Cells within the adult vasculature were originally considered to be terminally differentiated. Although vascular integrity underpins normal tissue physiology, vascular plasticity, including new vessel formation (vasculogenesis) and sprouting (angiogenesis) contributes to both healthy tissue repair and also disease development, highlighted by its importance in atherogenesis and tumorigenesis. Investigations of the dynamic nature of blood vessels have defined the vasculature as a reservoir for multipotent stem/progenitor cell populations. At present, knowledge in this nascent field is emerging and uncertainties abound. Although threads of data have increasingly implicated vascular wall progenitor cells (VW-PCs) in maintaining normal vessel homeostasis and mounting reparative and pathological responses in disease, there remains much to be learnt about their precise identities, developmental and postnatal origins, distribution, regulation, biological properties, lineage fates, and physiological and pathogenic roles. This review discusses the current body of evidence for the existence of vascular wall progenitor cell subpopulations from development to adulthood and addresses the gains made and significant challenges that lie ahead in trying to accurately delineate their identities, origins, regulatory pathways, and relevance to normal vascular structure and function, as well as disease. (Circ Res. 2015;116:1392-1412. DOI: 10.1161/CIRCRESAHA.116.305368.)

Key Words: atherosclerosis ■ endothelial progenitor cells ■ macrophage progenitor cells ■ mesenchymal stem cells ■ smooth muscle progenitor cells

Vascular Wall Progenitor Cells in Health and Disease

Peter J. Psaltis, Robert D. Simari

Abstract: The vasculature plays an indispensible role in organ development and maintenance of tissue homeostasis, such that disturbances to it impact greatly on developmental and postnatal health. Although cell turnover in healthy blood vessels is low, it increases considerably under pathological conditions. The principle sources for this phenomenon have long been considered to be the recruitment of cells from the peripheral circulation and the re-entry of mature cells in the vessel wall back into cell cycle. However, recent discoveries have also uncovered the presence of a range of multipotent and lineage-restricted progenitor cells in the mural layers of postnatal blood vessels, possessing high proliferative capacity and potential to generate endothelial, smooth muscle, hematopoietic or mesenchymal cell progeny. In particular, the tunica adventitia has emerged as a progenitor-rich compartment with niche-like characteristics that support and regulate vascular wall progenitor cells. Preliminary data indicate the involvement of some of these vascular wall progenitor cells in vascular disease states, adding weight to the notion that the adventitia is integral to vascular wall pathogenesis, and raising potential implications for clinical therapies. This review discusses the current body of evidence for the existence of vascular wall progenitor cell subpopulations from development to adulthood and addresses the gains made and significant challenges that lie ahead in trying to accurately delineate their identities, origins, regulatory pathways, and relevance to normal vascular structure and function, as well as disease. (Circ Res. 2015;116:1392-1412. DOI: 10.1161/CIRCRESAHA.116.305368.)

Key Words: atherosclerosis ■ endothelial progenitor cells ■ macrophage progenitor cells ■ mesenchymal stem cells ■ smooth muscle progenitor cells

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cell populations that can generate vascular cell progeny but principally reside outside the vessel wall, namely bone marrow (BM) and circulating progenitor cells, induced pluripotent stem cells (iPSCs) and embryonic stem cells (ESCs). Special focus is given to the niche-like properties of the vascular wall, most notably its adventitial layer, which functions as a complex repository and processing compartment for progenitor cells and their interactions with the other constituents of the vessel wall. Finally, we consider the untapped potential for implementing VW-PCs into cardiovascular therapeutics.

**Embryonic Vasculature as a Stem Cell Source**

Vasculogenesis is the de novo process by which blood vessels develop during embryogenesis, giving rise to the aorta and its main branches. Cellular components of the vasculature originate from multiple embryonic sources whose progeny converge on the same cell fate. Although most vascular endothelial cells (ECs) and smooth muscle cells (SMCs) are mesoderm-derived, the neural crest also provides some of the cells in the great arteries and central nervous system blood vessels. Intramembranous vasculogenesis begins with the establishment and migration of strands of angioblasts (EC precursors) from the lateral regions of the embryo toward the midline, where they coalesce to form the endocardium, dorsal aorta, and cardinal vein. The limb vessels also arise from angioblasts that migrate from mesodermal somites, while vascular connections are formed in many tissue beds between sprouts of the major vessels and locally generated angioblasts. During development, the endothelial and mural cells of the vasculature are intricately linked with the presence and regulation of stem or progenitor cells, most notably hematopoietic stem cells (HSCs) and multipotent mesoangioblasts that are described in detail in the Online Data Supplement and summarized in Figure 1. The first implication of this association is of symbiotic ontogeny, whereby vascular integrity is fundamental to organogenesis, and equally the local presence of ancestral cells crucially regulates vascular development. Second, there is the notion that these vascular-resident stem/progenitor cells may persist after birth, influencing postnatal growth, aging, and disease.

**Vascular Wall Progenitor Cells in Postnatal Life**

The development of new vessels from progenitor cells continues beyond embryogenesis. Throughout postnatal life, blood vessels are exposed to potentially harmful influences that can threaten their structural integrity and function. The vasculature, therefore, needs to have capacity for cell turnover, growth, and repair to maintain normal homeostasis. Although it could be reasonably argued that this is accounted for by the ability of terminally differentiated vascular cells, namely ECs and SMCs, to re-enter cell cycle and proliferate,

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**Nonstandard Abbreviations and Acronyms**

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
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<tbody>
<tr>
<td>AMPCs</td>
<td>adventitial macrophage progenitor cells</td>
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<tr>
<td>BM</td>
<td>bone marrow</td>
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<tr>
<td>BMP</td>
<td>bone morphogenetic protein</td>
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<tr>
<td>CFUs</td>
<td>colony-forming units</td>
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<tr>
<td>ECs</td>
<td>endothelial cells</td>
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<td>EPCs</td>
<td>endothelial progenitor cells</td>
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<tr>
<td>ESC</td>
<td>embryonic stem cells</td>
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<td>HSCs</td>
<td>hematopoietic stem cells</td>
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<tr>
<td>iPSCs</td>
<td>induced pluripotent stem cells</td>
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<tr>
<td>MSCs</td>
<td>mesenchymal stem/stromal cells</td>
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<tr>
<td>MVSCs</td>
<td>multipotent vascular stem cells</td>
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<tr>
<td>PDGF</td>
<td>platelet-derived growth factor</td>
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<tr>
<td>Shh</td>
<td>sonic hedgehog</td>
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<tr>
<td>SMCs</td>
<td>smooth muscle cells</td>
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<td>SPCS</td>
<td>smooth muscle progenitor cells</td>
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<tr>
<td>TGF</td>
<td>transforming growth factor</td>
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<tr>
<td>VW-EPCs</td>
<td>vascular wall endothelial progenitor cells</td>
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<tr>
<td>VW-MPSCs</td>
<td>vascular wall multipotent stem cells</td>
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<tr>
<td>VW-MSCs</td>
<td>vascular wall MSCs</td>
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<td>VW-PCs</td>
<td>vascular wall progenitor cells</td>
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<tr>
<td>VW-SPCs</td>
<td>vascular wall smooth muscle progenitor cells</td>
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**Figure 1. Vascular origins of definitive hematopoiesis during embryogenesis.** Definitive hematopoiesis begins prenatally with the emergence of hematopoietic stem cells (HSCs) from hemogenic endothelium in the ventral floor of the dorsal aorta in the para-aortic splanchnopleura and subsequent aorta-gonad-mesonephros (AGM) region (embryonic days, 9.5–12.5 in mice). This Runx1-dependent process involves intermediary pre-HSCs that aggregate as intra-aortic hematopoietic clusters (IAHs). Soon after their emergence, HSCs enter the embryonic circulation and robustly seed fetal liver and ultimately postnatal bone marrow. Mesoangioblasts, which lack hematopoietic potential, reside in the roof and lateral walls of the dorsal embryonic aorta and may possibly originate from extraembryonic endothelium. For detailed discussion see Online Data Supplement. SP indicates side population.
it has emerged during the past decade that there exist an array of ancestral progenitor cells resident within the mural layers of macro- and microvessels. As discussed in the following sections, these consist of lineage-committed endothelial progenitor cells (EPCs) and smooth muscle progenitor cells (SMCs). Despite reliance on surface antigens, such as CD34, the EPC banner, without adherence to the necessary criteria for clonal expansion, stemness characteristics, including self-renewal and high proliferative capacity, and ability to adhere to matrix molecules and differentiate into functional, mature ECs, have been the most intensely studied of the different vascular progenitor cell subtypes. The great majority of this work has related to peripheral blood and BM-derived progenitor cells, with numerous publications showing their contribution of endothelial progeny to neovessels at sites of tumorigenesis, wound healing, and ischemia and to intimal re-endothelialization after vessel wall injury. However, throughout the 2000s, the study of EPCs became complicated and hampered by inconsistencies in isolation, characterization, and definition of different proangiogenic cell subsets that were all labeled under the EPC banner, without adherence to the necessary criteria stated above.

Among the numerous controversies surrounding the true identity of EPCs, were revelations that some so-called EPC populations were actually circulating monocytes contaminated with platelet microparticles, or injured or senescent ECs sloughed from the vessel wall into the blood stream. Such confusion has been fueled by the lack of EPC-specific markers. Despite reliance on surface antigens, such as CD34, vascular endothelial growth factor receptor (VEGFR) 2 and CD133 to identify and isolate EPCs, these markers are shared by progenitors of hematopoietic lineage. Similarly, culture-based techniques to isolate EPCs have resulted in distinct cell populations, of which early outgrowth ECs or endothelial colony-forming cells (CFU-Hill) actually consist of hematopoietic colony-forming units (CFU-Hill) in murine and human melanoma cells and breast cancer samples. The authors then verified that the clonal progeny of single c-kit+ ECs could generate opinion has urged that to avoid ongoing confusion, the term EPC should be strictly reserved for the progenitors of late outgrowth endothelial cells or endothelial colony-forming cells, that do not express blood cell lineage markers and have more robust clonal, replicative, and endothelial-forming potency.

Ambiguities aside, data have emerged to indicate that reconstitution of ECs may predominantly occur from local vascular-resident progenitor cells, rather than those originating in BM or recruited via peripheral blood. This was first inferred from small animal studies of EC turnover in allograft atherosclerosis, endothelial dysfunction, wound and tissue healing, and tumorogenesis. Second, resident nonhematopoietic, endothelial-specific precursor cells were isolated from different tissues (eg, muscle, adipose, dermis, liver, and intestine) and shown to contribute to the circulating progenitor cell pool. Several groups have now reported on VW-PCs with endothelial or angiogenic potential. Although in the majority of cases these progenitors have been multipotent with plasticity for nonendothelial lineages also, a few studies have been dedicated to the rigorous characterization of endothelial-specific VW-PCs (Table 1).

On the basis of clonal analysis of human umbilical vein endothelial cells (HUVECs) and human aortic endothelial cells (HAECs), Ingram et al made landmark observations to indicate that the renewal of vessel wall ECs can be attributed to their content of a complete hierarchy of low- and high-proliferative endothelial colony-forming cells. These intima- derived progenitor cells expressed EC surface markers (CD31, CD141, CD105, CD146, CD144, von Willebrand factor, and fetal liver kinase [Flk]-1) and displayed similar clonogenic potential to umbilical cord blood EPCs. Subsequent data have supported this notion that EC turnover is not a stochastic property of mature ECs, but rather the result of a hierarchical organization of different endothelial subpopulations discriminated by their clonogenic potential. Bearzi et al demonstrated that all three mural layers of human coronary arteries contain small clusters of clonogenic c-kit+/VEGFR2+/CD45−/tryptase− progenitor cells that contact neighboring mature cells via gap junctions, and can differentiate toward ECs, SMCs, and in part, cardiomyocytes. The functional capacity of these human VW-PCs was illustrated by their integration into newly formed small, intermediate, and large-sized blood vessels after in vivo transfer in a model of coronary artery stenosis in immunosuppressed dogs. Fang et al later identified a population of Lin−CD31−CD105−Sca-1−/c-kit+ ECs from adult mouse pulmonary vessels that contained a rare (≈0.4%) subset of endothelial colony-forming cells. The term vascular endothelial stem cells was used to describe this clonal subpopulation of c-kit+ ECs, which was shown by transgenic reporter tracing to be of probably endothelial and nonhematopoietic origin. By in situ immunostaining, Lin−CD31−CD105−Sca-1−/c-kit+ ECs were observed at different frequencies in the capillaries, arteries, and veins of a range of organs (lung, liver, kidney, and subcutaneous tissues), as well as in neoangiogenic vessels in subcutaneous matrigel plugs and in murine and human melanoma cells and breast cancer samples. The authors then verified that the clonal progeny of single c-kit+ ECs could generate...
See the image for the complete text.
 genomomic sequences. They then undergo cleavage in the cell nucleus by the RNase III endonuclease Drosha to shorter precursor miRs, which are exported to the cytoplasm and further cleaved by a complex containing the endonuclease Dicer to mature miRs of 18 to 25 nucleotide length. Each miR binds to the 3’ untranslated regions of its target mRNA to bring about post-transcriptional gene silencing and repression of protein production. Individual miRs target several genes, and conversely individual mRNAs can be targeted by multiple miRs, resulting in a high level of complexity of miR regulatory potential. Although there is little published information that specifically relates to miR regulation of hematopoietic and endothelial commitment during the differentiation of pluripotent stem cells. In one study, microarray profiling during different stages of human ESC differentiation to ECs identified roles for miR-181a, miR-181b, and miR-181b. Overexpression of these miRs potentiated generation of ESC–ECs and improved their angiogenic capacity in a murine model of hindlimb ischemia.44 More recently, miR-21 has also been highlighted as mediating EC generation from iPSCs, through upregulation of transforming growth factor (TGF)–β2 pathway signaling.45

Just as specialized ECs in prenatal development give rise to hematopoietic and possibly mesenchymal progeny (Online Data Supplement), there is also the intriguing postnatal phenomenon of endothelial-to-mesenchymal transformation, whereby ECs detach from neighboring cells before undergoing morphological and cytoskeletal transformation to mesenchymal phenotypes. ECs have been observed to generate SMCs during in vitro clonal assays,46 myofibroblast-like cells in pathologic states in vivo and adipogenic, osteogenic, and chondrogenic progeny.48,49 In a recent lineage tracing study, Yao et al49 reported that intimal ECs can give rise to multipotent osteoprogenitor cells that produce vascular calcification during atherosclerosis, especially in the presence of dysregulated bone morphogenetic protein (BMP) activation as occurs in diabetes mellitus. Similarly, a pro-osteogenic phenotype has also been described for circulating CD34+ progenitor cells in human patients with diabetes mellitus.50

Vascular Wall Smooth Muscle Progenitor Cells
Vascular SMCs impart mechanical stability for newly generated vessels and support and regulate vascular tone and blood flow through their contractile properties. Both atherosclerosis and in-stent restenosis are characterized by the accumulation of SMCs in the neointima. A long-held view was that this occurred via a process of dedifferentiation, by which mature SMCs were transformed from their quiescent, contractile state in the tunica media to a proliferative, synthetic phenotype in the subendothelial intima. However, considerable evidence also indicates the importance of various progenitor cell populations to the origins of postnatal vascular SMCs. As for vascular endothelium, chimeric small animal models of atherosclerosis and graft vasculopathy have revealed mixed sources for vascular SMCs. Early studies indicated a predominant role for BM-derived cells, with some demonstrating the SMC plasticity of mononuclear cell subsets from BM and blood. In contrast, others have suggested that there is negligible long-term contribution of BM-derived cells to vascular SMCs, instead pointing to their local origin.55,56 To this end, there are now several reports identifying SMC-relevant progenitor/stem cells in the vasculature itself (Table 2). These encompass multipotent and adventitial SPCs, microvascular pericytes and adventitial MSCs,7 and vascular ECs capable of endothelial-to-mesenchymal transformation.46,48

By their nature, SPCs are specified for SMC fate but do not express differentiated SMC marker proteins, such as αSMA (Acta2), SM22α (Tagln), SM-calponin (Cnn1), and SM-myosin heavy chain (Myh11).51 Although there are currently no established SPC-specific antigens, stem cell antigen-1 (Sca-1, Ly6a) has been used to identify SPCs in the adventitia of murine vasculature.52,60,61,63 Hu et al53 reported on the prominent expression of well-established progenitor markers (Sca-1, c-kit, CD34, and Flk-1) in the adventitia of aortic roots from apolipoprotein E (ApoE)–deficient (ApoE−/−) mice and showed that cultured explants of vascular adventitia gave rise to SMC marker negative, Sca-1+ progenitor cells that could be differentiated into SMCs in response to platelet-derived growth factor (PDGF)-BB. Lineage tracing determined that these cells were not of hematopoietic origin and, therefore, most probably constitutively resident within the vascular wall itself. Transfer of LacZ-marked Sca-1+ SPCs to the adventitial side of irradiated vein grafts in ApoE−/− mice resulted in their mobilization to the media by 2 weeks and to the neointima by 4 weeks, at which time the donor cells expressed SM22α and made up 30% of neointimal cell content. Notably, this was not the case after transfer of adventitial Sca-1− fibroblasts, whose participation in neointimal formation had previously been described.44

Subsequently, it was determined that Sca-1+CD34+ progenitor cells were predominantly confined to a domain of sonic hedgehog (Shh) signaling in the inner adventitia of large- and medium-sized arteries and veins in mice. This region corresponds to the progenitor-enriched vasculogenic zone that has been described for human adventitia.6,30 Passman et al46 reported that the aortic root of Shh-deficient mice contained markedly diminished numbers of adventitial Sca-1+ cells, which otherwise first appeared in normal development from E15.5 to E18.5, between the ascending aorta and pulmonary trunk, and were not of neural crest origin. Postnatal adventitial Sca-1+ cells expressed markers of Shh signaling (Ptc1, Ptc2, Smo, Gli1, Gli2, and Gli3), transcription factors crucial to SMC differentiation (Srf, myocardin family members), and others thought to maintain the progenitor phenotype by repressing SMC differentiation (Klf4,
Msx1, and Fox04). Furthermore, their multipotency was demonstrated for mural cell (pericytes and SMCs) lineages in the presence of PDGF-BB, and to a lesser extent for ECs and osteogenic cells. Msx1+ cells isolated from neointima were clonogenic and differentiated to ECs and SMCs. Migration and differentiation were mediated by SDF-1/CXCR4 and EGFR signaling, respectively, while they also displayed angiogenic properties in matrigel implants in vivo.

Recently, Xu et al have provided updated findings to build the case that Sca-1+ progenitor cells are involved in vascular lesion development. Further analyses of their mouse vein graft model showed that remodeling adventitia contained a significant number of adventitial Sca-1+ cells in close proximity to vasa vasorum, ≈5% to 10% of which could form primary colonies in culture and were heterogeneous in size and morphology, suggesting different subpopulations of Sca-1+ progenitor cells.62 These adventitia-derived cells possessed multipotent differentiation capacity giving rise to adipocytes, osteoblasts, chondrocytes, and SMCs under specific culture conditions, with the latter found to be dependent on integrin/collagen IV interactions. Moreover, Sca-1+ cells were also isolated from neointimal lesions and these too could be induced toward EC and SMC lineages in culture.60 Evaluation of adventitial Sca-1+ cells seeded onto decellularized vascular scaffolds in a bioreactor system revealed that their migratory ability was mediated by the stromal cell–derived factor-1/CXCR4 chemokine axis and their SMC differentiation potential via signaling through epidermal growth factor receptor, followed by activation of extracellular signal-regulated kinase 1/2 and nuclear translocation of β-catenin.61,62 Interestingly, the drug sirolimus was shown to augment both progenitor cell migration and differentiation through these respective pathways, raising a new potential mechanism for restenosis formation after drug-eluting stent treatment.61

In addition to adventitial progenitor cells, distinct subpopulations of mural SPCs have also been identified in the media of blood vessels.7,29 The first of these described was a side population of Lin−Sca-1+c-kit−/loCD34−/lo cells from murine thoracic and abdominal aorta, with bipotency for EC and SMC differentiation but no hematopoietic-forming capacity.29 A few years later, Tang et al reported the discovery of MVSCs in rat, mouse, and human arteries and veins. Initially identified from enzymatic digests of the tunica media from rat carotid arteries and then during tissue explant culture as small, migratory, and highly proliferative SM-MHC−CNN1−SMALo cells, this population uniformly expressed markers for neural crest (Sox10, Table 2.

<table>
<thead>
<tr>
<th>Authors/Publication</th>
<th>Species/Source</th>
<th>Mural Location</th>
<th>Population Described</th>
<th>Isolation Method</th>
<th>Main Findings</th>
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<tbody>
<tr>
<td>Hu et al5</td>
<td>Mouse thoracic aorta</td>
<td>Adventitia</td>
<td>Adventitial Sca-1+ progenitor cells</td>
<td>Immunoselection of cultured aortic explants</td>
<td>Culture-isolated adventitial Lin−Sca-1− cells differentiated to SMCs, were not of BM origin, and contributed to neointimal SMCs when applied externally to irradiated vein grafts. Sca-1+ cells isolated from neointima were clonogenic and differentiated to ECs and SMCs. Migration and differentiation were mediated by SDF-1/CXCR4 and EGFR signaling respectively, with both stimulated by sirolimus.</td>
</tr>
<tr>
<td>Passman et al6</td>
<td>Mouse embryonic and adult arteries</td>
<td>Adventitia</td>
<td>Adventitial Sca-1+ progenitor cells</td>
<td>Immunoselection of fresh digests</td>
<td>Adventitial Sca-1+ cells differentiated into SMCs and to a lesser extent ECs and osteogenic cells. Shh signaling colocalized with Sca-1 along media-adventitia interface. Adventitial Sca-1+ cells were absent or diminished in Shh-deficient mice.</td>
</tr>
<tr>
<td>Sainz et al29</td>
<td>Mouse aorta</td>
<td>Media</td>
<td>SP cells</td>
<td>Immunoselection of fresh digests</td>
<td>SP cells comprised 6% of cells in media, were Lin−Sca-1−c-kit−/loCD34−/lo, differentiated into ECs and SMCs, and formed vascular-like tubes in matrigel.</td>
</tr>
<tr>
<td>Tang et al7</td>
<td>Mouse, rat, and human vessels</td>
<td>Media</td>
<td>MVSCs</td>
<td>Fresh digests and tissue explant culture</td>
<td>MVSCs were small, migratory, and proliferative SM-MHC− cells, that had clonal and self-renewal capacity and differentiated into mesodermal and ectodermal lineages, including SMCs. MVSCs were responsible for neointimal formation in endothelial denudation model.</td>
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BM indicates bone marrow; EC, endothelial cell; EGFR, epidermal growth factor receptor; MVSC, multipotent vascular stem cell; SDF, stromal cell–derived factor; Shh, sonic hedgehog; SMC, smooth muscle cell; and SP, side population.
Sox1, Snail, vimentin, and nestin), endoderm (Sox17), neural cells (NFM, peripherin, Brm3a, and Phox2b), glia (S100β), and MSCs (CD29 and CD44), but not Sca-1 or CD146, distinguishing them from previously described VW-PCs.5,29,56,65 MVSCs displayed clonal and self-renewal properties in culture, during which they maintained multipotency, which was shown by inducing their differentiation into different ectodermal and mesodermal lineages. Notably, differentiation into SMCs occurred via an intermediate stage of MSC-like progenitor cells, which responded differently to stimulation by vascular growth factors (basic fibroblast growth factor, PDGF-B, and TGF-β1) compared with the starting MVSC population. The authors determined by fate-mapping that MVSCs did not arise from dedifferentiation of contractile SMCs, and that MVSCs from carotid artery but not jugular vein may have a neural crest origin. Finally, they concluded from analysis of a carotid artery endothelial denudation model, that proliferating MVSCs and not dedifferentiated SMCs were responsible for neointimal formation, contributing to newly formed synthetic SMCs, extracellular matrix deposition, and even chondrogenesis.7 This latter finding provocatively contradicted the long-standing dogma that contractile SMCs re-enter cell cycle and undergo phenotypic modulation into proliferative, synthetic SMCs after vascular injury or atherosclerosis. Subsequent criticisms highlighted deficiencies in the experimental design and data analysis performed in Tang’s study, most notably, the use of a nonconditionally regulated lineage tracing system and the possibility that the endothelial denudation model was too severe.7,51

In summary, there is much evidence for ancestral diversity of SMCs in adult vasculature, which may partly account for the phenotypic and functional heterogeneity that these cells exhibit in both health and disease.52 Although preliminary data indicate a possible role for vascular wall SPCs (VW-SPCs) and MVSCs in vascular remodeling and neointima formation, this still requires further investigation, especially in the context of traditional paradigms about SMC dedifferentiation. Some of the known mechanisms that regulate VW-SPC biology include (1) integrin/collagen IV interactions, (2) growth factor and chemokine signaling (eg, PDGF, TGF-β, and stromal cell–derived factor-1), and (3) transcription factor-mediated expression of SMC-specific genes via binding of the myocardin/serum response factor complex to the CArG (CC(AT)6GG) element of their promoter regions.62,66 An excellent review by Majesky et al52 clarifies the critical role of coactivators (myocardin family, eg, myocardin-related transcription factors) and corepressors (eg, Msn1, KLF4, and FoxO4), of serum response factor-CArG binding in SMC promoter/enhancer regions, along with its epigenetic regulation by histone acetylation and methylation, and ATPase-dependent chromatin remodeling complexes, and the influence of miRs (eg, miR-143, miR-145, miR-221, and miR-222) and other intracellular signaling pathways (eg, Rhoa/ROCK1/LIMK1 and Nr3b/Nos4).

Vascular Wall Hematopoietic Progenitor Cells
The fact that definitive HSCs first arise from embryonic aorta (Online Data Supplement) prompts consideration as to whether postnatal blood vessels also harbor ancestral cells of hematopoietic lineage. Robust leukocyte populations are present in the adventitia of healthy and lesion-prone arteries,67 while hematopoietic cells are central in the angiogenic responses to wound healing, ischemia and tumorogenesis, and during all stages in the evolution of vascular diseases, including atherosclerosis and aneurysms. In particular, macrophages contribute critically to atherogenesis, both by collecting in atheroma lesions where they transform into lipid-rich foam cells, and by accumulating rapidly in the adventitia, where they facilitate formation of vasa vasorum and plaque microvessels, key regulators of atheroma burden and stability.68,69 As in other tissues, vascular macrophages are heterogeneous, comprising proinflammatory M1, profibrotic, proangiogenic M2, and other subtypes. M1 cells have been found to dominate in rupture-prone shoulder regions of atheroma, whereas M2 macrophages are more common in adventitia, in early lesions and after plaque regression.68

Traditional teaching has asserted that the sole source of vascular macrophages is through the recruitment and differentiation of BM or spleen-derived circulating monocytes.68 Although macrophage proliferation has long been recognized in atherosclerosis,70 it has generally been overlooked as a source of plaque formation and growth. Recently Robbins et al re-evaluated the relative importance of monocyte influx and macrophage proliferation in atheroma.71 Intriguingly they elucidated a switch in the predominant source of rapidly accumulating plaque macrophages from monocytes in early lesions to local macrophage proliferation without monocyte recruitment in established ones. However, in long-term adoptive transfer experiments, their results still indicated that during the course of 5 months lesional aortic macrophages were primarily replenished via the circulation by donor-derived leukocytes.72 Other groups have also suggested the participation of locally derived macrophages in angiogenesis. Although not a gold standard for specific depletion of monocytes, one study used treatment with cyclophosphamide to induce BM toxicity and thereby reduce circulating monocyte numbers in a rodent model of hindlimb ischemia.72 The fact that this did not negatively affect the accumulation of macrophages in collateralizing vessels was used as evidence for the activation of tissue-resident macrophages or their local vascular precursors.72 Similarly, analyses of the ex vivo arterial ring sprouting assay have revealed early accumulation and outgrowth of CD45+CD68+ macrophages and this too was not affected by cyclophosphamide-induced BM depletion, leading to the inference that the vasculogenic zone of adventitia may contain local macrophage progenitor cells that reside in proximity to other progenitor subtypes.30,39

These observations coincide with a recent paradigm of dual developmental pathways to explain the origins of postnatal tissue macrophages, with some arising from circulating precursors and some by local production.73 Whereas most macrophages and their monocyte precursors descend from the pathway of definitive Runx1 and c-Myb-dependent hematopoiesis that begins in embryonic aorta-gonad-mesonephros (AGM) and ultimately continues in postnatal BM, some subsets (eg, brain microglias, epidermal Langerhans cells, hepatic Kupffer cells) arise from yolk sac (YS)
erythromyeloid progenitors that are dependent on Pu.1 and Csf1r. These macrophages persist after birth and are maintained by local renewal, independently of BM hematopoiesis and monocyte trafficking. Although recent studies of microglia and Langerhans cells have proposed their origins from tissue-resident macrophage progenitors, current expert opinion continues to emphasize the view that local macrophage turnover is because of the self-renewal and proliferation of mature macrophages without loss of functional differentiation.

In addition to the conclusions drawn from arterial ring experiments, three murine studies have used traditional hematopoietic assays to more definitively validate the existence of postnatal vascular wall hematopoietic progenitor cells (Table 3). More than a decade ago, Montfort et al interpreted that vascular intima contains proliferative cells with hematopoietic capacity, after observing long-term chimeric for CD3 lymphocytes when segments of thoracic aorta and inferior vena cava were surgically transplanted beneath the renal capsule of irradiated mice. Recently, two studies from our group have reported that the adventitia of postnatal murine arteries contains a hierarchy of HSCs/HPCs, comprising rare numbers of multilineage progenitor cells and a markedly enriched content of macrophage-dedicated progenitors. Freshly isolated single cell disaggregates of adult C57BL/6 mouse aorta generated the full spectrum of hematopoietic CFUs in methylcellulose-based assays. Compared with BM, spleen and blood, the aorta’s clonal capacity was uniquely predisposed toward macrophage colonies (CFU-M), and this was much greater than that of other nonhematopoietic tissues. Specifically, >90% of aortic CFUs were of macrophage subtype, with a yield of 15 CFU-M per 106 cells or 200 to 300 CFU-M per 12-week-old aorta. Although adoptive transfer experiments confirmed the aorta’s content of rare multilineage HSCs (1 per 4×106 aortic cells), the overwhelming signal was for durable macrophage renewal in primary and secondary transplants. After whole body irradiation and transfer of GFP-labeled BM or splenic cells, the majority of the aorta’s low content of non-M CFUs (CFU-GEMM, -GM, -G and BFU-E) were GFP+, consistent with circulatory surveillance of multipotent HSCs/HPCs, as has been described for other tissues. In contrast, only 3% of its CFU-M and 50% of its macrophages were replenished by donor cells, suggesting that the enriched population of vascular wall macrophages is maintained locally in the vessel wall.

Arterial CFU-M were specifically derived from a subpopulation of Lin−Sca-1+CD45+ adventitial cells (Figure 3), and importantly could not be generated from CD11b+ monocytes or macrophages, nor was their yield affected by clodronate-induced or genetic deletion of circulating monocytes or vascular macrophages. This indicates that the CFU-M capacity of blood vessels cannot be ascribed to monocytes or mature macrophages and is therefore because of a population of AMPCs. Furthermore, the uniqueness of AMPCs as a tissue-resident macrophage ancestral population was emphasized by their expression of Sca-1, which contrasts the absence of this marker in lineage-specified BM macrophage-dendritic cell progenitors (MDPs) and common monocyte progenitors (cMoPs).

The prevalence of AMPCs displayed spatial and temporal heterogeneity, being higher in aorta than other arteries, and higher in weaning age mice than adults, hinting that these progenitors may be seeded in vascular adventitia during prenatal development. Consistent with previous descriptions of culture-isolated Sca-1+CD45+ SPCs, freshly isolated Sca+CD45+ arterial cells had no hematopoietic capacity. This underlines the heterogeneity of the adventitial Sca-1 compartment. Notably its Sca-1+CD45+ subset displayed the highest cell cycle activity in both healthy and atherosclerotic arteries, and further subanalysis determined that this population expressed other markers of macrophage ancestral relevance, including a highly proliferative Lin−c-kit−CD135−CD115+CX1, CR1+CD11b+Ly6C− fraction.

Whether vascular wall hematopoietic progenitor cells and AMPCs specifically contribute to macrophages in atherosclerotic vessels requires further evaluation, including the use of definitive molecular fate-mapping models and vascular graft transposition studies. Robbins et al claimed to exclude a progenitor source for their findings of local macrophage turnover in neointimal plaque, but did so partly based on analysis of vascular wall CFU-GM, which are extremely rare, without assessing for the far more prevalent CFU-M progenitors. We observed that CFU-M-forming AMPCs were more frequent in aortas of atheropane and atherosclerotic ApoE−/− and LDLR−/− mice, and that adventitial transfer of GFP-marked Sca-1+CD45+ cells to carotid arteries of ApoE−/− recipients resulted in long-term engraftment and contribution of macrophage progeny, especially in the adventitia and to a lesser extent in atheroma lesions. One working hypothesis is that AMPCs help to maintain certain subpopulations of vascular macrophages (eg, M2) and participate in adventitial remodeling in pathological states, including the formation of vasa vasorum.

Besides their relevance to normal vascular physiology and disease pathogenesis, other key topics that need to be addressed for the newly described population of AMPCs include (1) their developmental origins, particularly in the context of YS progenitors; (2) the identification and characterization of corresponding macrophage progenitor cells in human vessels; and (3) the regulatory factors involved in AMPC self-renewal, expansion, maturation, and differentiation toward polarized (pro- and anti-inflammatory) macrophages and dendritic cells. Macrophage colony-stimulating factor is of particular interest because it is expressed in the adventitia of murine vessels, and at higher levels in chow-diet and western-diet fed ApoE−/− mice, while its receptor (colony-stimulating factor 1 receptor, CD115) is present on a subset of proliferative adventitial Sca-1+CD45+ cells. It has been shown that macrophage colony-stimulating factor–induced differentiation of BM HSCs toward monocytes/macrophages is regulated by tumor necrosis factor (TNF)−/− mice released from MSCs, which in turn, is under the influence of angiotensin II. Notably, all three of these soluble factors readily stimulate adventitial macrophage accumulation and induce or aggravate vascular remodeling in atherosclerosis and aneurysm formation.
Broadly speaking, postnatal vasculature contains two multipotent mesenchymogenic populations whose similarities have led to conjecture as to their respective identities and ontogenic relationship. For the purpose of this review, we have simplified these into (1) microvascular pericytes and (2) vascular wall MSCs (VW-MSCs).

Pericytes

Despite being first described in the late 1800s and named in the 1920s, the identity, ontogeny, and progeny of pericytes remain somewhat enigmatic.84 Not to be confused with other cells that occupy periendothelial locations in blood vessels (eg, vascular smooth muscle cells, fibroblasts, and macrophages) pericytes are best regarded as mural, and not perivascular cells of the microvasculature. These contractile cells surround and appose microvascular ECs, with which they share a basement membrane that separates much of the pericyte–endothelial interface, except at discrete intercellular contact points, which vary markedly in number. New information is coming to light about the importance of pericytes to the regulation of vascular morphogenesis in development, the maintenance of postnatal vascular homeostasis, and disease pathogenesis.84 In angiogenesis, they serve to pattern vascular networks and facilitate EC growth and differentiation, while regulating vessel tone, caliber, and permeability and providing mechanical stability in established vessels.85 Despite some consistent properties, pericytes are ontogenically, morphologically, and phenotypically heterogeneous. Their embryonic origins in different organs remain unclear but most probably mirror the diversity of mesodermal and neural crest origins for vascular SMCs (Online Data Supplement) and tissue fibroblasts.84 Furthermore, there is uncertainty as to their derivation during postnatal angiogenesis and how this relates to proliferation of pre-existing pericytes, SMCs on neighboring large vessels or differentiation of MSCs. This emphasizes the notion that these three cell types may represent a continuum with shared ancestry, although their hierarchical arrangement requires further elucidation.

At the heart of the ambiguity that has confounded pericyte research is the lack of a single molecular marker that can distinguish pericytes from vascular SMCs or other mesenchymal cells. Contemporary studies define pericytes by a set of criteria that combines their location, morphology, and gene/protein expression profile. The latter typically involves the presence of ≥2 markers, that are variably expressed and not altogether pericyte-specific, including CD146, PDGFR-β, NG2, CD13, αSMA, and desmin.84 During the last decade, numerous studies have indicated that pericytes may constitute multipotent stem/progenitor cells, such as MSCs,58,86,87 white adipose progenitors,88 muscle stem cells,89 neural stem cells,90 and precursors for myofibroblasts.91 Although results from Pdgfrb-Cre and inducible ng2-Cre fate mapping experiments have suggested that pericytes may be an ancestral source of white adipocytes and odontoblasts,87,88 such data are

### Table 3. Studies on Postnatal Vascular Wall Progenitor Cells With Hematopoietic Potential

<table>
<thead>
<tr>
<th>Authors/Publication</th>
<th>Species/Source</th>
<th>Mural Location</th>
<th>Population Described</th>
<th>Experimental Method</th>
<th>Main Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Montfort et al79</td>
<td>Mouse thoracic aorta and inferior vena cava</td>
<td>Intima</td>
<td>Lymphocyte precursors</td>
<td>Vascular tissue transplants</td>
<td>Vascular transplants contained proliferating cells in intima and provided radioprotection to irradiated recipients, with low-grade long-term blood chimerism for CD3+ cells</td>
</tr>
<tr>
<td>Zengin et al30</td>
<td>Human internal thoracic artery and vessels from other organs</td>
<td>Vasculogenic zone between media and adventitia</td>
<td>Macrophage precursor cells</td>
<td>Arterial ring assays</td>
<td>Table 1; CD68+ macrophages newly accumulated in arterial ring adventitial outgrowths, and in segments of ligated femoral artery in monocyte-depleted rats</td>
</tr>
<tr>
<td>Zorzi et al92</td>
<td>Rat aorta</td>
<td>Adventitia</td>
<td>Immature immunocytes</td>
<td>Aortic ring assays</td>
<td>M-CSF induced adventitial outgrowths of proliferating CD45+CD68+CD163+CD169αSMA+ cells, which lacked endothelial markers and differentiated to macrophages and DCs with phagocytic and proangiogenic properties</td>
</tr>
<tr>
<td>Psaltis et al50,11</td>
<td>Mouse aorta, femoral, and carotid arteries</td>
<td>Adventitia</td>
<td>AMPCs</td>
<td>Immunoselection of fresh digests</td>
<td>CFU-M were enriched in adventitial Lin′Sca-1′CD45′ compartment. Sca-1′CD45′ AMPCs displayed macrophage and DC commitment, were not reconstituted from BM or spleen, were increased in atherosclerotic mice and generated adventitial and neointimal progeny after adventitial transfer</td>
</tr>
</tbody>
</table>

AMPC indicates adventitial macrophage progenitor cell; BM, bone marrow; CFU-M, colony-forming unit–macrophage; DC, dendritic cell; and M-CSF, macrophage colony-stimulating factor.
There are clearly similarities between pericytes and MSCs, beginning with the fact that the latter reside in perivascular niches in almost all tissues. Second, pericytes exhibit expression of several MSC markers, such as CD44, CD73, CD90, CD105, and CD146, at the exclusion of hematopoietic, myogenic and most endothelial markers. Finally, once detached from their EC contacts, pericytes adopt other MSC-like properties in culture, including spindle-shaped morphology, proliferative kinetics, clonal self-renewal capacity and multilineage differentiation potential for adipocytes, osteoblasts, chondrocytes, SMCs, and other cell types that may be specific to their tissue of origin (eg, skeletal muscle and cardiomyocytes). They also possess MSC-like propensity to provide immune modulation and mechanical and paracrine support of other cell types, promote angiogenesis and mediate tissue repair. However, it is still unclear how these properties relate to pericyte function in situ and moreover, how pericytes and MSCs are inter-related in their natural state.

Although studies have reported that MSCs in adipose tissue (adipose-derived stem cells [ADSCs]) are perhaps pericytes, the validity of such results have been questioned based on the issue of in situ CD34 expression. Increasingly, phenotypic and genotypic differences have been demarcated between MSCs and microvascular pericytes, and just as not all MSCs can be considered to be pericytes, it has also become apparent that only a subset of pericytes express MSC markers and possess their multilineage plasticity.

As distinct from some earlier reports, the prevailing view is that pericytes are classically CD34−CD146−CD31+, whereas ADSCs are CD34−CD146+CD31+, a phenotype also shared by MSCs from vascular adventitia. Although ADSCs and VW-MSCs typically display negligible expression of pericyte markers (eg, CD146, CD140b, NG2, and αSMA) in situ, these markers may be acquired during culture as they downregulate CD34 and adopt pericyte functional properties.

Further evaluation is still required to delineate if there is indeed a hierarchical relationship between VW-MSCs and pericytes in situ. Converging lines of evidence seem to indicate that pericytes are unlikely to be the source of ADSCs/VW-MSCs. Alternative theories are that pericytes and MSCs are simply discrete cell types residing in close proximity in the vasculature, or that mesenchymogenic pericytes do differentiate naturally from their CD34+ neighbors, especially as the latter seem to be more clonogenic, proliferative, and multipotent. These cells could, therefore, represent a postnatal ancestral source analogous to embryonic CD34+Flk-1+ mesangioblasts.

**Vascular Wall Mesenchymal Stem/Stromal Cells**

Along with confusion that has plagued the distinction between pericytes and MSCs, the field of MSC research itself has been complicated by inconsistencies in nomenclature used to describe overlapping cell populations, that have almost universally been characterized under ex vivo culture conditions. Multilineage MSCs have been isolated from virtually all postnatal tissues, and together with their shared properties with pericytes and the presence of MSC markers lining blood vessels, there is a strong case that they ubiquitously occupy a perivascular niche. Despite a paucity of information directly available for their native state in situ, various functions have been ascribed to perivascular MSCs, including their trophic capacity to support hematopoiesis, regulate immune tolerance, and facilitate angiogenesis, and their propensity to differentiate into tissue-specific and mesenchymal cells to mediate tissue homeostasis and repair.

MSCs have been cultured from each of the mural layers of larger arteries and veins in different species, particularly from adventitia (Table 4). As per BM MSCs, isolation of VW-MSCs has been performed by either traditional plastic adherence-based culture or prospective immunoselection. Although CD34 is not expressed on cultured MSCs, several groups have demonstrated its presence on vascular adventitial MSCs in situ. Campagnolo et al isolated CD34+CD31− cells from fresh enzymatic digests of human saphenous vein to derive a highly proliferative population that expressed pericyte and mesenchymal antigens in culture, exhibited clonogenic and multilineage differentiation potential, and interacted bidirectionally with ECs in angiogenic networks in vitro and in vivo.
CD34+CD31− adventitial cells were also thought to contain MSC-like stromal vascular cells in a study that compared explant cultures of human arteries, veins, and small vessels from adipose tissue. 114 Although morphologically similar, these populations exhibited different functional properties, with arterial stromal cells inducing substantially more mineralization and those from venous and small vessel explants having greater potency for capillary-like network formation. Another study has compared CD34−CD146−CD45−CD31+ (pericytes) and CD34+CD146+CD45−CD31− cells from human white adipose tissue. 65 The latter were identified in the outermost layer of adipose blood vessels of larger size than capillaries, and were therefore, anatomically distinct from pericytes. They natively expressed MSC markers (CD44, CD73, CD105, and CD90) before culture and gave rise to heterogeneous clones, which displayed phenotypic and differentiation properties of MSCs. Notably, CD34+CD146− derived cells proliferated faster than pericytes and only expressed pericyte markers in culture (CD146, PDGFR-β, NG2, and αSMA) when stimulated with angiopoietin-2. 65

Distinctly, Klein et al 9 isolated a population of CD44+ vascular wall multipotent stem cells (VM-MPSCs) from the adventitial vasculogenic zone of human internal thoracic artery. 115 These cells were initially identified as migratory during arterial ring assays, where they coexpressed αSMA, CD90, and nestin, but not CD34 or CD146. Cultured CD44+VM-MPSCs expressed Oct-4 and Sox2, exhibited typical vascular stem cell markers in comparison to mature ECs and SMCs, and epigenetically silenced these genes downregulated their invasive sprouting capacity and induced them toward SMC differentiation. 115

It is unknown to what extent different depictions of VW-MSCs/MSCs have in fact been overlapping cell populations, and how they might relate to other vascular wall cells (eg, pericytes, VW-PCs, and VW-SPCs). VW-MSCs seem to be divided into mature pericytes (αSMA+CD105−), nestin+ progenitors (CD105+αSMA−), and CD105− progenitors. The adventitial Sca-1+ progenitors in situ, they are downregulated with angiopoietin-2 10−11. The latter were identified in the outermost layer of adipose blood vessels of larger size than capillaries, and were therefore, anatomically distinct from pericytes. They natively expressed MSC markers (CD44, CD73, CD105, and CD90) before culture and gave rise to heterogeneous clones, which displayed phenotypic and differentiation properties of MSCs. Notably, CD34+CD146− derived cells proliferated faster than pericytes and only expressed pericyte markers in culture (CD146, PDGFR-β, NG2, and αSMA) when stimulated with angiopoietin-2 65

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The notion that stem/progenitor cells occupy anatomically defined and highly organized niches, has been characterized for the endosteal and vascular niches of HSCs in BM. 117 Here, hierarchies of hematopoietic stem, progenitor, and precursor cells are crucially dependent on support and regulation by neighboring cells (eg, ECs, MSCs, pericytes, osteoblasts, and osteoclasts) and their extracellular and soluble products. Together, these different components of the niche microenvironment provide a finely tuned network that maintains the survival of stem cells and controls the balance between their self-renewal and differentiation, quiescence and proliferation, adhesion and deadhesion, chemoattraction and retention, and mobilization under both steady-state and pathological conditions. In the case of HSCs and MSCs distributed throughout different tissues, the microvasculature itself is a fundamental constituent of the stem cell niche.

Vascular Wall Stem Cell Niche

There are substantial difficulties in trying to compare subsets of VW-PCs across different studies because of differences in species, vascular tissues, isolation strategies, and assays used, along with the nonspecific nature of surface marker profiles ascribed to these populations. The adventitial Sca-1+ (mouse) and CD34+ (human, mouse) compartments are heterogeneous, containing mixtures of progenitor and mature cell subsets. Multipotent MSC-like cells have been characterized as both CD34+ and CD34−, with the CD34 marker also associated with adventitial EPCs. Similarly in mice, Sca-1 has been used to study both culture-isolated SPCs and freshly isolated AMPCs, although coexpression of the panleukocyte antigen, CD45, can be used to distinguish between the two 5,6,11. Sca-1+CD45− lymphocytes are also seeded in the adventitia from peripheral circulation, making the focused study of locally resident AMPCs even more challenging. Another confounder is that although both Sca-1 and CD34 are expressed on adventitial progenitors in situ, they are downregulated with culture, as evident during expansion of MSCs and SPCs. 5,8
this time, it is also unclear as to the degree of overlap between adventitial CD34+ progenitors described in human vessels and Sca-1+ cells in mice. Although reported in at least one study,118 the study of Sca-1+ counterparts in human arteries is significantly hampered by the lack of a human ortholog for the Sca-1 marker.119 Notably, there is coexpression of CD34, Sca-1 and c-kit in murine adventitia,5,10,11 suggesting that their combined use may help to identify progenitor subpopulations with higher precision, and this may be further improved by inclusion of other lineage-specific markers (eg, PDGFR-β for SPCs; CX3CR1 and CD115 for AMPCs; and CD133 and VEGFR2 for EPCs).

To summarize, considerable work is still required to fully characterize the different subpopulations of VW-PCs and

<p>| Table 4. Selected Studies on Postnatal VW-MSCs |</p>
<table>
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<tr>
<th>Authors/Publication</th>
<th>Species/Source</th>
<th>Mural Location</th>
<th>Population Described</th>
<th>Isolation Method</th>
<th>Main Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tintut et al111</td>
<td>Bovine aorta</td>
<td>Media</td>
<td>CVCs</td>
<td>Dilutional cloning of SMCs</td>
<td>CVCs were CD29+CD44+CD4-5+CD14-, self-renewing, had chondrogenic, osteogenic, leiomyogenic but not adipogenic potential, and supported hematopoietic colonies</td>
</tr>
<tr>
<td>Covas et al110</td>
<td>Human saphenous vein</td>
<td>Intima</td>
<td>MSCs</td>
<td>Culture of inner surface of veins</td>
<td>Culture-isolated VW-MSCs were CD13+CD29+CD44+CD90+CD45+CD34-HLA-DR+, showed classical trilineage differentiation potential and had higher expression of angiogenic genes than other MSCs</td>
</tr>
<tr>
<td>Pasquinelli et al138</td>
<td>Human thoracic aorta, aortic arch, and femoral artery</td>
<td>Media-adventitia interface</td>
<td>MSCs</td>
<td>Culture of whole arterial wall digests</td>
<td>Culture-isolated VW-MSCs expressed stem (Stro-1, Notch-1, and Oct-4) and mesenchymal (CD44, CD90, CD105, CD73, CD29, and CD166) markers, displayed chondrogenic, adipogenic and leiomyogenic but less osteogenic potential, and formed capillary-like tubes in vitro</td>
</tr>
<tr>
<td>Hoshino et al113</td>
<td>Human pulmonary artery</td>
<td>Adventitia</td>
<td>MSCs</td>
<td>Culture of adventitial fibroblasts</td>
<td>Cultured adventitial fibroblasts expressed vimentin, collagen I, CD29, CD44, and CD105 and had osteogenic, adipogenic, and leiomyogenic potential</td>
</tr>
<tr>
<td>Campagnolo et al34</td>
<td>Human saphenous vein</td>
<td>Mixed</td>
<td>SVPs</td>
<td>Immunoselection of fresh digests of total vessel wall</td>
<td>Cultured CD34+CD31+ cells were clonogenic, expressed MSC and pericyte markers, displayed MSC and NSC-like differentiation potential, and acted as pericytes to integrate with ECs in vascular networks in vitro and in vivo</td>
</tr>
<tr>
<td>Pasquinelli et al59</td>
<td>Human thoracic aorta, aortic arch, and femoral artery</td>
<td>Media-adventitia interface</td>
<td>MSCs</td>
<td>Immunoselection of fresh digests of SVF</td>
<td>CD34+CD146+ adventitial cells natively expressed MSC markers and gave rise in culture to clonogenic multipotent MSCs with potential to also acquire pericyte traits</td>
</tr>
<tr>
<td>Klein et al8,115</td>
<td>Human internal thoracic artery</td>
<td>Adventitia</td>
<td>MPSCs</td>
<td>Immunoselection of fresh digests</td>
<td>Cultured CD44+ cells expressed stem (Oct-4 and Sox2) and MSC markers and showed clonal capacity and multipotency, including for pericytes in vivo</td>
</tr>
<tr>
<td>Zaniboni et al112</td>
<td>Porcine aorta</td>
<td>Media</td>
<td>MSCs</td>
<td>Culture of tunica media digests</td>
<td>Culture-isolated MSCs were CD44+CD56+CD90+CD105+CD34+CD45+, with mesenchymal differentiation potential and formed capillary-like networks with HUVECs</td>
</tr>
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</table>

CVC indicates calcifying vascular cell; EC, endothelial cells; HUVEC, human umbilical vein endothelial cell; MPSC, multipotent stem cells; MSC, mesenchymal stromal/stem cell; NSC, neuronal stem cell; SMC, smooth muscle cells; SVF, stromal vascular fraction; SVP, saphenous vein progenitor cell; and VW-MSC, vascular wall MSC.
elucidate their respective hierarchical organizations, as well as the different mechanisms responsible for their activation, differentiation, and mobilization in their local environment and potential release into the peripheral circulation. Although some of the purported regulators of VW-PCs have been discussed in the sections above, it must be emphasized that relatively few studies have specifically examined these in the context of the vascular wall niche itself. One important exception is that of Shh signaling, which strikingly localizes to the progenitor-rich adventitia in murine aortic root and thoracic aorta, and has been shown to be integral to the regulation of adventitial Sca-1+CD45− SPCs. Although it is not yet known if Shh has similar importance for Sca-1+CD45+ AMPCs, it is worth noting that this pathway does modulate primitive hematopoiesis via downstream BMP signaling, along with monocyte/macrophage chemotaxis, with a recent study showing that its Patched receptor is strongly expressed in macrophages in human atherosclerotic lesions. Extrapolating from stem/progenitor cell niches in other tissues, it is anticipated that the Wnt, Notch, and BMP signaling pathways may also play roles in the adventitial niche.

In addition to their interactions with each other, it is probable that different VW-PCs are also under the regulatory control of other vascular wall cell populations that help to maintain the stem cell niche, namely adventitial fibroblasts, adventitial and perivascular adipocytes, resident and migratory inflammatory cells (macrophages, mast cells, neutrophils, and lymphocytes), and adventitial neurons (Figure 3). Considerable preclinical and clinical research has identified an association between epicardial adipose tissue and cardiovascular disease, which seems at least, in part, to be mediated via the paracrine effects of adipokines on coronary atherosclerosis. Adipocytes are already known to contribute to stem cell niches in other tissues, including as negative regulators of BM hematopoiesis. Although still speculative, it is easy to extrapolate that perivascular adipocytes might impart some of their influence on vascular wall health and disease through their close proximity with the adventitial stem cell niche.

Recent reviews have aptly described the adventitia as a dynamic and complex biological processing center for the retrieval, integration, storage, and release of key regulators of vessel wall function. It provides an interface connecting the rest of the vessel wall to the highly cellular and cytokine-rich perivascular adipose and connective tissue, as well as the peripheral circulation by virtue of its content of thin-walled microvessels. Thus, it is well situated to serve as an injury sensor for the rest of the vessel wall, and this is reflected by its early activation in different vascular disease models, such as atherosclerosis, ligation and balloon injury, aneurysm formation, and pulmonary hypertension. Manifestations of adventitial activation include increased cell proliferation, upregulation of extracellular matrix, matricellular, contractile

![Figure 3. Mural distribution of postnatal vascular wall progenitor cells (VW-PCs).](http://circres.ahajournals.org/)

Different types of VW-PCs have been identified within the mural layers of arteries and veins. Clonogenic progenitor cells with endothelial potential (EPCs) occupy the intima, while the presence of EPCs in the so-called vasculogenic zone of the inner adventitia has been inferred from arterial ring sprouting assays. The latter region is enriched with a variety of distinct and overlapping ancestral populations, notably adventitial Sca-1+ smooth muscle progenitors (Adv SPCs) and Sca-1+CD45− adventitial macrophage progenitor cells (AMPCs) in mice, and CD34+CD31− mesenchymal stromal/stem cells (MSCs) and CD44+ multipotent stem cells (MPSCs) in humans. MSCs have also been isolated from the intima and media, with the media also harboring multipotent vascular stem cells (MVSCs) and side population (SP) progenitors, both of which have smooth muscle cell-forming potential. The presence of subendothelial pericytes is well established for microvessels, including adventitial vasa vaso, and is also described for intima. Although some progenitor populations seem to be locally derived or maintained in the vessel wall itself (eg, Adv SPCs and AMPCs), multilineage hematopoietic stem cells (HSCs) and hematopoietic progenitor cells (HPCs) are recruited to the vasculature in rare numbers via the peripheral circulation (illustration credit: Ben Smith).
and adhesion proteins, and secretion of chemokines, cytokines, growth, and angiogenic factors. Among the latter are numerous mediators of vascular inflammation and remodeling, such as TNF-α, TGF-β, interleukins (ILs), VEGF, stromal cell-derived factor-1, macrophage colony-stimulating factor, monocyte chemoattractant protein (MCP)-1, fractalkine (CX3CL1), matrix metalloproteinases, and angiotensin II. Each of these soluble factors may influence VW-PC responses, with one such example being the effect of stromal cell–derived factor-1 on the migration of adventitial Sca-1+ SPCs. Other adventitial stimuli that may also be relevant to progenitor cell biology include the augmentation of reactive oxygen species and extracellular purines and pyrimidines in response to hypoxia and inflammation, and the effect of biomechanical forces caused by vascular pulsations and adventitial stretch.

Research efforts must also focus on the developmental origins of postnatal VW-PCs, addressing unanswered questions relating to the embryonic formation of the adventitia and its interface with the media, and the selective and architectural seeding of this territory with progenitor cells. Interest also centers on the heterogeneous prevalence of VW-PCs in different vascular beds. To date, studies have shown that progenitor marker expression is especially prominent in the aortic root, discrepant between aorta, femoral, and carotid arteries, while AMPCs are also less prevalent in some peripheral arteries compared with aorta. Although entirely speculative, these observations raise the possibility that if VW-PCs are involved in disease pathogenesis, their nonuniform distribution may partly account for anatomic variations in the susceptibility of different vessels to disease.

Clinical Implications

Two postulates of importance to clinical medicine have arisen in the context of VW-PCs. The first is that by virtue of their unique localization and functional properties, VW-PCs somehow contribute to the initiation, acceleration, or perpetuation of disease states, including those that involve the vessel wall itself, and those whose pathogenesis depends on microvascular integrity and angiogenesis. This, in turn, raises prospects for the endogenous manipulation of VW-PCs to therapeutically target such diseases. Second, it can be anticipated that the isolation, expansion, and exogenous delivery of VW-PCs may be used to facilitate tissue repair and regeneration, as has been evaluated during the past two decades for stem/progenitor cells derived from BM and other sources.

VW-PCs in Disease

Vascular injury and disease are accompanied by cellular proliferation in all three layers of the vessel wall. Experimental models of atherosclerosis, restenosis, and graft vasculopathy have offered conflicting interpretations as to the sources of EC turnover, and macrophage and SMC accumulation in vascular lesions. A variety of techniques have been used to separate the local contributions of vascular wall resident cells, from those recruited via blood from remote tissues (eg, BM and spleen). These consist of (1) irradiation and adoptive cell transfer, (2) parabiosis, (3) vascular allograft transplantation, and (4) genetic fate-mapping. Although discrepant results between studies may be partly explained by these methodological differences and the limitations of each model system, they also probably indicate that some vascular cell subtypes have diverse origins, and this may further vary depending on the nature of the disease process and its stage in evolution. Beyond differentiating between circulating and local sources for cells in the vasculature, another challenge is to distinguish between bona fide progenitor cell ancestry and re-entry of mature cells into cell cycle. The latter phenomenon has been used to explain the proliferation of tissue-resident macrophages and has relevance to SMC dedifferentiation, SMC-to-macrophage transdifferentiation and endothelial-to-mesenchymal transformation. Investigators have also described the transformation of mature ECs and SMCs into multipotent MSC/progenitor cells that may mediate vascular calcification and fibrosis.

VW-PCs are likely to have mixed roles in vascular disease (Figure 4). Intimal EPCs seem ideally placed to rapidly initiate re-endothelialization after intimal injury, while proteomic and metabolomic analyses of adventitial Sca-1+ SPCs indicate that these cells share similarities with mature SMCs and could, therefore, provide a source of rapid replacement if needed after insult to the tunica media. Conversely, it is easy to envisage pathologic sequelae resulting from VW-PC activation and specification, including their participation in adventitial and plaque inflammation, neointimal hyperplasia, vascular calcification, and growth of adventitial and plaque microvessels. Consistent with a pathogenic role for VW-PCs, is an emerging outside-in paradigm of vascular disease that recognizes the importance of adventitial inflammation and vasa vasorum expansion in initiating and perpetuating deleterious remodeling changes, which extend to the other mural layers. Several groups have corroborated that there is early proliferation and subsequent migration of adventitial cells to the neo-intima, in response to intimal denudation injury. In one such study, reported that neo-intimal cell expansion was preceded by a 5-fold increase in the number of adventitial cells and a 4-fold increase in the number of adventitial microvessels, while observing from explant cultures an influx of undifferentiated adventitial precursor cells. However, the authors also concluded that cell migration more than proliferation contributed to neointimal hyperplasia. Although early reports indicated that adventitial fibroblasts were the probable candidate for this migratory phenomenon, subsequent studies have largely rejected this notion on the way to demonstrating roles for adventitial SPCs, medial MVSCs, and intimal and adventitial pericytes in mediating neointimal thickening.

The mechanisms by which adventitial VW-PCs are released from their niches and migrate to the other mural layers are far from resolved. Recent bioreactor experiments have used decellularized vascular grafts to show the capacity for adventitial Sca-1+ SPCs to mobilize directly across the vessel wall, presumably through breaches in the external elastic lamina, under the regulatory control of chemokines and extracellular proteases. Another obvious conduit for VW-PC delivery to vascular lesions is via adventitial vasa vasorum that expand under pathological conditions, and extend into adjacent plaque...
as thin-walled and leaky microvessels because of deficient pericyte coverage. It has been well demonstrated that development of atheroma and its complications are closely related to the density and structural integrity of vasa vasorum, dependent on the stage of disease. As highlighted by the activation of locally resident macrophage progenitors, EPCs and MSCs during arterial ring assays, VW-PCs are likely to contribute centrally to vasa vasorum formation, both by direct differentiation into ECs and pericytes, and by paracrine proangiogenic signaling.9,30,34,39,111 In return, the adventitial microvasculature itself provides a niche-like repository for different VW-PCs, partly accounting for increases that have been observed in the prevalence of VW-PCs in atherosclerotic arteries compared with healthy vessels.11,118 In addition, this also increases the contact area that VW-PCs have with the systemic circulation, exposing them to a variety of other cell types and stimulatory cues, that may induce disease-modifying responses.

It is unclear whether the angiogenic properties of different VW-PC subtypes act beneficially or detrimentally in atherogenesis, although incomplete pericyte maturation and coverage results in vasa vasorum leakiness and predisposes to plaque hemorrhage and rupture. Inhibition or stabilization of plaque angiogenesis remains an enticing therapeutic option toward

Figure 4. Proposed roles for vascular wall progenitor cells in vascular disease. A, Vascular wall endothelial progenitor cells may replace lost endothelial cells (ECs) after intimal injury, under regulatory signals, such as vascular endothelial growth factor (VEGF) and shear stress. B, Adventitial and medial smooth muscle progenitor cells (SPCs) and multipotent vascular stem cells (MVSCs) may provide smooth muscle cells (SMCs) to the media and neointima under paracrine stimulation from adventitial fibroblasts, MSCs, macrophages, and nerve signaling. Soluble mediators of SMC differentiation include transforming growth factor (TGF)-β and platelet-derived growth factor (PDGF), whereas stromal cell–derived factor (SDF)-1 stimulates SPC migration. Sonic hedgehog (Shh) is involved in maintenance of the adventitial SPC niche. MVSC differentiation to SMCs may occur via an intermediate MSC-like progenitor. Mature SMCs have capacity to dedifferentiate into proliferative, synthetic SMCs and possibly to transform into lesional macrophages. C, Adventitial macrophage progenitor cells (AMPCs) proliferate and differentiate in response to growth factors (eg, macrophage colony-stimulating factor [M-CSF]) to generate macrophages and dendritic cells locally in the adventitia, although it is currently unknown to what extent they provide inflammatory cells to atheroma. D, MSCs have numerous possible roles, including trophic support of other vascular cells, differentiation into SMCs, pericytes and adventitial fibroblasts, and production of mineral and fat deposits, collagen and extracellular matrix in both adventitia and atheroma. Subendothelial pericytes and endothelial-to-mesenchymal transformation (EndMT) may also contribute similarly. E, The adventitial vasculogenic zone is enriched with EPCs, AMPCs, and MSCs that may participate in angiogenic responses, including vasa vasorum expansion and invasion, which in turn, influence plaque growth and stability. MPSC indicates multipotent stem cells (illustration credit: Ben Smith).
plaque modification and regression, and has been successfully targeted in animal studies by both conventional (eg, statins)\textsuperscript{136} and experimental agents (eg, angiostatin).\textsuperscript{137} However, little is known about whether the effects of such antiangiogenic treatments are mediated via adventitial progenitor cells.

Another important consideration is how the aging process impacts on VW-PCs and whether degenerative vascular changes are mediated by age-related senescence and depletion of the endogenous VW-PC pool or disruptions in their niche-dependent signaling. Preclinical studies have to date described a 3-fold reduction in the presence of aortic AMPCs\textsuperscript{10} and a 40% reduction in aortic ring sprouting capacity\textsuperscript{138} in mature mice compared with young ones. One mechanism that may account for age- or stress-induced senescence of EPCs is cathepsin-induced cleavage and depletion of vimentin-1, which results in lysosomal dysfunction and autophagy.\textsuperscript{139} The effect of aging on endothelial and microvascular dysfunction has broad relevance to both macrovascular health and normal organ function, as it may cause critical disturbances to organ-specific stem cell niches.

**Therapeutic Implications for VW-PCs**

As our understanding of how VW-PCs function in healthy and diseased tissues grows, there will be greater scope to target these cells and their regulatory mechanisms in the treatment of different diseases. This will first involve the design and investigation of new therapeutic modulators against critical VW-PC markers and signaling pathways. Second, there will be a new framework to reconsider whether traditional risk factors their deleterious effects on vascular health by disturbing the VW-PC niche, and how this, in turn, is mitigated by conventional pharmacological and nonpharmacological interventions. Finally, there will also be opportunities to directly tailor treatment to the vessel wall itself, using catheter-based adventitial delivery systems\textsuperscript{140} or other novel means (eg, nanodiamonds).\textsuperscript{141}

The potential of stem cell delivery for cardiovascular repair has long been recognized. For the past two decades, numerous preclinical and clinical studies have evaluated the therapeutic application of different cell preparations in the settings of acute coronary syndrome, chronic myocardial ischemia, cardiomyopathy, cerebral, and peripheral vascular disease. Many of the cell types tested have been shown to mediate their reparative properties through pathways influencing angiogenesis and neovascularization, with vascular progenitor cells, such as blood-derived EPCs and SPCs, also being used for ex vivo tissue engineering of vascular conduits.\textsuperscript{142} More recently, breakthroughs in pluripotent stem cell research and particularly iPSC technology have heralded exciting new opportunities for patient-specific stem cell–based therapies.\textsuperscript{143}

Differentiation of iPSCs to hematopoietic and vascular lineages has been achieved using a variety of cell substrates, reprogramming factors and culture protocols. Elcheva et al \textsuperscript{144} applied comprehensive gain-of-function screening to identify two optimal combinations of transcription factors capable of inducing discrete types of hematopoietic progenitors from human pluripotent stem cells through a hemogenic endothelium stage: pan-myeloid (ETV2 and GATA-2) and erythromegakaryocytic (GATA-2 and TAL1). Overexpression of Gata2, along with GifIb, cFos, and Env6 has also been used to directly reprogram hemogenic cells from mouse fibroblasts with bypass of the pluripotency stage.\textsuperscript{145} Other work has revealed the phenotypic and functional heterogeneity of iPSC-derived ECs, and has found that iPSCs can be more selectively differentiated into specific EC subtypes (arterial, venous, and lymphatic) by modifying the concentrations of inductive factors in media, such as VEGF-A, VEGF-C, angiopoietin-1, and BMP-4.\textsuperscript{146} Transplantation of iPSC-derived ECs and vascular progenitors has augmented tissue regeneration in a variety of preclinical ischemic models,\textsuperscript{147–150} while in reverse, vascular ECs,\textsuperscript{151} and blood-derived EPCs\textsuperscript{152} have also been used to generate iPSCs with high angiogenic and regenerative capacity.

In contrast to vascular progenitor cells from other sources, the therapeutic application of VW-PCs has been relatively understudied to date. This is despite the feasibility of accessing fragments of human blood vessels as the stromal vascular fraction of adipose aspirates,\textsuperscript{143} or as buttons of aorta and segments of saphenous vein, radial artery or internal thoracic artery at coronary artery bypass surgery.\textsuperscript{153} The pleiotropic properties of VW-MSCs and pericytes are especially attractive for therapeutic application, given their capacity for culture expansion and their immunoprivileged nature, which enables allogeneic use. The therapeutic capacity of pericytes was first verified for skeletal muscle injury\textsuperscript{89} and subsequently harnessed for cellularizing tissue-engineered vascular scaffolds.\textsuperscript{154} Recently, pericytes from human saphenous vein\textsuperscript{155} and skeletal muscle\textsuperscript{157} have been used in murine studies of myocardial infarction, resulting in increased neovascularization, reduced myocardial fibrosis, inflammation and vascular permeability, attenuation of left ventricular dilatation, and improved cardiac contractility. These effects have been attributed predominantly to their extensive paracrine secretome, which involves the expression of immunoregulatory molecules (IL-6, leukemia inhibitory factor, cyclooxygenase-2, and heme oxygenase-1), vascular-relevant growth factors (VEGF-A, PDGF-β, TGF-β1, and angiopoietin-1) and miR-132.\textsuperscript{97,98}

Maddu and colleagues\textsuperscript{153,155,156} have taken steps toward the therapeutic use of human saphenous vein–derived adventitial progenitor cells with the ultimate objective of refining these cells into a clinical grade product to treat ischemic heart disease. Using in vitro and preclinical model systems, they demonstrated the resilience of adventitial progenitor cells to oxidative stress through upregulated expression of antioxidant and detoxifier enzymes (eg, superoxide dismutase and catalase), which may facilitate their capacity to promote angiogenesis and to improve tissue perfusion in the face of ischemia.\textsuperscript{153} They have also recently performed comparisons of numerous adventitial progenitor cell lines from left over saphenous vein tissue at coronary bypass surgery and varicose vein removal.\textsuperscript{156} These lines were expanded to therapeutic levels of 30 to 50×10\textsuperscript{6} cells within ≈10 weeks, with conservation of phenotypic and functional properties and low levels of replicative senescence during subculture. In vivo transplantation into ischemic mouse hindlimbs improved blood flow recovery and revascularization. Furthermore, whole genome screening revealed correlations between the DNA methylation profile of transplanted cells, especially involving genes involved in
the VEGFR angiogenic network, and their ability to augment microvascular density and blood flow recovery. This indicates the potential for epigenetic screening to help guide cell-based vascular therapeutics.

Others have shown the feasibility of preparing high numbers of human VW-MSCs from segments of fresh thoracic aorta harvested from multiorgan donors. An interesting objective for future studies is now to determine how the reparative properties of pericytes and VW-MSCs vary from different vascular sources, and how this is further affected by age and comorbidities. However, in theory such concerns can be circumvented by the use of young, healthy, allogeneic donors. By comparison, the clinical applicability of VW-EPCs is likely to be hindered by the need for autologous supply and for high initiating quantities of source tissue, as these cells are neither immunotolerant nor as easily expanded.

Conclusions

The discovery of the vessel wall as a reservoir for multipotent and lineage-specific progenitor cells has introduced a new frame-work, with which to consider vascular development, health, and disease, and more broadly the maintenance and dysregulation of tissue homeostasis. The notion that vessel wall pathology may be mediated from the outside–in, at least in part by VW-PCs, has considerable implications for new treatment strategies, especially for atherosclerosis, which remains one of the leading causes of morbidity and mortality worldwide. However, the information that is presently available about the identity, origins, and regulation of different ancestral cells in the vasculature is still preliminary and many critical issues need to be resolved before these cells can be targeted or harnessed for clinical benefit.

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Disclosures

None.

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