Editorial

Intimal Smooth Muscle Cells
The Context-Dependent Origin

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“Know the enemy and know yourself; in numerous battles you will never be in peril.”
—Sun Tzu (The Art of War, Chapter III)

The sixth-century BC Chinese military general Sun Tzu underscored the dangers of overconfidence in strategy. A similar view has been shared by other thinkers and applied further to various contexts other than the military. A major goal of the medical sciences is to develop effective, well-refined therapies. Accomplishing this task requires a comprehensive understanding of the biology of each component in the disease mechanism and realization of the limits of current knowledge. This view can apply not only to the development of new therapies but also to advanced diagnostics (eg, imaging and biomarkers).

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The biology of vascular smooth muscle cells (SMC) has been central to cardiovascular research. Many investigations have focused on the mechanisms of SMC accumulation in the tunica intima, an inner layer of the vessel wall between the endothelium and the media. Evidence suggests that, unlike arteries in other mammals, those in humans spontaneously develop intima of substantial thickness. In addition to higher cholesterol levels (even in normolipidemic individuals compared with other mammals) and other human-specific risk factors (eg, smoking), the intima may serve as a soil for the future development of atherosclerotic plaques. Coronary atherosclerosis leads to acute thrombotic complications, the leading cause of death in many countries.

High plasticity represents a unique feature of SMC compared with other muscle cell types. A classic theory proposes that, on activation in response to injury or inflammatory stimuli, medial SMC migrate into the subendothelial space, proliferate, and produce extracellular matrix, forming the tunica intima. Once relocated to the intima, migrated SMC regain a quiescent phenotype over time.2,3 This phenomenon suggests that SMC can transiently modulate their phenotype, travel, and repair injuries. However, human arteries often contain modulated SMC even in physiological intimal thickening with no complex components of atherosclerosis (eg, macrophage accumulation),4 which may be a precursor to the development of advanced plaques.

Activated SMC undergo phenotypic modulation, as gauged by reduced machinery of fully differentiated cells specialized in contraction, and produce higher levels of growth factors, cytokines, chemokines, and matrix-degrading enzymes than quiescent SMC. Several proteins serve as markers of SMC differentiation/maturation, including α-smooth muscle actin (α-SMA), calponin, SM22, and smooth muscle myosin heavy chain isoforms (SM-MHC; SM1 and SM2). The combined detection of such molecules helps to determine diversity of SMC differentiation.4,5 Although SMC express α-SMA from the early stage of development, expression of SM-MHC isoforms is strictly limited in fully differentiated SMC. The expression profile of α-SMA+SM-MHC+ thus identifies differentiated SMC such as those found in the uninjured medial layer, whereas α-SMA+SM-MHC− indicates undifferentiated or modulated SMC. Such SMC heterogeneity is often associated with the development of vascular disease.6,7 Although the theory of intimal formation through phenotypic modulation and migration of medial SMC has been generally accepted, another classic view suggests that an immature SMC subpopulation resides in the media and contributes to intimal lesion formation.7 Furthermore, several lines of recent evidence suggest that intimal SMC (or intimal cells with phenotypes similar to SMC, often called SM-like cells) originate from sources other than the tunica media, a topic that we will discuss in this editorial. In addition to such intriguing hypotheses on the recruitment of intimal SMC from traditionally unanticipated origins, the question of whether such cells can fully differentiate into SMC in the vessel wall has spurred many investigations. Here we summarize the current understanding of the origins and characterizations of intimal SMC and review several animal models for different vascular diseases.

Evidence suggests that intimal SMC are heterogeneous and have at least 3 origins: (1) the tunica media; (2) circulating precursors (eg, mesenchymal stem cells [MSC] and monocytes); and (3) the adventitia (Figure). However, the relative contribution of each of these sources remains obscure and may also largely depend on the disease context, experimental models, or species (eg, human versus mouse). The intima develops in human arteries from the very early stages and throughout the life span, but spontaneous development of the intima rarely occurs in metabolically normal animals (eg, rabbits on a regular diet,
Potential sources of intimal smooth muscle cells (SMC).

1. On activation in response to injury or inflammatory stimuli, medial SMC undergo phenotypic modulation, migrate into the subendothelial space, and form the tunica intima. SMC progenitor cells may also reside in the media and contribute to intima formation. 2. Circulating precursor cells including MSC and monocytes may engraft in the intima and contribute to lesion development. Other organs, including spleen, fat, and muscle, may also release SMC precursors. 3. The adventitia contains possible SMC precursors, such as MSC, and may contribute to intima formation, particularly after mechanical injury.

wild-type mice). These findings have driven the development of various experimental models for investigation of the pathophysiology of intimal formation. Most common methods include high-cholesterol/high-fat feeding, mechanical vascular injury, and arterial or heart transplantation. However, the nature of the intima and underlying mechanisms involved in its formation differ substantially among these models, and no model can completely mimic human disease. For example, the rate of intimal SMC growth after mechanical injury of the vessel wall in rodents is generally greater than that of human lesions after percutaneous coronary intervention. Some models cause massive damage and substantial loss of medial SMC due to mechanical injury or irradiation and thus may favor repopulation by cells of blood-borne origin. Tanaka et al demonstrated that the relative contribution of bone marrow–derived cells to lesion formation varies among 3 different mechanical injury models in mice. The evidence from previous studies indicates that the origin of intimal cells (eg, monocytes versus MSC) may depend on the type of vascular disease or injury. Furthermore, whether a substantial population of SMC-like cells of blood-borne origin accumulates in the intima in chronic atherosclerosis, causing acute coronary events in humans, or in a chronic phase of vascular injury remains elusive. Nevertheless, investigators have used creative methods to provide insights that contribute to the development of this new paradigm of intimal SMC biology. An extended list of such studies is available in the online-only Data Supplement.

The comprehensive study by Iwata et al, published in this issue of Circulation, used several mouse models of vascular diseases as well as original unique reagents to address critical aspects of intimal SMC biology. In particular, this landmark study attempted to identify the cell type that serves as the origin of intimal SM-like cells; to determine the extent to which cells of nonmedial origin can differentiate toward a SMC lineage in vivo; and to further examine the functional characteristics of such intimal cells. Taking advantage of an antibody specific for mouse SM-MHC (SM1), a definitive marker of fully differentiated SMC, and 2 reporter mouse strains that genetically tag cells expressing α-SMA and SM-MHC, the authors have provided data that fill in some missing links. Using 3 mouse models (mechanical injury, aortic graft vasculopathy, and chronic atherosclerosis), the authors concluded that bone marrow–derived monocytes participate in the accumulation of SM-like cells in the intima. The percentage of intimal cells of blood-borne origin was greater in acute mechanical injury than in more chronic atheromata, suggesting that acute vascular injury enhances engraftment of circulating cells.

Since the discovery of bone marrow–derived SM-like cells in the intima, several in vivo studies have identified these cells as α-SMA–expressing cells. While serving as a general marker for the identification of SMC, α-SMA is also expressed by immature or modulated SMC. Furthermore, other non-SM cell types that share similar morphology and behaviors, such as myofibroblasts, can also express α-SMA. Therefore, whether bone marrow–derived cells can fully differentiate into SMC in vivo remains controversial. In their study, Iwata et al used a newly developed antibody for SM-MHC, and α-SMA and SM-MHC reporter mice, to explore this question more rigorously. The data indicated that, in 3 mouse models, engrafted cells of bone marrow origin, presumably monocytes, did not fully differentiate into the SMC lineage, as demonstrated by the lack of reporter gene signal in the neointima of mice that received bone marrow cells of SM-MHC reporter mice. In contrast, a series of seminal in vivo studies from Campbell and Campbell et al, with peritoneum used as a bioreactor, demonstrated that macrophages can form a layer in the scaffold of vascular graft that contains muscle cells and elastic fibers. Interestingly, these cells appear to gain a phenotype of well-differentiated SMC, which is consistent with some in vitro studies. Collectively, these conflicting findings should lead to further studies on this topic.

Some studies have proposed various potential sources of intimal SMC other than the media or bone marrow–derived monocytes (Figure). The study by Iwata et al did not rule out the possibility that MSC are important in vascular lesion progression. MSC can originate from bone marrow stroma. Alternatively, MSC may exist in adult vasculature (media and
adventitia) and migrate and differentiate into SM-like cells in the intima.\textsuperscript{16} Furthermore, MSC, or even pericytes, may travel from non–bone marrow/nonvasculature remote organs, including adipose tissue and skeletal muscles, to the intima. Swirski et al\textsuperscript{17} established that the spleen is a critical reservoir of monocytes that delivers these proinflammatory cells to the site of acute injury. A portion of the monocytes that form SM-like cells in the intima may originate from the spleen. Again, the relative contribution of cells from each source appears to be context dependent, and the mixture of intimal SMC of various origins may contribute to the complex heterogeneous nature of these cells. Thus, a better understanding of the origins of intimal SMC in each context may help to develop more refined disease-oriented therapies.

Iwata et al identified the blood-borne cells that contribute to vascular lesions as monocytes with a proinflammatory phenotype (CD11b+Ly-6C\textsuperscript{hi}). This is an intriguing finding. A subpopulation of leukocytes expressing high levels of Ly-6C (Ly-6C\textsuperscript{hi}) represents proinflammatory monocytes that promote tissue destruction.\textsuperscript{17} Although it is difficult to directly compare expression levels of Ly-6C in the study of Iwata et al and in the previous literature, the possible association between monocyte subpopulations and their fate in the intima is interesting. In the present study, these monocytes appear to express higher levels of matrix metalloproteinase (MMP)-2, MMP-9, and MMP-13, which are potent matrix-degrading enzymes that likely contribute to plaque instability. These monocyte-derived SM-like cells do not comprise a large population in the \textalpha-actin\textsuperscript{+} intimal cells in each of the 3 models, indicating that the majority of intimal SMC may have originated from the media or cell types other than bone marrow–derived monocytes. Nonetheless, the detection of intimal cells with enhanced proteolytic activity may indicate a unique functional role of these cells, compared with other SMC subpopulations, in the pathogenesis of acute thrombotic complications of atherosclerosis.

Despite many in vivo and in vitro investigations, several questions remain unanswered. The relative contribution of hematopoietic versus mesenchymal lineage to the formation of intimal SMC remains unclear, and the functional differences between cells from different origins need to be addressed in each vascular disease. Iwata et al found that the population of neointimal \textalpha-SMA–positive cells bearing monocyte/macrophage lineage markers decreased at a later time point, likely indicating that cells of blood-borne origin play an active role in the acute phase. However, we do not know the fate of this subpopulation of SMC-like cells. A previous study reported that “dedifferentiated” intimal SMC after coronary intervention in humans gained a more mature phenotype over time, as demonstrated through the expression of \textalpha-actin and SM-MHC.\textsuperscript{4} Another study demonstrated that, although SMC in the fibrous cap of hypercholesterolemic rabbits had an immature phenotype and produced MMPs, similar to the population reported by Iwata et al, lipid lowering promoted SM-MHC expression and reduced MMP expression.\textsuperscript{3} Such dynamic reversible changes of differentiation may account for the high plasticity of SMC, but it may also result from reduced recruitment of SMC precursors of blood-borne origin into the intima as a result of injury repair, normalized lipid profile, or reduced endothelial cell activation. A less inflammatory microenvironment may also prevent engrafted progenitor cells from differentiating into SM-like cells. Dissecting these mechanisms for recruitment, engraftment, adjustment, and transdifferentiation of SMC-precursor cells may lead to a better understanding of various vascular diseases. For example, Fukuda et al\textsuperscript{18} reported that sirolimus inhibited the differentiation of peripheral blood mononuclear cells into SM-like cells.

Tissue engineering, particularly the development of vascular grafts, has led to a greater interest in SMC biology. Some groups have reported that grafts recruit SM-like cells within the scaffold.\textsuperscript{13,14,19} Because SMC phenotype influences matrix production, mechanical properties, and the expression of proinflammatory factors, the contributions of monocyte/macrophage, MSC, and pericyte transdifferentiation to the SMC lineage in engineered vessels should affect clinical outcomes. Finally, the levels of circulating SMC precursors may serve as biomarkers for the prediction of vascular lesion progression and clinical events. Sugiyama et al\textsuperscript{15} demonstrated that CD14\textsuperscript{+}CD105\textsuperscript{+} monocytes isolated from human peripheral blood differentiate into SMC, and the circulating levels of these were greater in patients with coronary artery disease. Thus, a better understanding of the biology of intimal SMC should lead to more effective treatment of atherosclerosis and to new paradigms of preventive vascular medicine.

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Disclosures

None.

References


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Supplemental Data (Fukuda and Aikawa, Origin of Intimal SMC)

Extended reference list


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