E.M. Zeisberg et al., 2007a), a finding consistent with a previous report that TGF-β1-neutralizing antibody administration prevents myocardial fibrosis (Kuwahara et al., 2006).

While the cells undergoing EndMT obviously exhibit some progenitor-like features, we reiterate that the consensus view, at the present time, is that EMT/EndMT falls short of explaining the definition of a progenitor cell phenomenon. In particular, it has been demonstrated that EndMT can occur in the absence of cell division (Paranya et al., 2001; Frid et al., 2002). Nevertheless, the previously described finding that EPCs may reside in the intimal endothelial layer raises the possibility that EPCs may be involved in EndMT. Also, it was very recently suggested that the process of EMT may result in the generation of cells with many of the properties of self-renewing stem cells (Mani et al., 2008). Metically designed studies are now required to delineate fully the progenitor cell status (or otherwise) of EndMT and, most importantly, which aspects of this process might be exploited or manipulated for therapeutic gain.

Conclusion

A remarkable biological story is emerging: a surprising number of stem and progenitor cells reside within the embryonic, developing, and mature vasculature (Table 1). Further to this, certain endothelial cells or EPCs may be capable of a hitherto unappreciated degree of cellular plasticity in the form of EndMT/EMT. A particularly compelling aspect of many of the vascular stem, progenitor, and other cells discussed in this review is that, as a component of the vasculature, they have quasi-omnipresence throughout the body, with a virtual pan-organ distribution. This, along with the fact that many of the mature cell types that these vessel-resident progenitor cells are able to give rise to relate to host tissues (skeletal muscle, adipose tissue, etc.), rather than the vasculature per se, suggests a significant role(s) in developmental and adult biology.

With embryonic HSCs as an obvious exception, currently the true importance of these vessel-resident stem and progenitor populations is largely unknown. We must be mindful that several of these vascular stem/progenitor cells have only just been described and that our overall knowledge and understanding are limited. Indeed, the possible role of vessel-resident progenitor cells in the evolution of atherosclerotic vascular disease, the No. 1 killer in Western societies, is poorly understood. Nonetheless, the prospect that the therapeutic manipulation of vessel-resident stem and progenitor cells may be used for the treatment of any number of a broad range of clinical disorders is particularly exciting. A further understanding of the function and contribution of resident vascular progenitor cells to development, homeostasis, and disease should provide a rational platform for the successful clinical exploitation of these cells.

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