Myofibroblast-Mediated Adventitial Remodeling
An Underestimated Player in Arterial Pathology
Matteo Coen, Giulio Gabbiani, Marie-Luce Bochaton-Piallat

Abstract—The arterial adventitia has been long considered an essentially supportive tissue; however, more and more data suggest that it plays a major role in the modulation of the vascular tone by complex interactions with structures located within intima and media. The purpose of this review is to summarize these data and to describe the mechanisms involved in adventitia/media and adventitia/intima cross-talk. In response to a plethora of stimuli, the adventitia undergoes remodeling processes, resulting in positive (adaptive) remodeling, negative (constrictive) remodeling, or both. The differentiation of the adventitial fibroblast into myofibroblast (MF), a key player of wound healing and fibrosis development, is a hallmark of negative remodeling; this can lead to vessel stenosis and thus contribute to major cardiovascular diseases. The mechanisms of fibroblast-to-MF differentiation and the role of the MF in adventitial remodeling are highlighted herein. (Arterioscler Thromb Vasc Biol. 2011;31:2391-2396.)

Key Words: atherosclerosis ■ restenosis ■ α-smooth muscle actin ■ fibroblast

The adventitia has been long considered an essentially supportive tissue compared with the intima and media layers; however, several observations in recent years have contributed to changing this paradigm. Experimental evidence has unequivocally shown that injury to the adventitia induces intima and media pathological changes similar to those elicited by endothelial damage; moreover, it has been reported that early lesions of the adventitia are involved in the evolution of intima and media restructuring. The myofibroblast (MF), a cell crucial for wound healing and fibrosis development, has been shown to play a role in adventitia remodeling (an adaptation process consisting of structural and functional reorganization). This review attempts to summarize the cross-talk between adventitia and intima or media layers, as well as the mechanisms involved in adventitia adaptation to pathological stimuli, with special interest in the role of MFs. We suggest that adventitia remodeling plays a role more important than previously assumed in the evolution of lesions such as atheroma formation and restenosis.

The Adventitia: Structure, Embryological Origin, and Features
The intima, separated from the media by the internal elastic lamina, is composed of a monolayer of polarized endothelial cells (ECs), which mediate vascular tone and provide an antithrombogenic surface. The media consists of diagonally oriented smooth muscle cells (SMCs) surrounded by a basal lamina and embedded in variable amount of extracellular matrix (ECM) including elastin. Separated from the media by the external elastic lamina, the outermost layer of the arterial wall, the adventitia, appears less organized. It contains sparsely localized fibroblasts (Fs) surrounded by a connective tissue mainly composed of collagen type I and proteoglycans, as well as small vessels (vasa vasorum, present only in large arteries) providing nourishment to the arterial wall; unmyelinated nerve fibers (nervi vasorum); and rare macrophages, lymphocytes, and mast cells. The existence of adventitial progenitor cells, capable of mesenchymal differentiation, has been demonstrated in animal models and humans. Moreover, recent work has demonstrated the presence of neuronal cell bodies in the adventitia of the rat mesenteric artery. The adventitia lacks a clear boundary with the surrounding fat and stromal connective tissue that form the periadventitial layer. Recently, the role of the periadventitial fat in vessel remodeling and atherosclerosis has gained importance.

Early in development, large vessels such as the dorsal aorta and aortic arches are formed by vasculogenesis, a process in which ECs arise from isolated splanchnic mesodermal cells called angioblasts. ECs then form the primitive vascular network. The other arteries develop by angiogenesis, ie, sprouting of preexisting vessels. The spatiotemporal development of the endothelium determines the organization of the vascular tree. Subsequently, SMCs are recruited from the...
local mesenchyme or distally from the neural crest (great vessels) or from the proepicardium (coronary arteries) to form the tunica media. SMC proliferation and differentiation are associated with production of the ECM components, such as elastin, that contribute to vessel wall maturation. Adventitial Fs are thought to arise locally from the mesenchyme. Except for the coronary artery, in which a common proepicardial origin has been demonstrated for medial SMCs and adventitial Fs, it is not clear whether the 2 cell types share common progenitors in large vessels.

Adventitia/Media and Adventitia/Intima Cross-Talk

Although long considered a mere supporting tissue, the adventitia plays a major role in the modulation of the vascular tone by complex interactions with cells located within the other wall layers (particularly SMCs). The adventitia contains sympathetic nerve terminals discharging noradrenaline, which diffuses into the medial layer and induces SMC contraction. In coronaries and skeletal muscle arteries, parasympathetic (cholinergic) nerves are present in the adventitia as well: once released, acetylcholine diffuses to the endothelium where it causes the release of nitric oxide (NO) that supports SMC relaxation and vasodilatation. In a recent work, the existence of an adventitial neuronal population expressing calcitonin gene–related peptide has been demonstrated in the rat mesenteric arteries. Calcitonin gene–related peptide, primarily located in sensory nerves and widely distributed in the central and peripheral nervous system, exerts potent vasodilatory activity. Calcitonin gene–related peptide might contribute to the regulation of the vascular tone. Experimental evidence has suggested a direct role of adventitial cells in regulating the vasomotor response. Adventitial macrophages and Fs can produce NO on certain stimuli (eg, exposure to lipopolysaccharide), thus affecting vessel contraction. Adventitial Fs are also implicated in the synthesis and release of endothelin-1 on stimulation with angiotensin II; in turn, endothelin-1 modulates medial SMCs contraction, exerts an effect on ECM organization (eg, stimulating collagen type I synthesis), increases the release of reactive oxygen species, and attracts white blood cells. Furthermore, adventitia is the primary site of reactive oxygen species production in different animal models; reactive oxygen species can inactivate NO and cause the loss of ECs, thus affecting endothelium-dependent relaxation.

The adventitia has been indicated as the principal “injury-sensing tissue” within the artery, as it is capable of responding to different stimuli in an “outside-in” manner by originating and coordinating changes that progress toward the intima and finally end up in vessel reshaping. Among the different cell types present in the adventitia, the F plays a pivotal role in vascular remodeling owing to its remarkable plasticity. Once activated by different stimuli, the adventitial F undergoes phenotypic changes ending up in MF differentiation. It should be noted, however, that adventitial Fs represent a heterogeneous population as defined by several criteria such as cell surface markers (eg, Thy-1), cytoskeletal protein expression, lipid content, and cytokine produc-

The MF

MF was first described in experimental wound healing by means of electron microscopy, as a fibroblastic cell located within granulation tissue and exhibiting bundles of microfilaments. Since then, evidence from many laboratories has indicated that the MF plays a pivotal role in tissue repair and remodeling; moreover, it is a key player in different pathological conditions such as hypertrophic scars, fibromatoses, systemic sclerosis, organ fibrosis, and stroma reaction to epithelial tumors.

Although the main MF progenitor cell is likely to be the locally residing F, MFs may derive from many other sources, including epithelial cells and ECs (through the phenomenon called epithelial-mesenchymal transition), SMCs, pericytes, hepatic perisinusoidal cells, mesenchymal stem cells, and bone marrow-derived circulating precursor cells called fibrocytes. For these reasons, it has been suggested that the MF describes more a functional status than a fixed cell type.

MF differentiation follows a well-established sequence of events. On tissue injury, the local release of cytokines from inflammatory blood-borne and resident cells or from malignant epithelial cells activates Fs, which acquire a migratory phenotype and populate the damaged tissue. This process takes place in the arterial adventitia after stretch injury due to balloon angioplasty. Here they synthesize several components of ECM and organize into a mechanically supportive structure, which eventually produces a significant contractile force. This activated F, characterized by the development of contractile bundles or stress fibers composed of β- and γ-cytosplasmic actins, is referred to as the proto-MF. The increased stress within the ECM resulting from the proto-MF remodeling activity culminates in the transition toward the “differentiated MF,” characterized by the de novo expression of α-smooth muscle actin, the actin isoform typical of SMCs that has become its most widely used marker. In addition to the mechanical environment, the expression of α-SMA is promoted by the production of transforming growth factor-β1 from local inflammatory cells in the presence of the cellular fibronectin splice variant extra domain-A. Finally, once tissue repair is achieved, MFs undergo a massive wave of apoptosis, a process lacking in conditions such as fibrosis and fibromatoses. Very recent evidence also suggests that the process of MF differentiation/dedifferentiation can also be regulated by myocardin. In a recent review, a figure schematizing the crucial points of the F-MF transition is available.

Unlike SMCs, characterized by a rapid and transient contraction, MFs exert a long-lasting isometric tension resulting in a slow, irreversible retraction as observed in experimental and human wound granulation tissue or tissue strips isolated from several fibrotic lesions (for a review, see ); such contraction mechanism should also take place in vascular fibrosis. In SMCs, the rise in intracellular Ca²⁺ results in Ca²⁺/calmodulin-mediated activation of the myosin light chain (MLC) kinase that in turn phosphorylates the MLC,
thus promoting actin-myosin contraction. This type of contraction is rapid and reversible, because phosphate is continuously removed from the MLC by the MLC phosphatase. Instead, MF contraction is essentially regulated by the activity of the small GTPase RhoA that activates the Rho- (associated) kinase, which in turn inactivates the MLC phosphatase and directly phosphorylates the MLC, thus resulting in continued phosphorylation of MLC and persistent actin-myosin contraction.28

Experimental evidence has demonstrated that α-SMA is instrumental in tension production by MFs both in vitro and in vivo through its N-terminal amino acid sequence Ac-EEED, which is likely implicated in α-SMA organization and function, in particular in its polymerization along stress fibers.29 Moreover, it has been proven that the intracellular delivery of the sequence Ac-EEED inhibits the incorporation of α-SMA in MF stress fibers, thus reducing force production, as well as, remarkably, collagen type I synthesis, and inhibiting experimental wound contraction in vivo.30 This finding suggests that the sequence Ac-EEED could represent a target for strategies aimed at reducing connective tissue retraction and remodeling.21

Role of Adventitia in Arterial Remodeling

A plethora of pathological settings, eg, hypertension,31 as well as various types of vascular injury, either intraluminal (eg, atherosclerotic plaque formation,32 vessel overdistention due to balloon dilatation)33 or perivascular (eg, perivascular collar application),34 can compromise the structure and function of the vessel wall. In response to these stimuli, relatively standardized mechanisms of adaptation and repair processes, known as remodeling, take place in the adventitia (Figure).

Adaptive vascular remodeling (also known as positive remodeling or Glagov phenomenon)32 was originally described in the early stages of coronary atherosclerosis, where a compensatory vessel enlargement takes place to maintain a constant flow despite increased plaque burden; this adaptation depends on the dynamic interactions between growth factors, vasoactive substances, and hemodynamic stimuli (eg, flow, stretch, shear stress) and results eventually in sustained changes of the lumen or of the relative composition of the vessel wall (positive remodeling).35 If this reaction escapes self-limiting control, remodeling can cause a maladaptive response, resulting in a reduction in vessel lumen size (negative or constrictive remodeling) that contributes to the pathogenesis of major cardiovascular diseases (eg, atherosclerosis, restenosis and vein graft failure)36 and can eventually lead to clinical complications, such as myocardial ischemia and stroke.

Positive remodeling is dictated principally by inflammation-induced protease activity (mainly matrix metalloproteinases) in the cap and shoulder regions of atheromatous plaques.
phenomenon induces a loss in collagen and a reduction of SMC number\textsuperscript{35} that produce medial and adventitial thinning,\textsuperscript{37} thus allowing an outward expansion of the vessel wall. A similar process, albeit exaggerated, may occur in arterial aneurysm formation.\textsuperscript{38} It is worth noticing that matrix metalloproteinases also contribute to plaque vulnerability.\textsuperscript{29} This type of remodeling therefore hides a paradox: although it delays lumen narrowing, it may enhance the risk of plaque rupture and thrombosis with subsequent acute lumen reduction or even occlusion.\textsuperscript{35} F-MF transition has not been observed in positive remodeling.

Negative remodeling is a time-dependent phenomenon closely resembling wound healing. In balloon-injured porcine coronary artery, it has been shown that negative remodeling is mostly mediated by a rapid proliferation of adventitial Fs (within 3 days from vessel injury) and the switch from adventitial Fs to MFs (peak at 7–14 days after injury), resulting in the formation of a thickened adventitia rich in MFs and collagen fibers. Eventually, adventitial MFs undergo rapid apoptosis before maximal arterial shrinkage, producing a scar-like tissue.\textsuperscript{6} Besides active retraction, other factors, eg, realignment of collagen fibers, intermolecular cross-linking, and the persistent synthesis and deposition of elastin, play a role in late remodeling.\textsuperscript{40}

The negative remodeling due to adventitial modifications (see above) is a major determinant of restenosis after balloon angioplasty in addition to elastic recoil and intimal hyperplasia.\textsuperscript{41} Unlike positive remodeling, negative remodeling often correlates with high-grade stenosis associated with stable cardiovascular diseases.

**Vascular Diseases Related to Adventitial F-MF Transition**

Besides restenosis and vessel shrinkage in atherosclerotic arteries, the appearance of adventitial MFs characterizes many common vascular diseases: systemic\textsuperscript{42} and pulmonary\textsuperscript{43} hypertension, vein graft remodeling,\textsuperscript{44} coronary transplant vasculopathy,\textsuperscript{45} and inflammatory abdominal aortic aneurysms (IAAAs).\textsuperscript{46} The role of F-MF transition in these different situations is detailed below.

In all forms of pulmonary hypertension the early appearance of adventitial MFs is an event that precedes intimal and medial changes. Hypoxia is recognized as a pivotal factor in vascular remodeling, leading to pulmonary hypertension. It can induce F-MF transition, both directly and indirectly, by upregulating adhesion and ECM production; it can also stimulate the production of growth factors and cytokines, eg, transforming growth factor-\(\beta\), thrombin, endothelin.\textsuperscript{43} In addition, adventitial cells expressing mesenchymal stem cell markers could be a source of MF and contribute to arterial remodeling.\textsuperscript{47}

The role of MFs in systemic hypertension is poorly known. A recent study using a pig model of renovascular hypertension has shown that adventitial remodeling, characterized by an increase in collagen III and MF appearance, precedes intima and media modifications, suggesting a role for adventitia in hypertension-mediated atherogenesis.\textsuperscript{42}

Coronary transplant vasculopathy is a major cause of death among heart transplant recipients. It results from a progressive narrowing of the arterial lumen resulting in ischemic failure of the allograft. It is characterized by intimal hyperplasia, increased media tone, and adventitial fibrosis.\textsuperscript{48} Adventitial remodeling can be induced by the alloreactive inflammatory cells in the perivascular space\textsuperscript{48} and is related to early vessel modifications (shrinkage)\textsuperscript{49,50}; moreover, it may correlate with the clinical severity of the stenosis.

The role for adventitial MFs in neointima formation both in restenosis after balloon angioplasty or stent placement, as well as in the development of atherosclerosis, has long been controversial. Although some early studies suggested that the migration of adventitial MFs to the media and intima was pivotal to neointima formation,\textsuperscript{6,51} recent evidence has corroborated the notion of a negligible contribution of MFs to this process.\textsuperscript{24,52,53} Nevertheless, the study of SMC differentiation markers in different well-characterized human coronary lesions showed that coronary intimal SMCs exhibit a phenotypic profile, suggesting that they have modulated into MFs during their migratory and replicative processes. SMC-to-MF differentiation may contribute to plaque remodeling and facilitate plaque fissuration and thrombosis through the continuous production of retractive force (see above).\textsuperscript{54}

Autologous saphenous vein graft is commonly used to perform aorto-coronary bypass and arterial reconstructions. It is associated with important structural remodeling, frequently culminating in occlusion.\textsuperscript{55} These changes differ from atherosclerotic changes both in terms of chronology and histology. The importance of neointima formation (within 1 year) on vein graft fate has been resizrd: strategies aimed at inhibiting early neointimal proliferation have not translated into improved long-term vessel patency.\textsuperscript{56} An important role of adventitial MFs in vein remodeling after grafting has been suggested: in response to surgical trauma, adventitial Fs can modulate into MFs and induce perivascular shrinkage.\textsuperscript{56}

IAAAs make up 5% to 10% of all abdominal aortic aneurysms and develop almost exclusively in men. In contrast with atherosclerotic abdominal aortic aneurysms, IAAAs are frequently symptomatic, tend to occur at a younger age, and are often associated with elevated systemic inflammatory markers.\textsuperscript{57} Little is known about IAAA etiology and pathogenesis; nevertheless, its development appears to involve an immune response localized into the adventitia.\textsuperscript{58} Recent findings have suggested that adventitial MF proliferation can contribute to IAAA development via collagen deposition and subsequent fibrosis.\textsuperscript{56} Among the triggers capable of provoking the F-to-MF transition are adventitial macrophage cytokines and the hypoxic microenvironment observed in the adventitia of these aneurysms.

**Conclusions and Perspectives**

Until recently, most of the attention of basic and clinical research interested in arterial remodeling and pathology has been focused on intima and media layers. The finding of the pivotal role of the adventitia in the pathogenesis of many vascular disorders (ie, atherosclerotic plaque complications, restenosis, systemic and pulmonary hypertension, vein graft
remodeling, coronary transplant vasculopathy, and IAAAs) can furnish the basis for new therapeutic strategies. Inducing MF dedifferentiation or disappearance \(^21\) and influencing the cross-talk between the adventitia and the other arterial layers appear to be promising targets to inhibit the negative remodeling process and its consequences.

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**Disclosures**

None.

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