Vascular Smooth Muscle Cells

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Decades of work have shown that vascular smooth muscle cell (SMC) phenotypes are controlled by cues received from the local environment.[1–3] When nestled into a cage of cross-linked collagen and elastin of its own making,[4] medial SMCs exhibit a fully differentiated phenotype conferred by the transcriptional activity of myocardin and serum response factor (SRF) and reinforced by the stabilizing roles of miR-143/145.[2,3,5–7] The central MADS (MCM1, Agamous, Deficiens, SRF) box of SRF is an ancient DNA-binding regulatory platform[8,9] that formed a partnership with the basic and polyglutamine domains of myocardin family proteins early in metazoan evolution.[10–12] This evolutionary innovation allowed for the formation of an extremely versatile type of muscle cell now found in the walls of almost all lumen-containing organs and tissues in adults and as a transitional form in the embryonic heart.

Vascular SMCs Adopt Macrophage-Like Phenotypes

Recent studies, many of which have been published or discussed in the pages of ATVB, extend the range of phenotypes exhibited by vascular SMCs to those previously thought to be found only in cells of myeloid origin.[13–17] These papers use modern genetic tools to build upon observations made during the past 30 years on SMC responses to injury and cholesterol loading.[18–21] These early studies identified and characterized the SMC foam cell, but investigators lacked modern transgenic methods to assess the frequency of SMC foam cell formation in atherogenesis. The surprising result described in recent reports is not that SMC to macrophage-like cell transitions occur in atherosclerosis, but rather the evidently large extent to which a SMC origin can account for cells that express common macrophage markers in the plaque.[14–17,22] The high frequency of SMC-derived plaque macrophages may begin to explain how human atherosclerotic plaques appear as clonal lesions[23] because the 2 main cell types in the lesion are now shown to have a common origin.

One question that arises from these observations is why do SMCs so readily express macrophage-like phenotypes?[17,24,25] Is there an inherent sloppiness in SMC regulatory networks with the purposeless outcome that some macrophage marker genes become expressed as SMCs cope with environmental stress? Or is it possible that this property of SMCs has been maintained by selection during vertebrate evolution? Insight into this issue comes from the work of Vengrenyuk et al[16] who showed that in addition to upregulation of the macrophage markers CD68, Lgals3, Abca1, and Abcg1, cholesterol loading of SMCs also increased expression of Ccl2, Ccl7, and TLR4, which initiates proinflammatory responses to endogenous and microbial ligands. It is possible, therefore, that the dysfunctional macrophage phenotype adopted by SMC foam cells[13–16] may, at least in part, represent an attempt at initiating a wound-healing response by injured SMCs. This notion is supported by evidence from multiple groups that disruption of myocardin expression or function in SMCs leads to increased expression of proinflammatory cytokines, chemokines, and adhesion molecules.[26–28] The fact that SMCs can also adopt other mesenchymal phenotypes, such as chondrogenic transformation accompanied by downregulation of Wnt16[29] or produced by Cox2 inhibitors,[30] suggests that permissiveness of SRF MADS box–binding interactions with many different activator and repressor cofactor proteins may underlie these phenotype transitions.[8,31–33]

Control of SMC Phenotypes by Transforming Growth Factor β and Actin Polymerization

Vascular SMC phenotypes are also controlled by transforming growth factor (TGF) β signaling and the state of actin polymerization.[34–37] One factor that has been shown to function in a pathway for actin polymerization and SMC differentiation in vitro is the diaphanous-related formin mDia.[38,39] To determine whether mDia has a similar role in promoting the differentiated phenotype of SMCs in vivo, Weise-Cross et al[40] expressed a dominant-negative form of mDia driven by SM22α-Cre (SM22α-DNmDia) in transgenic mice. Animals expressing SM22α-DNmDia exhibit defects in SMC investment in developing arteries and reduced SMC migration leading to inhibition of neointimal formation after vascular injury in adult mice. In experiments using Myh11creERT2, the authors found that inducible expression of DNmDia in adult arteries reduced the levels of expression of SMC differentiation marker proteins in vivo. To identify additional genes regulated by actin polymerization in SMCs, Turczyńska et al[41] stimulated SMCs in vitro with the F-actin–promoting agent jasplakinolide and identified upregulated genes by microarray analysis. Two genes that were identified in this screen were dystrophin and synaptopodin, both of which promote the differentiated phenotype of vascular SMCs in vitro and whose expression is downregulated after arterial injury in vivo.

In normal healthy arteries, SMCs are organized within medial layers into electrically coupled clusters. Balint et al[42] showed that SMCs are actually self-organized within normal media via TGFβ- and p38-dependent assembly of novel

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(*Arterioscler Thromb Vasc Biol*. 2016;36:e82-e86.
DOI: 10.1161/ATVBAHA.116.308261.)

***Arterioscler Thromb Vasc Biol* is available at http://atvb.ahajournals.org**
DOI: 10.1161/ATVBAHA.116.308261

adherens junction structures composed of hybrid nanotracks of N-cadherin and cadherin-11. Blocking either N-cadherin or cadherin-11 inhibited formation of SMC collectives and reduced expression of a mature SMC phenotype. The authors concluded that maintenance of SMC collectives via specialized hybrid adherens junctions may be necessary for homeostasis and SMC quiescence in vivo. The organization of SMC collectives in the adult tunica media may be a remnant of the clusters of migrating neural crest-derived SMC progenitor cells in the embryo as they move toward and assemble around endothelial cells in the pharyngeal arch complex around embryonic day E9.5. This behavior, called the community effect, is thought to reinforce progenitor cell identity as cells move through a complex and changing mixture of signaling molecules and extracellular matrix (ECM) proteins.[43] In addition to making contacts with other SMCs, it is also important for SMCs to make contacts with the ECM via interactions of focal adhesions (FAs) with ECM proteins and with the SMC cytoskeleton. TGFβ stimulates vascular SMCs to increase the number of FAs on their surface and thereby promotes connection of the SMC cytoskeleton to the ECM. Fernandez et al[44] showed that TGFβ increases the expression and interaction of Hic5 and Hsp27 with FAs in cultured SMCs and that this interaction occurs via a Nox4-dependent pathway. These authors found that localization of Hic5/Hsp27 complexes to SMC FAs promotes the adhesion and migration of SMCs to native substrates.

TGFβ signaling is inherently complex with multiple ligands, latent growth factor activation pathways associated with ECM-binding sites, and both canonical signaling mediated by SMAD proteins and noncanonical signaling mediated via Erk1/2 and p38-MAPK pathways.[45] Despite the upstream and downstream complexity, essentially all SMC signaling is mediated by activation of the type II TGFβ receptor (TβRII). Using 2 different tamoxifen-inducible SMC-specific Cre lines to delete TβRII in adult mice, Hu et al[46] reported that the absence of SMC TβRII leads to development of severe aortic pathology that progresses with age. Loss of SMC TβRII was associated with aortic ulceration, medial dissection, hemorrhage, elastolysis, accumulation of macrophage-like cells, increased proteoglycan content, and aberrant SMC gene expression.[46] These structural and phenotypic changes were most severe in the ascending thoracic aorta, a region composed mostly of neural crest-derived SMCs with a subset of second heart field-derived SMCs concentrated around the aortic root and proximal ascending aorta.[47] The results reported by Hu et al and similar results reported by Li et al[48] and by Chen et al[49] suggest that ongoing TGFβ signaling is required to maintain normal artery wall structure and function in the adult and that pharmacological antagonists of this signaling pathway may have deleterious effects on major conduit arteries in vivo. Loss of trophic TGFβ signaling is also implicated in the increased incidence of ascending aortic aneurysms seen in patients with bicuspid aortic valve (BAV).[50] Principle component analysis performed on gene expression profiles from aortic SMCs and aortic valve fibroblasts identified 217 TGFβ-regulated genes whose expression significantly differed in samples from BAV versus normal tricuspid aortic valve patients.[51] Moreover, latent TGF β–binding protein content was higher in cells from BAV patients, whereas levels of free TGFβ were lower in BAV cells, suggesting that reduced TGFβ signaling in BAV cells contributed to the development of ascending aortic aneurysm in these patients.

At the same time, studies of ascending aortic aneurysms in patients with Marfan or Loeys–Dietz syndromes reach the opposite conclusion—that chronic ongoing TGF β signaling actually promotes the formation of these aortic wall lesions.[52] For example, dilated aortas from Marfan patients showed increased expression of SMC differentiation markers including ActA2, SM22α, calponin-1, and smoothelin along with nuclear accumulation of phospho-Smad2 to levels greater than healthy aortas.[53] Elevated expression of these SMC differentiation markers was maintained in aortic medial explant–derived cell cultures along with increased expression of myocardin. Further analysis showed that SMCs obtained from Marfan patients displayed more robust actin stress fibers, increased rhoA-GTP levels, nuclear accumulation of MRTF-A, greater numbers of FAs at the cell surface, and increased levels of nuclear pSmad2 and pSmad3 compared with similar SMC cultures from healthy aortas. Pharmacological inhibition of TGF β signaling in Marfan SMCs with the TβR1 inhibitor LY364947 (LY) strongly reduced the high pSmad2/pSmad3 levels, SMC differentiation marker expression, and mRNA levels for myocardin and SRF.[53] Atomic force microscopy was used to show that aortic SMCs from Marfan patients were stiffer than counterparts from healthy aortas, and similar results were found for the secreted ECM from these SMC cultures. The authors suggest that upregulated TGFβ signaling in Marfan SMC cultures may result from reduced TGF β–binding capacity of defective fibrillin-1 microfibrils in the ECM and the consequent increased levels of free, biologically-active TGFβ in the medium. Although this is a logical conclusion, it is possible that the increased stiffness of aortic cells and matrix in Mafan patients may be a response to aortic wall dilation, rather than a cause of it, and the enhanced expression of a SMC-differentiated phenotype may be a feature of lesion progression, rather than lesion initiation. Clearly, it is not a simple matter to tease out cause and effect relations in complex systems in general and in the aortic wall specifically. More work is required to understand how aortic dilation progresses during aneurysm formation.

Vascular SMCs and Adaptive Wall Remodeling

Artery wall–remodeling responses to intraluminal injuries (catheters, stents, and downstream ligation) involves changes at virtually all levels of regulation of SMC gene expression and function. Once identified, some of these regulatory changes offer the potential of a shortened path to therapeutics. One example of this is found in the report by Wang et al[54] exploring the regulatory role of microRNAs (miRs) in a humanized animal model in which balloon catheter–injured human internal mammary arteries were transplanted into nude rats and subjected to miR profiling. miR-21 was identified as a significantly upregulated target. Experiments using intravenous fluorescein-tagged locked nucleic acid antagomirs (anti-miR21) showed inhibition of neointimal thickening was obtained

along with significant off-target effects on liver, heart, lung, and kidney. The authors then tested anti-miR21–coated stents and compared with bare metal stents, anti-miR21 stents inhibited in-stent restenosis without the off-target effects observed with intravenous injection.[54] miR-21 has many targets whose inhibition effectively promotes growth factor signaling pathways.[55,56] Continuing the theme, Xu et al[57] reported that miR-15b/16 inhibits neointimal formation in a rat carotid balloon injury model by promoting the SMC-differentiated phenotype, attenuating arterial SMC migration, and inhibiting SMC proliferation. In part, these effects are mediated by the ability of miR-15b/16 to downregulate the expression of the oncoprotein YAP (yes-associated protein). This group previously reported that TEAD1, a DNA-binding partner of YAP, acts to repress SMC differentiation by competing with myocardin for binding to SRF and thereby downregulating expression of the SMC-differentiated phenotype.[58] A role for X-box-binding protein-1 (XBP1)–mediated increases in miR-1274B in neointima formation after femoral artery injury was reported by Zeng et al.[59] In these experiments, miR-1274B was found to target calponin h1 (CNN1) mRNA for inhibition thereby contributing to the PDGFRβ-mediated increases in SMC migration and proliferation.

Additional novel regulators of vascular SMC migration were identified by Williams et al.[60] These investigators showed that Wnt2 mRNA and protein levels were specifically increased in migrating aortic SMCs.[60] Wnt2 increased Wnt1-inducible signaling pathway protein-1 (WISP1) via βcatenin/TCF-dependent pathways in cultured SMCs. Silencing of WISP1 reduced SMC migration in vitro and carotid artery ligation in WISP1$^{-/-}$ mice led to greatly reduced neointima formation compared with WISP1 wild-type mice.[60] Vascular SMC migration is also associated with production of a proteoglycan and hyaluronan (HA)-rich provisional ECM.[61] Kiene et al[62] reported an important role for hyaluronic acid synthase (HAS)-3 during neointimal formation in mice. When examined 28 days after carotid ligation injury in HAS3-deficient mice, HA content of the artery wall was greatly reduced, and formation of a neointima was strongly inhibited compared with wild-type animals.[62] Transcriptome analysis in HAS3-deficient carotid arteries suggested that genes involved in cell migration including FA proteins, SMC integrins, and PDGF-BB signal pathway mediators were downregulated. The authors conclude that HAS3-mediated hyaluronan production supports important steps in SMC migration and neointimal formation in vivo.

Vascular SMCs, Inflammation, and Atherosclerosis

Throughout the long history of research on the pathogenesis of atherosclerosis, the close association between intimal SMCs and inflammatory cells has been the subject of intense investigation.[63,64] As discussed earlier in this article, recent studies shed new light on the relation between vascular SMC phenotype transitions and expression of proinflammatory genes.[16,26,27] This relation can also be extended to senescent vascular SMCs that are present within atherosclerotic plaques. Rather than being inert bystanders in plaque progression, senescent human SMCs exhibit a proinflammatory senescence-associated secretory phenotype characterized by release of multiple cytokines and chemokines and driven by autocrine-acting interleukin-1α.[65] Senescent SMCs release MMP9, secrete less collagen, promote mononuclear cell chemotaxis, and stimulate adjacent endothelial cells to express a proadhesive and proinflammatory phenotype. Thus, senescent SMCs, possibly through the same downregulation of myocardin-SRF regulated SMC gene expression,[27] can contribute to plaque progression by assuming a proinflammatory and cap-destabilizing phenotype in vivo. As discussed above, transition of SMCs to macrophage-like foam cells in a hyperlipidemic environment is associated with coexpression of a proinflammatory phenotype.[14–16] She et al[66] reported that the conversion of SMCs to foam cells may be accelerated by expression of neural/glial antigen-2 (NG2) proteoglycan on the surface of synthetic SMCs. Despite the fact that ablation of NG2 proteoglycan in mice leads to hyperlipidemia and obesity, both risk factors for the progression of atherosclerosis, when tested on an ApoE-null background loss of NG2 expression unexpectedly results in reduced plaque development.[66] These results suggest that local acquisition of a macrophage-like proinflammatory phenotype by plaque SMCs plays a more predominant role in plaque progression than systemic hyperlipidemia or obesity. Finally, an emphasis on the role of local factors in the control of macrophage-like phenotype transition of plaque SMCs emphasizes the role of SMC-derived ECM proteins in plaque progression. Schwanekamp et al[67] examined the role of periostin, a secreted ECM protein, on the development of atherosclerosis in an ApoE$^{-/-}$ model. These authors found that periostin-deficient, ApoE-null mice developed reduced plaque areas, fewer plaque macrophages, disorganized plaque ECM, thinner fibrotic caps, and increased MMP-2 and MMP-13 levels than ApoE-null control mice.[67] It would be interesting to determine if the reduced number of plaque macrophages observed in the absence of periostin was the result of reduced plaque SMC transition to a macrophage-like phenotype now that the necessary mouse genetic tools are available to test this possibility.

Summary

The studies reviewed above suggest that the diverse origins of vascular SMCs in the embryo[47] are mirrored by the diverse fates that these cells exhibit in response to injury or disease.[13–16,29–31,68–70] An understanding of these phenotype transitions will most likely ultimately lead back to control of the SMC CArG box by myocardin and SRF together with the constellation of corepressors and coactivators that have been described to interact with this SMC differentiation platform.[32,33,71,72] Whether these phenotype transitions to macrophage-like or osteoblast-like cell types are reversible in vivo, and what role resident SMC progenitor cells play in the homeostasis of the artery wall[73–77] are among the important questions going forward.

Sources of Funding

Research in my laboratory is supported by the National Institutes of Health grants RO1HL123650, RO1HL121877, RO1HL133723, the

Loie Power Robinson Stem Cell and Regenerative Medicine Fund, and the Seattle Children's Research Institute, Seattle, WA.

Disclosures

None.

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KEY WORDS: aneurysm ■ atherosclerosis ■ collagen ■ elastin ■ smooth muscle

Arteriosclerosis, Thrombosis, and Vascular Biology

JOURNAL OF THE AMERICAN HEART ASSOCIATION

Vascular Smooth Muscle Cells

Mark W. Majesky

Arterioscler Thromb Vasc Biol. 2016;36:e82-e86
doi: 10.1161/ATVBAHA.116.308261
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 1079-5642. Online ISSN: 1524-4636