Adventitial Biology
Differentiation and Function
Yanhua Hu, Qingbo Xu

Abstract—Recent evidence indicates that stem/progenitor cells are present in the adventitia and participate in vascular repair and the formation of neointimal lesions in severely damaged vessels. Data have also demonstrated that these resident stem/progenitor cells could differentiate into endothelial or smooth muscle cells in response to different stimuli. Under pathological conditions, adventitial inflammation results in releasing a panel of cytokines, such as stromal cell-derived factor-1 and tumor necrosis factor-α, that may lead to local stem/progenitor mobilization and differentiation. Overall, these data support the impact of the adventitial progenitors in pathophysiological processes of lesion development in the arterial wall. In the present review, we aim to summarize the data concerning the presence of the resident stem cells and discuss the pathological impact of the adventitia in vascular diseases. We will also discuss the possible signal pathways orchestrating stem cell differentiation toward vascular lineage and highlight controversial issues related to the role of adventitial progenitors. (Arterioscler Thromb Vasc Biol. 2011;31:1523-1529.)

Key Words: vascular progenitors ■ stem cells ■ angiogenesis ■ restenosis ■ vascular biology

Adventitia is the outermost connective tissue that surrounds an artery. It comes from the Latin adventicius, meaning foreign, strange, extraneous, or coming from abroad or from outside. The adventitia is composed of a network of connective tissue, including collagen fibers, vasa vasorum, nerve endings, a few quiescent resident inflammatory cells, and fibroblasts.1 Adventitial vasa vasorum constitutes a complete vascular tree-like structure,2 including arterioles, capillaries, and veins. It functions in the supply to the outer part of the arterial wall, molecule exchanges, and drainage of the wall soluble components.3 The vascular adventitia acts as a biological processing center for the retrieval, integration, storage, and release of key regulators for the vessel wall function, and in this role it has been extensively discussed in other review articles.4–7 It is the most complex compartment of the vessel wall and comprises of a variety of cells. Especially notable is the recent finding of the abundant presence of resident stem/progenitor cells,6,8,9 which attracted much interest from researchers.

Under pathological conditions, the arterial adventitia undergoes remodeling in response to a variety of arterial injuries, in which resident adventitial cells are often the first to be activated and reprogrammed and then to influence tone and structure of the vessel wall, as reviewed by Michel et al.9 For instance, in restenosis after balloon angioplasty, adventitial fibrosis, thickening, and shrinkage are common and contribute to constrictive remodeling and luminal narrowing.10 In pulmonary vascular remodeling, adventitial cells may act as a key regulator of the vascular function and structure from the outside in (for review, see4). In atherosclerosis, adventitial inflammation and increased vascularization via the vasa vasorum (angiogenesis) are prevalent, particularly in advanced plaques that are considered vulnerable to rupture and thrombosis.11 These capabilities of the vasa vasorum underscore its dynamic role in the regulation of vascular wall perfusion.12 In addition, activated adventitia cells can also release reactive oxygen species, chemokines, cytokines, growth factors, and metalloproteinases that collectively affect medial smooth muscle cell tone and growth directly and stimulate recruitment and retention of circulating inflammatory and progenitor cells to the vessel wall.7–13 Concomitantly, the adventitial stem/progenitor cells can be activated and undergoes changes that include proliferation, differentiation, and migration.7 They may actively participate in the lesion formation. This review presents the current evidence demonstrating that the adventitia acts as a key regulator of arterial wall function and has a pathological impact. Specifically, it focuses on the mechanisms of stem/progenitor cell involvement in the process of vascular diseases.

Adventitial Cell Components: From Fibroblasts to Stem Cells
The adventitia is a complicated tissue or segment that contains a variety of cell types.7 It has been proposed that a vascular myoblast–type cell exists in the adventitia, which can migrate and differentiate to smooth muscle cells.14,15
Labeled myofibroblasts added to the adventitia migrate to the neointima in a rat balloon injury model, with appearance in the media at day 5 and in the intima at day 7. Other models have also indicated fibroblast movement and differentiation as a source of neointimal smooth muscle cells. This is supported by phenotypic differences between medial and neointimal smooth muscle cells. The myoblast is described as a progenitor cell with the potential to become a myofibroblast or smooth muscle cell. In 2004, it was demonstrated that the adventitia contains abundant stem/progenitor cells that may differentiate into several types of cells participating in the pathogenesis of atherosclerosis. The early report demonstrated that stem/progenitor cells are present (2% to 5% of total cells) in the adventitia of aortas from apolipoprotein E−/− mice. Immunohistochemistry to detect a panel of progenitor cell markers (Sca-1, c-kit, CD34, and FLK-1) was performed. Cells expressing each of the progenitor markers were identified in the adventitia, particularly in the region of the aortic root. To determine the source of the adventitial progenitor cells, Hu et al used ROSA26 mice, which express LacZ in almost all cell types. In this model, it was demonstrated that the resident progenitor cells are not bone marrow derived, as shown by the absence of β-galactosidase cells in the adventitia. Interestingly, Passman et al found a restricted domain of sonic hedgehog signaling localized to the media-adventitia boundary. Sonic hedgehog signaling is involved in arterial-venous identity of endothelial cells, remodeling of yolk sac blood vessels, and recruitment of mural cells. Sonic hedgehog−/− mice have a reduced number of Sca-1+ cells in the aortic root, suggesting a role for sonic hedgehog signaling in Sca-1+ function. The existence of a vasculogenic zone within the vascular adventitia has recently been identified in adult human vessels. This niche-like zone is believed to act as source of progenitors for postnatal vasculogenesis. Consequently, many reports from different laboratories confirmed the presence of stem/progenitor cells in the adventitia (Table).

Several groups reported the presence of mesenchymal stem/progenitor cells in the adventitia of human vessels by culturing adventitial tissues and identified progenitor cells expressing vimentin, collagen I, CD29, and CD105, which had adipogenic and osteogenic potential. In addition, Campagnolo et al isolated CD34+/CD31− cells from the adventitia of human saphenous vein, which gave rise to a proliferative population with multilineage potential. In vivo, these cells improved neovascularization after they were injected intramuscularly into murine ischemic limbs. Similarly, Fang et al determined that CD105+, CD34−, FLK-1+ cells isolated from human aorta had vasculogenic potential. Thus, adult arteries contain cells with characteristics of ancestral stem cells (Table). Traditionally, pericytes are the ubiquitous periendothelial cells that surround capillaries and microvessels. These contractile cells are closely juxtaposed to their overlying endothelial cells, with which they share a basement membrane and are connected by elongated processes and intercellular junctions. Besides the progenitors described above, pericytes have been identified in the adventitia of the vessel wall. They are morphologically and functionally heterogeneous and have a function similar to that of stem/progenitor cells in terms of differentiation potential. It was demonstrated that pericytes express common progenitor cell markers, such as CD44, CD73, CD90, CD105, CD146, stromal precursor antigen-1, platelet-derived growth factor (PDGF)-β receptor, the neural glial antigen NG2, and alkaline phosphatase. It was reported that rat aortic wall cells, when cultured in vitro, form spheroid colonies and express a range of pericyte markers. Analysis of human saphenous vein revealed CD34+/CD31− cells that can differentiate to pericytes and incorporate into vascular networks. Both studies describe CD34+ cells with pericyte potential within the vessel wall. In addition, Howson et al investigated whether the vessel wall contains pericyte progenitor cells by isolating nonendothelial mesenchymal cells from rat aorta for in vitro analysis. It was found that in the presence of basic fibroblast growth factor, the cells form spheroidal colonies in culture suspension. The cells express CD34 and Tie-2 but not mature endothelial or smooth muscle markers. Importantly, they also expressed NG2, nestin, and PDGF receptor, which is characteristic of pericytes. Coculture of the spheroid cells in collagen with vascular outgrowths from the aorta induces differentiation to a pericyte phenotype. This suggests that primitive cells with pericyte capability reside within the vessel wall.

These cells appear well placed to serve as another stem/progenitor source of vascular endothelial and smooth muscle cells and to participate in local vasculogenesis, such as in the emergence and expansion of the vasa vasorum and the neovascularization of atheroma. In fact, pericytes were shown to exert a key role during angiogenesis as regulators of vascular development, stabilization, maturation, and remodeling. Recent histological observations showing that subendothelial pericytes from the large vessel intima produce a large amount of immunoreactive tissue factor imply the possible presence of those cells in plaque instability. During ex vivo culture, pericytes from different fetal and adult sources also display a stem cell-like phenotype with respect to morphology, surface antigen profile, proliferative kinetics, and colony capacity for self-renewal and trilineage differentiation for bone, fat, and cartilage. Therefore, pericytes can contribute to plaque formation by differentiating into adipocytes contributing to the lipid core, chondrocytes found in the fibrous cap, or osteochondrogenic lineages responsible for calcification in late-stage atherosclerotic lesions. Pericytes per se may serve as vascular stem/progenitors.

Adventitial Stem Cells Differentiate Into Vascular Lineage

As described above, stem/progenitor cells and pericytes are present in the adventitia and have the ability to differentiate into endothelial and smooth muscle cells. It has been shown that the vessel wall progenitor cells have a clonogenic potential similar to that of circulating progenitor cells. Progenitor cells with endothelial potential were also found in the media-adventitia border of vessels from a range of organs. In vitro cell culture studies indicate that FLK1+ progenitors are endothelial precursors derived from stem cells. FLK1 is the earliest marker of angioblast precursors, specifically marking a subset of Brachyury-positive cells that
migrate into the extraembryonic yolk sac to form the vascular plexus during murine development. The phosphorylation of the intracellular domain of FLK1 and its interactions with phospholipase Cγ1 and phosphatidylinositol 3-kinase are fundamental for the survival/proliferation of FLK1 progenitors and their commitment into the endothelial lineage. It was found that vascular endothelial growth factor (VEGF) can rapidly activate the VEGF receptor–Akt–endothelial nitric oxide synthase pathway in stem cell–derived progenitors, in which histone deacetylase (HDAC) 3 is also involved in Akt phosphorylation. Novel evidence provided recently indicates a functional interaction between Akt and HDAC3 that identifies a key role for HDAC3 in maintaining baseline Akt activation levels. In a similar manner, transient overexpression of HDAC3 using an adenoviral vector resulted in increased Akt phosphorylation and enhanced kinase activity. HDAC3 controls Akt activation through regulation of phosphatidylinositol 3-kinase activity. Taken together, VEGF is a positive signal for progenitor cell differentiation into endothelial cells via pathways involving VEGF receptor–phosphatidylinositol 3-kinase–HDAC3/Akt.

Concerning smooth muscle differentiation from the stem/progenitors, accumulating evidence indicates the involvement of multisignal pathways from stimulation to the nuclear programming. There is evidence showing the impact of integrin, PDGF, and transforming growth factor-β (TGF-β) in stem cell–derived Sca-1+ progenitor differentiation into smooth muscle cells. The initial signal is sensed by progenitor cells via their cell surface receptors, namely PDGF receptors, TGF-β receptors, and integrins (Figure 1). The activation of integrin receptors via tyrosine phosphorylation of β-subunits is essential for their function, whereby signal transmission through these complexes can affect various aspects of cell physiology, including smooth muscle differentiation. Dur-

### Table. Summary of Published Reports on Progenitor Cells Found in the Adventitia

<table>
<thead>
<tr>
<th>Publication</th>
<th>Source/Species</th>
<th>Location of Progenitor Cells</th>
<th>Progenitor Cell Differentiation Potential</th>
<th>Cell Marker Expression</th>
<th>Summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hu et al (2004)</td>
<td>ApoE−/− mouse</td>
<td>Adventitia</td>
<td>SMC</td>
<td>Sca-1+</td>
<td>Sca-1+ cells added to adventitia, migration to intima observed.</td>
</tr>
<tr>
<td>Passman et al (2008)</td>
<td>Mouse embryonic/adult arteries</td>
<td>Media-adventitia</td>
<td>SMC</td>
<td>Sca-1+</td>
<td>Cells at media-adventitia have an Shh signalling domain, in Shh−/− mice adventical Sca-1 cells reduced, Sca-1+ cells express SMC differentiation markers.</td>
</tr>
<tr>
<td>Pasquini et al (2010)</td>
<td>Human arteries</td>
<td>Media-adventitia</td>
<td>Adipogenic, chondrogenic, leiomyogenic</td>
<td>Oct-4, Sca-1, Notch-1</td>
<td>Oct-4, Sca-1, Notch-1 found in vasculogenic niche. Total vessel wall isolated showed expression of stem (Stro-1, Sca-1, Oct-4) and MSC lineages (CD44, CD90, CD105, CD73, CD29, and CD166).</td>
</tr>
</tbody>
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Apo indicates apolipoprotein; SMC, smooth muscle cell; EC, endothelial cell; PDGFR, PDGF receptor; VEGFR, VEGF receptor; vWF, von Willebrand factor; MSC, mesenchymal stem cell; Shh, sonic hedgehog.
ing this stage of cell differentiation, Nox4 can be activated by TGF-β1 autosecreted from differentiating cells.42 Then, activated Nox4 generates reactive oxygen species (H₂O₂ and O₂⁻). Nox4-derived H₂O₂ upregulates serum response factor (SRF) gene transcription and protein translation, which leads to phosphorylation of SRF in cytoplasm and drives activated SRF to translocate from cytoplasm into the nucleus.42 It has been well established that phosphorylated SRF binds to CArG elements within the promoter-enhancer region of smooth muscle–specific genes, recruits coactivator myocardin and other transcription factors, and then regulates smooth muscle gene expression.

Adventitial Inflammation and Stem Cells in Vascular Pathologies

The vascular adventitia acts as a biological processing center for the release of key regulators of vessel wall function. In response to stress, atherosclerotic plaques, or injury, resident stem and progenitor cells can be activated and specified to exhibit different functional and structural behaviors.4 Based on these findings, we can hypothesize that stem/progenitor cells, normally involved in physiological vascular homeostasis, might also act as a reservoir of undifferentiated cells ready to supply the cellular demands and acquiring local phenotypic characteristics during development of neointimal lesions or atherosclerosis in response to pathological conditions.

If the adventitia serves as a reservoir for undifferentiated stem/progenitor cells in a niche-like area, these cells have to be mobilized and released from their niches. So far there is no direct evidence available for the presence of stem cell niches and stem cell mobilization from the adventitia. However, accumulating data indicate that adventitial inflammation results in cytokine production that may stimulate stem cell mobilization. In this respect, it is well known that adventitial inflammation occurs in injured arteries and atherosclerotic vessels, which produce a panel of cytokines or enzymes, eg, tumor necrosis factor-α, TGF-β, granulocyte colony-stimulating factor, granulocyte macrophage colony-stimulating factor, monocyte chemoattractant protein-1, matrix metalloproteinase-9, and stromal cell–derived factor-19,45–47 (Figure 2). These cytokines may directly or indirectly stimulate the cells within adventitia to mobilize the stem cells to the intima via the vasa vasorum to accumulate in the intima. Subsequently, they can differentiate into endothelial or smooth muscle cells. GCS-F indicates granulocyte colony-stimulating factor.
cells. As described for bone marrow, the initial step in mobilization involves activation of matrix metalloproteinase-9, which catalyzes the conversion of membrane-bound Kit ligand to a soluble Kit ligand. The subsequent c-Kit-positive progenitor cells can then move from the niche to the vascular zone. This process is enhanced by elevated levels of stromal cell–derived factor-1 and VEGF and appears to be nitric oxide dependent. Indeed, a number of studies have shown that VEGF and stromal cell–derived factor-1, in addition to granulocyte colony-stimulating factor, granulocyte macrophage colony-stimulating factor, estrogen, erythropoietin, statins, and physical exercise, enhance mobilization of proangiogenic progenitors in the circulation. Furthermore, this is also the case in patients displaying onset of acute ischemia following myocardial infarction, coronary artery bypass grafting, or stent implantation.

During the inflammatory response within the adventitia induced by vessel injury, atherosclerotic lesions, or atheroma, microvessels (vasa vasorum) were significantly increased. Antiangiogenic treatment can limit experimental atherogenesis, suggesting that the density of microvessels in lesions is positively correlated with the development of atherosclerosis. Previous studies from our laboratory demonstrated that endothelial cells of microvessels within the lesioned vessel wall are derived, at least in part, from progenitor cells. Several reports indicated that adventitia-derived stem/progenitor cells have the ability to differentiate into endothelial cells to form a capillary-like structure in vitro (reviewed in). Additionally, it has been found that the adventitial pericytes/progenitor cells can also envelop endothelium and thus contribute to the stabilization of vasa vasorum. Importantly, under pathological conditions (eg, arterial injury and hypertrophy or atherosclerosis), the vascular cells undergo hypoxia, resulting in activation of a transcriptional regulator, hypoxia-inducible factor-1α. Hypoxiainducible factor-1α can activate VEGF gene transcription to produce VEGF locally. Possibly, VEGF could bind to its high-affinity tyrosine kinase receptor FLK-1, which exists on adventitial progenitor cells, leading to conversion of progenitors to the angiogenic phenotype. Therefore, local mobilized stem/progenitor cells might have a function for angiogenesis to form microvessels within the vessel wall.

In the absence of atherosclerosis, normal vessel walls have a microvasculature that is confined to the adventitia. In atherosclerotic lesions, abundant microvessels have been observed. Besides the function of vasa vasorum for supplying the nutrition, we postulate that these vessels may serve as a way to transport mobilized stem/progenitor cells into the intima, where they proliferate and differentiate into smooth muscle cells. There is evidence that progenitor cells applied to the adventitial side of vessel graft can be found in the intima. In this experiment, Sca-1+ cells carrying the LacZ reporter gene were isolated from the aortic root of apolipoprotein E−/− mice. The vena cava was grafted into the carotid artery of another animal, and the donor Sca-1+/LacZ cells were subsequently applied to the adventitia. LacZ–smooth muscle cells were found in the resultant vessel graft atherosclerotic lesion, suggesting migration of the Sca-1+ cells from the adventitia. This implies that adventitial progenitor cells have the potential to be mobilized to move across the vessel wall, which might involve the vasa vasorum, and subsequently differentiate to smooth muscle cells.

**Perspectives and Future Study**

In summary, recent evidence shows the implications of the presence of stem/progenitor cells in the adventitia that is linked by the vasa vasorum. These vascular progenitor cells have the potential to differentiate into either endothelial cells, to participate in angiogenesis, or smooth muscle cells, to accumulate in neointimal lesions. It is therefore plausible that the microenvironment in which the progenitor cells reside in is an important determinant of their subsequent differentiation into either endothelial or smooth muscle cells. The signal pathways mediating endothelial differentiation mainly involve VEGF–phosphatidylinositol 3-kinase–endothelial nitric oxide synthase–HDAC3–p21, whereas smooth muscle differentiation is largely mediated by integrin/collagen IV, PDGF, and TGF-β; they subsequently activate downstream signaling and transcription factor SRF and myocardin (Figure 1). All of the evidence listed here (Table) indicates that stem/progenitor cells that reside in the vascular adventitia are likely to play a direct or indirect role(s) in the pathology of atherosclerosis. However, it is important to be aware that there are no known specific and confined molecules/markers that certainly define endothelial or smooth muscle progenitors. The investigators reported their results to call the cell “vascular stem/progenitor” according to a combination of several markers (eg, Sca-1, c-kit, CD34, or FLK-1), which is specifically a marker for vascular progenitors, although these cells can differentiate into endothelial- or smooth muscle–like cells in vitro.

As discussed above, there are several types of cells present in the adventitia, eg, myofibroblasts/fibroblasts, vascular progenitors, and pericytes. It has been shown that myofibroblasts/fibroblasts can differentiate into smooth muscle cells, but no report indicates endothelial differentiation potential of fibroblasts in the adventitia, which could serve as a criterion to distinguish mature fibroblasts from stem/progenitors and pericytes. Although cell markers for pericytes have been repeatedly mentioned, it is hard to completely distinguish them from stem/progenitor cells in adventitia. There is a possibility that stem/progenitor cells might be a source of all types of cells in the adventitia, ie, they differentiate into pericytes and myofibroblasts/fibroblasts when needed. However, further investigation is needed to confirm this speculation.

A number of challenges and questions remain to be answered before we can fully understand the potential role of these cells. In the first instance, how can it be proved that these cells are resident in the vessel wall and not transiently located at that site? What is the likely origin of the resident vascular progenitor cells? Are the stem/progenitor cells isolated from the adult aortic wall an extension of the stem cells in the embryonic aortic wall? Are they present from developmental stages and activated during the adult lifetime as and when required? How are these cells mobilized? What is the molecule switch to direct stem/progenitor differentiation to either endothelial or smooth muscle cells?
these questions, we would be able to manipulate vascular progenitor cells to control their trafficking to the intima to retard the development of atherosclerosis. Thus, there is a possibility in the future for investigators to direct progenitors to the cell type beneficial for the vessel wall.

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None.

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