Diverse Origin of Intimal Cells
Smooth Muscle Cells, Myofibroblasts, Fibroblasts, and Beyond?
Andrew Zalewski, Yi Shi, Anthony G. Johnson

The formation of vascular lesions is invariably associated with the accumulation of mesenchymal cells and their products in the intima, which either compromise the vessel lumen or contribute to retention of atherogenic molecules (reviewed in References 1 and 2). As a result, pathological intimal hyperplasia is pivotal in the development of a wide range of clinical conditions, which are associated with increased cardiovascular morbidity and mortality. Nevertheless, the origin of intimal cells has remained a controversial issue in vascular biology and clinical cardiology. In addition to the expansion of preexisting intimal cells, the initial hypothesis argued for phenotypic modulation of medial smooth muscle cells (SMCs) from a contractile to a synthetic phenotype (dedifferentiation), resulting in their migration into the intima. Several recent investigations, however, have shed new light on the mechanisms of arterial remodeling, coronary restenosis after transcatheter interventions, and vein graft changes after arterialization, which are accompanied by marked alterations in cellular composition of the affected vessel. Understanding how the vasculature alters its composition holds the key to discerning vascular responses under physiological and pathological conditions (Figure 1). In a recent issue of Circulation Research, Hu and colleagues join this quest, focusing on the origin of intimal cells during vein graft remodeling. In contrast to numerous observations after arterial injury, there are relatively few studies of vein graft remodeling under dyslipidemic conditions, making the analysis of vein grafts in apoE-deficient mice clearly relevant. In different types of transgenic animals, the authors demonstrated that intima of venous isografts appeared to contain SMC-like cells originating from both donor and recipient, with no apparent contribution of bone marrow-derived cells. These findings suggest vascular-bed dependent differences in the mechanisms of repair and remodeling. They also underscore the diverse cellular origin of intimal hyperplasia, which may originate from medial SMCs, poorly differentiated vascular fibroblasts, or even nonvascular sources.

Diversity of Vascular SMCs
Intravessel Differences
Mature tunica media contains layers of cells that express several SMC markers acquired during development (eg, α-SM actin, SM myosin heavy chain). Despite the appearance of cellular homogeneity in situ, isolation of medial cells yields subsets with different morphology, growth, and varying expression of growth regulatory genes (eg, ERK1/ERK2). For example, the subendothelial layer of bovine pulmonary artery contains cells that exhibit robust proliferation and autonomous growth, which is reminiscent of the fetal SMC phenotype. A small fraction (≈10%) of medial SMCs lacks muscle markers (nonmuscle cells), resembling fibroblasts that are particularly responsive to growth stimuli. Because direct isolation of distinct populations of SMCs is fraught with technical difficulties, others have characterized SMC clones originating from individual cultured cells. This approach identified distinct subsets of SMCs, which differ in regard to morphology, growth, and migration. The above observations confirm cellular heterogeneity of normal vessels, as assessed by independent methodologies, in different vascular beds (eg, aorta, pulmonary artery, saphenous vein) and among several species. The involvement of selected cellular components in intimal hyperplasia has been inferred from the ability of only some cells to respond to stimuli in vitro, their accumulation in injury-induced intima, and the differences in gene expression between neointimal cells and medial SMCs. In this context, the findings by Hu et al would benefit from further identification of specific progenitor(s) of SMC-like cells noted in the intima of remodeled vein grafts.

Intervessel Differences
Site-specific differences in SMC characteristics have been reported among noncoronary vascular beds, although only a few studies specifically focused on the attributes of coronary SMCs. Coronary arteries have a distinct developmental origin, whereby their SMCs and adventitial/perivascular fibroblasts arise from common proepicardial progenitors, which undergo epithelial to mesenchymal differentiation in developing hearts in situ (Figure 2). Postnatal coronary SMCs display a highly differentiated phenotype, retaining several muscle markers in culture. Comparisons of freshly iso-
lated coronary and noncoronary SMCs reveal differences in growth, collagen synthesis, and LDL degradation, which all suggest quantitatively lesser responses of coronary SMCs. Low expression of $\alpha_\beta_1$ integrin (fibronectin receptor) on coronary SMCs, compared with noncoronary counterparts, may confer some coronary SMC characteristics (eg, low growth, advanced differentiation).20 The highly differentiated phenotype of coronary SMCs offers a protective mechanism, preventing early formation of occlusive lesions in the coronary vasculature. Other homeostatic properties of the intact coronary media include high expression of superoxide dismutase and tissue inhibitors of matrix metalloproteinase (TIMP-1/-2), which reduce oxidative stress and cell migration, respectively.21,22 Because the development of a more permissive SMC phenotype is associated with the increase in $\alpha_\beta_1$ integrin, it remains to be determined whether the preselection of individual clone(s) of coronary SMCs takes place under clinically relevant conditions (eg, dyslipidemia, diabetes mellitus).20

Vascular Fibroblasts

Adventitial Remodeling

Adventitial fibroblasts generate high levels of superoxide anion, regulated by a key vascular oxidase, NAD(P)H oxidase.23-25 The augmented oxidative stress may represent an important mechanism by which the coronary fibroblast phenotype is modified, resulting in redox-dependent growth and gene expression.25 Observations from a porcine model suggest that adventitial fibroblasts exhibit intense proliferation followed by extracellular matrix synthesis after dissecting medial injury.26, 27 The activation of adventitial fibroblasts is not unique to coronary vasculature, as it has been also noted in other models of arterial repair. Not unlike wound fibroblasts, they acquire SMC markers (eg, $\alpha$-SM actin), becoming myofibroblasts. The accumulation of a collagenous matrix and stress fiber containing adventitial myofibroblasts may contribute to constrictive remodeling of nonstented arteries.

Fibroblast Migration

The most controversial issue to date has been the possibility that activated fibroblasts migrate from the sites of their origin. Adventitial involvement in vascular remodeling has been implicated even in the absence of a direct endoluminal injury (Table).29-34 Labeling of proliferating adventitial fibroblasts with bromodeoxyuridine allowed for more direct tracing of their translocation to the intima.26, 27 Although suggestive of the adventitia-to-intima migration, this approach cannot exclude labeling of a few proliferating (and then migrating) clones of medial SMCs. Subsequent observations in noncoronary vascular beds have provided additional support for

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MCP-1 indicates monocyte chemoattractant protein-1.
fibroblast contribution to intimal formation. Labeling of perivascular fibroblasts days before surgical interposition of the saphenous vein graft in the arterial system is followed by the appearance of these cells in the intima.39 The loss of cellular content in the media due to apoptosis after grafting (particularly in the mouse model) makes attractive the hypothesis that at least some neointimal cells originate from perivascular/adventitial fibroblasts. Although increased expression of matrix metalloproteinase-2 (MMP-2) and MMP-9 observed in activated adventitial fibroblasts in other experimental models provides a mechanistic explanation for their migration due to gelatinolytic and elastolytic activities, this possibility has not been explored in the study by Hu et al.5,22,36 In addition, downregulation of constitutively expressed TIMPs in the media (eg, aging, response to grafting) may contribute to cellular remodeling of the vessel.22,37

Cytoskeletal Changes

The expression of several cytoskeletal proteins is often used to determine cell origin in the remodeled vessel. Unfortunately, a number of these initially believed to be SMC-specific stains (eg, α-SM actin, desmin) were later found in cells of fibroblastic origin during organ remodeling. The changes in cytoskeletal proteins in activated adventitial fibroblasts resemble the sequential expression of differentiation markers in the process of SMC maturation during development.17,38 The autocrine expression of TGFβ1 has been associated with the appearance of adventitial myofibroblasts, expressing α-SM actin, to a lesser degree desmin, and possibly other SMC markers (eg, SM22).39,40 Smoothelin, a novel marker acquired at the end of SMC maturation, has been found only in the media and neointima but not in the adventitia.41 These findings suggest that either fibroblast migration to the intima is negligible or that upon translocation of these cells, the process of transdifferentiation continues with the appearance of late markers of SMCs in the intima. Additional studies are required to define molecular mechanisms and local factors that override repressing activity that normally prevents transdifferentiation of fibroblasts to SMCs.42 Taking into consideration the common origin of coronary mesenchymal cells and the overall biological properties of coronary fibroblasts, it is suggested that the terms such as fibroblasts, myofibroblasts, or SMCs represent a continuum of the differentiation process.

Bone Marrow–Derived Intimal Cells

Additional sources of vascular myofibroblasts may include cells derived from the bone marrow. Mesenchymal stromal cells of the bone marrow have the ability to acquire characteristics of several more differentiated cells (eg, osteoblasts, adipocytes).43 Hematopoietic cells (monocyte-derived) or stromal cells of the bone marrow can also transdifferentiate to myofibroblasts in vitro.44,45 Whether this occurs in vivo remains the subject of disparate reports, with findings by Hu et al5 unable to confirm the presence of bone marrow–derived cells in intima of atherosclerotic vein grafts (mouse model). This contrasts with recent studies of arterial injury, heterotopic cardiac transplantation, and arterial atherosclerosis where SMC-like cells originating from hematopoietic stem cells were identified in intima (mouse models).46,47 If bone marrow cells participate in pathological remodeling in other species, including humans, then the understanding of molecular mechanisms underlying their homing in lesion-prone segments and transdifferentiation to proatherogenic phenotype would be of clear therapeutic value.

Conclusions

There is growing recognition that the cellular constituents of vascular beds display diverse intravessel and intervessel characteristics. Ample evidence indicates the biological uniqueness of intimal cells, which may originate from highly distinct subset(s) of medial SMCs or nonmuscle cells. Vascular remodeling appears to have unique characteristics in coronary and noncoronary arterial beds as well as venous bypass grafts. The understanding of these processes goes far beyond intellectual curiosity, because it raises the prospect of defining better potential therapeutic targets for the prevention of intimal lesion formation. There are, however, major challenges related to uncertain relevance of current animal models of atherosclerosis to clinical conditions. The latter clearly includes both coronary and noncoronary cell responses to long-term stimulation, such as low-grade inflammation, diabetes mellitus, dyslipidemia, and specific immune responses, which are often lacking in the preclinical studies. Only by launching an integrated basic and clinical research initiative (“from mouse to man”) can recent advances in the understanding of the mechanisms of vascular repair and remodeling be translated to the development of novel systemic therapies as well as locally applied therapeutics.

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