Recent Highlights of ATVB

Arterial Smooth Muscle
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The prime function of the arterial smooth muscle cell (SMC) in adult individuals is to contract and relax, thereby regulating blood flow to target tissues. However, in several vascular diseases, arterial SMCs in the adult vessel undergo major changes in structure and function. For example, SMCs can take on properties that allow them to proliferate and migrate, to promote calcification, to undergo apoptosis, and to orchestrate other cell types to take on different properties. These altered SMCs are often viewed as vascular troublemakers. However, these responses can also be seen as an attempt of the SMC to mend a diseased blood vessel. The plasticity and diversity of SMC populations and the presence of SMC progenitor cells contribute to the growing complexity of SMC biology. In the past couple of years, several new mechanisms that allow SMCs to proliferate, migrate, promote calcification, and communicate with and respond to other cell types have been discovered. Much of this work has been published in ATVB, and some of that body of work is highlighted in this article. We emphasize areas that have generated substantial interest, including novel mechanisms governing SMC phenotypic switching, SMC progenitor cells, noncoding RNAs as regulators of SMC function and phenotype, novel mechanisms of cross talk between SMCs and other cells and differences in regulation of SMCs and other vascular cells.

Plasticity of the Arterial SMC

The arterial SMC exhibits an impressive potential for plasticity. SMCs have been known to exist in different phenotypic states for decades, and the switch from a quiescent contractile phenotype to a synthetic proliferative phenotype is thought to play an important role in cardiovascular disease (for a historical perspective). Thus, switching to a synthetic phenotype is generally thought to enable SMCs to migrate and proliferate in atherosclerotic lesions, thereby forming a fibrous cap covering a core of macrophages and other immune cells, and in injured vessels after angioplasty. However, additional lineage-tracing studies are needed to address the origin of intimal lesion cells. Conversion of an early fatty streak atherosclerotic lesion to a fibrotic lesion is thought to cause permanent nonreversible changes in the artery wall. In that sense, SMC phenotypic switching can be considered to be detrimental.

However, at later stages of atherogenesis, fibrotic lesions are often more stable than acellular necrotic lesions, making them less likely to cause clinical symptoms. In such lesions, SMC phenotypic switching and survival could be considered beneficial. Different SMC phenotypes overlap and probably exist as a continuum in vivo.

Recent research on intracellular regulators of SMC phenotypic switching has focused on the role of kinases and downstream transcriptional regulators controlling expression of contractile proteins and proteins associated with the cytoskeleton in SMCs. These proteins, including smooth muscle α-actin, smooth muscle myosin heavy chain, calponin, and smooth muscle 22α, are often used as markers of the contractile SMC phenotype. It is now known that myocardin, a transcriptional coactivator of serum response factor (SRF), is a master regulator of the SMC contractile phenotype and is required for the expression of these contractile proteins.

The Hippo kinase pathway, which received its name from a Drosophila mutant with tissue overgrowth, was recently shown to control SMC phenotypic switching, perhaps through modulating the myocardin–SRF axis’ effects on gene expression. Yes-associated protein (YAP) is phosphorylated and inhibited by the Hippo kinase pathway. Phosphorylated YAP remains in the cytosol and is thereby prevented to mediate its effects on repressing gene expression of smooth muscle α-actin, smooth muscle myosin heavy chain, calponin, and smooth muscle 22α. YAP is induced in SMCs transitioning from a contractile to a synthetic proliferative phenotype in injured rat carotid arteries, YAP expression promotes SMC proliferation and migration, and deletion of YAP results in protection against neointimal formation after vascular injury. The findings that YAP interacts with myocardin and disrupts SRF binding and downstream effects on SMC contractile protein expression tie these observations into the myocardin–SRF axis.

Another kinase recently discovered to regulate SMC phenotypic switching is p38 MAPKα (mitogen-activated protein kinase; Mapk14). Knockdown of Mapk14, like YAP, results in increased expression of contractile proteins in SMCs. Interestingly, MAPK14 and YAP might both act, at least in part, within the myocardin–SRF pathway. MAPK14 acts through the myocardin paralog myocardin-related transcription factor A. However, the exact relationship between YAP and MAPK14 remains to be clarified.

There are many known extracellular factors that can activate the Hippo pathway and p38 MAPKα. One likely regulator is cell–cell or cell–matrix contact, which in turn modulates cytoskeletal organization. Cell adherence is crucial for SMC survival and function, and mice with SMC-targeted deletion of the β1 integrin exhibit a loss of SMCs. Other recent interest on extracellular regulators of SMC phenotypic switching include bioactive lipids and enzymes involved in regulation of bioactive lipid levels, such as lipid phosphate phosphatase.
SMCs can also take on properties of other cell types. For example, SMCs in atherosclerotic lesions have long been known to be able to accumulate cholesterol and thereby resemble macrophage foam cells. As much as 50% of oil red O–positive foam cells in human coronary artery lesions have been identified as SMCs, based on their expression of smooth muscle α-actin.11 Recently, beige adipocytes have also been identified as having a smooth muscle–like origin.12 Furthermore, during the process leading to vascular calcification, SMCs largely lose their expression of smooth muscle α-actin and smooth muscle 22α and gain expression of bone-forming proteins through a process, at least in part, dependent on the sodium-dependent phosphate cotransporters Pit-1 and Pit-2, which act through redundant mechanisms to promote calcification13 and through tumor necrosis factor–related protein-3,14 osteoprotegerin,15 and angiopoietin II.16

To complicate the identity of SMCs further, the origin of SMCs in the vasculature is diverse, and different arteries or segments of arteries contain SMCs of different developmental origin.17 In the mouse, neural crest-derived SMCs are found in the aortic arch and arteries branching off of the arch, such as the brachiocephalic artery often used for studies of atherosclerosis in mouse models. SMCs in the thoracic aorta are derived from somites, whereas SMCs in the abdominal aorta are derived primarily from splanchnic mesoderm.17 In addition, other lineages exist in other arteries, and stem cells and mesangioblasts also contribute to the composition of SMCs in different arteries. Although the phenotypic switching described above seems to be common to all SMCs regardless of origin, these SMCs respond differently to various stimuli.18,19 Building on this information, a recent study demonstrated that regional phenotypic variability of SMCs contributes to their regulation of nuclear factor-κB activity in response to tumor necrosis factor-α stimulation.18,19 These interesting findings might explain the differences in atherosclerosis susceptibility of different arteries under conditions in which systemic inflammation is elevated.

Another research area currently receiving considerable attention is that of SMC progenitor cells. These progenitor cells are now known to exist in the adult blood vessel wall20 and their differentiation into cells expressing SMC proteins is regulated by specific signaling pathways and transcription factors,21 as well as mechanical force.21 Interestingly, sirolimus, an inhibitor of the mammalian target of rapamycin has recently been shown to stimulate SMC progenitor cell differentiation, which might explain the recurrence of coronary artery restenosis in some patients who received sirolimus-eluting stents.22

In addition, endothelial cells and macrophages have been described to direct mesenchymal stem cells toward SMC fates.23,24 Therefore, loss of functional endothelial cells or accumulation of lesion macrophages could affect the differentiation of SMCs from progenitor cells.

The plasticity of the SMCs suggests that new treatment strategies for cardiovascular disease could target these processes.

Noncoding RNA and Micro-RNA as Regulators of SMC Phenotype

Noncoding RNAs are divided into 2 groups: the short (processed transcript length, <200 nucleotides) noncoding RNAs, including micro-RNAs, and the long (>200 nucleotides) noncoding RNAs. Several recent articles published in ATVB support the concept of noncoding RNAs as important regulators of SMC proliferation, migration, and calcification. For example, identification of long noncoding RNAs in human vasculature by RNA sequencing25 identified Smooth muscle and Endothelial cell–enriched migration/differentiation-associated long NonCoding RNA as a previously unannotated long noncoding RNA expressed in human coronary artery SMCs. By several in vitro studies, Smooth muscle and Endothelial cell–enriched migration/differentiation-associated long NonCoding RNA was shown to maintain SMCs in a contractile phenotype by maintaining expression of myocardin and SMC contractile protein genes.26 Conversely, myocardin was demonstrated to regulate the SMC response to injury, in part, through the micro-RNAs, miR-24 and miR-29a,27 and the expression of myocardin in injured blood vessels prevented neointimal thickening by reducing SMC proliferation and migration. Another micro-RNA that inhibits SMC proliferation in response to estrogen receptor-α activation in SMCs is miR-203.28 The ability of estrogen to induce miR-203 was shown to be because of the transcription factors, Zeb-1 and AP-1. Furthermore, in a study using a rat model of metabolic syndrome, miR-145 delivered to the arterial wall by adenovirus was identified as being able to maintain the contractile phenotype of SMCs after injury.29 Another micro-RNA, miR-663, was also recently found to regulate SMC phenotypic switching and neointimal formation.29 This micro-RNA is downregulated in SMCs stimulated by platelet-derived growth factor (PDGF), and its overexpression resulted in upregulation of SMC markers of the contractile phenotype and suppression of neointimal formation after injury in mice.29 Other micro-RNAs promote SMC proliferation, as has recently been shown to be the case for miR-130a in pulmonary SMCs.30

Micro-RNAs have also been identified as regulators of SMC calcification. Thus, miR-29 mediates vascular calcification through upregulation of ADAMTS-7 (a disintegrin and metalloproteinase with thrombospondin motifs-7) in rat SMCs.31

Another concept that has received much interest recently is that micro-RNAs can be released from cells and travel to other sites in circulation bound to particles, such as lipoproteins.32 Thus, it is possible that systemic changes in levels of micro-RNAs might influence SMC phenotype and response to local and systemic factors. It is also plausible that lipid particles might see a use as delivery tools of therapeutic noncoding RNAs.

Cross Talk Among SMCs and Other Cell Types

SMCs are continuously responding to signals derived from other cells and dysfunction of neighboring cells can lead to maladaptive SMC proliferation, migration, calcification, or apoptosis. Conversely, SMCs can signal to nearby SMCs or other cell types to direct the status of these neighboring cells. Much of the recent work on cross talk between SMCs and
other cell types in the vascular wall has been accomplished through the use of ex vivo coculture experiments, which can directly test the outcome of secreted factors from one cell type on another. In vivo models of cell-type–specific knockout mice are often done in conjunction with these studies to support the relevance of these findings.

Such coculture experiments recently suggested that matrix metalloproteinase 13 (MMP13), a protease that cleaves membrane-associated and extracellular proteins and is secreted by, for example, classically activated macrophages and probably also by lesion macrophages, promotes migration of SMCs after aortic endothelial denudation in endothelial nitric oxide (Nos3)—deficient mice.\(^{33}\) MMP13 acts as the predominant interstitial collagenase in mouse atherosclerotic lesions, and mice deficient in both Mmp13 and apolipoprotein E (ApoE) exhibit increased SMC accumulation in lesions of atherosclerosis, as well as increased intraplaque collagen when compared with ApoE\(^{+}\) control mice.\(^{34}\) Chemokines released from other cell types also affect SMCs. For instance, a recent perivascular adipose tissue (PVAT) transplantation study using PVAT from wild-type or monocyte chemoattractant protein 1 (CCL2)–deficient donor mice identified the PVAT as a driver of neointimal SMC accumulation through secreted monocyte chemoattractant protein 1.\(^{35}\) This work supports other recent findings in which monocyte chemoattractant protein 1, a known chemoattractant for monocytes, contributes to SMC proliferation.\(^{36}\) PVAT has 10- to 40-fold greater expression of monocyte chemoattractant protein 1 when compared with other depots of adipose tissue. Therefore, expanded PVAT as a result of risk factors, such as consumption of a high-fat diet, seems to contribute to neointimal SMC accumulation directly.\(^{35}\)

Beyond being responsive to signals from other cell types, SMCs can actively signal through paracrine and autocrine mechanisms to neighboring SMCs, creating a feed-forward loop and amplifying signals from their environment and, in disease models, deregulated migration, proliferation, and neointimal thickening can occur. SMCs produce chemokines that promote migration and recruitment of additional SMCs and remodeling of the vessel wall. Recently, the cytokines interleukin-6 and CXCL10 were found to promote SMC migration in this manner. Media from SMCs treated with a toll-like receptor 2 agonist promote migration of other SMCs in an interleukin-6–dependent manner,\(^{37}\) and deficiency in CXCL10 secretion from SMCs impairs SMC chemotaxis in a gradient of fetal calf serum.\(^{38}\) Similarly, MMP8 is released by SMCs and is correlated with atherosclerotic lesion progression.\(^{39}\) In addition to its known role in leukocyte trafficking,\(^{40}\) MMP8 was recently found to promote maturation of ADAM10 (a disintegrin and metalloproteinase domain-containing protein 10) in other SMCs by cleavage of its propeptide domain. Mature ADAM10 induces SMC migration and proliferation through the cadherin-β-catenin pathway.\(^{41}\) Other interactions are more complex. For instance, serum aldosterone is increased after endothelial injury. Aldosterone signals to mineralocorticoid receptors on the SMCs leading to increased placental growth factor secretion and increased expression of its receptor, vascular endothelial growth factor receptor 1. Placental growth factor signals to vascular endothelial growth factor receptor 1 leading to SMC proliferation and vascular remodeling.\(^{42}\) Beyond neighboring SMC-amplifying signals, cellular cross talk among multiple cell types can occur, activating these other cell types and enhancing proliferative behavior in SMCs. SMCs have been shown to activate monocytes,\(^{43}\) macrophages,\(^{44}\) and dendritic cells\(^{45}\) in this manner through release of soluble growth factors and cytokines. In turn, SMCs receive migration and proliferation signals from these neighboring cell types.

Finally, SMCs actively signal to other cell types to promote these cells to aid in vascular remodeling. Coculture experiments demonstrate that SMCs signal to endothelial cells both by direct contact and through paracrine interactions to modulate endothelial cell expression of the adhesion molecule VCAM1 (vascular cell adhesion molecule-1) and endothelial cell permeability through phosphorylation of β-catenin.\(^{46}\) Undoubtedly, we have just begun to uncover the autocrine and paracrine cross talk between SMCs, neighboring SMCs, and other cell types.

**Emerging Potential Drug Targets in SMCs**

With the many roles that SMCs have in normal and diseased vessels, they represent an exciting area for therapies that target vascular disease. Chemokines secreted by SMCs and other vascular cells are potentially promising therapeutic candidates. Biological therapies can be developed to target these chemokines before reaching their SMC target. Another area that has received continued attention is therapies to prevent restenosis after angioplasty. Life-saving surgeries, such as balloon-angioplasty and placement of stents, are used to restore blood flow in atherosclerotic arteries. However, endothelial cell damage inevitably occurs with these surgeries, promoting SMC proliferation and migration in an attempt to heal these new wounds. In turn, this narrows the artery and creates a vicious cycle. Drug-eluting stents are now being used to help control SMC proliferation after some of these surgeries. The drugs currently in use are antiproliferative agents as sirolimus or paclitaxel (taxol, a drug originally isolated from the Pacific Yew, inhibits SMC proliferation by microtubule stabilization). These drugs inhibit not only SMC proliferation but also endothelial cell proliferation, slowing the repair of the initial endothelial cell damage and leaving the patients at greater risk for thrombosis. Drug targets that can distinguish between SMC and endothelial cell proliferation are in need. As our understanding of the mechanisms of phenotype switching in SMCs deepens, specific targeting of these cells will be more attainable. We are seeing some recent progress in this area of research. In SMCs, CTP synthase 1 is increased after the stimulation of SMCs with PDGF. This enzyme catalyzes the CTP/pyrimidine biosynthesis required for cell proliferation. Blockade of CTP synthase 1 (by an inhibitor or shRNA) prevents in vitro proliferation of SMCs in response to PDGF and blocks in vivo neointimal formation after vascular injury but has less effect on endothelial cells.\(^{47}\) PDGF also increases the mitochondrial membrane potential, a phenomenon observed in vessel injury models. Treatment with the mitochondrial pyruvate dehydrogenase kinase inhibitor drug dichloroacetate, or knockdown of pyruvate dehydrogenase kinase isoform
2, reduces mitochondrial membrane potential through reduced association between hexokinase 2 and voltage-dependent anion channels. This negates the proliferative and antiapoptotic effects of PDGF treatment and reduces neointimal formation in models of vessel injury. Dichloroacetate does not inhibit endothelial cell migration or re-endothelialization, making it an interesting novel drug candidate.46 In addition, overexpression and knockdown studies have identified miR-203 as a micro-RNA with distinct effects on SMCs and endothelial cells. Although miR-203 slows SMC proliferation, it has no effect on endothelial cell growth.27

Targets that aid endothelial cell recovery without promoting SMC growth may also be good therapy options for restenosis. Recent research on Apoe−/− mice that lack the CXCL12 receptor CXCR4 indicates CXCR4’s requirement for normal healing after wire injury.49 The toll-like receptor 2/6 receptor CXCR4 indicates CXCR4’s requirement for normal healing after wire injury.49 The toll-like receptor 2/6

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None.

References


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