MicroRNA Regulation of Vascular Smooth Muscle Function and Phenotype

Early Career Committee Contribution

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In periods of health, the vascular smooth muscle cell (VSMC) predominantly functions to regulate vascular tone and to provide structural integrity to the vascular tree. However, the VSMC is also one of the most plastic cells in the body. Under periods of stress, or in response to disease stimuli, the VSMC deviates from its physiologic state and can become hyperproliferative, migratory and/or proinflammatory. Such de-differentiation is believed to contribute to a variety of maladaptive processes with relevance to vascular disease, including de novo atherogenesis, postangioplasty neointimal hyperplasia, aneurysmal degeneration, medial calcification, and even foam cell formation. In an effort to modulate these conditions, scientists have sought the master regulators of VSMC physiology and plasticity, and have made progress identifying a number of transcriptional cofactors that modulate expression of the contractile apparatus and genes which are activated in the perturbed VSMC. Recent work, however, has shown that several epigenetic factors also modify VSMC behavior and could similarly serve as translational targets or biomarkers of disease. In the review provided below, we will focus on the role of microRNAs as the best studied of these factors, and provide an overview of their important role in VSMC homeostasis and pathophysiology.

Physiology of the Vascular Smooth Muscle Cell

In the nondiseased artery, the tunica media is comprised almost exclusively of VSMCs and the connective tissues secreted by these cells. The VSMC serves to modulate vascular tone, and therefore, end-organ perfusion, by contracting and relaxing in response to sympathetic innervation and diffusible factors, such as nitric oxide released from the overlying endothelial cells of the tunica intima.1-3 Their related extracellular matrix proteins comprise the scaffolding, which provide the structural support necessary to withstand the radial force applied to the vessel during the cardiac cycle. At the molecular level, the VSMC is dominated by its contractile apparatus, including proteins, such as smooth muscle alpha actin (ACTA2), myosin heavy chain (MYH11), and transglutamin (TALGN). The expression of such SMC-specific proteins is used to identify the quiescent VSMC, although it is now appreciated that each of these factors can be expressed by various other cell types, in certain conditions.1 In general, the VSMC is a fully differentiated cell, and displays a slow turnover rate, rarely undergoes programmed cell death, does not migrate, and synthesizes little protein other than that required for its contractile machinery.1

Pathophysiology of the VSMC

Unlike the skeletal or cardiac muscle cell, the VSMC can respond to a variety of stressors and dedifferentiate.1,5 In general, this term is used to describe the phenomenon wherein a VSMC downregulates the expression of its contractile proteins, and increases its rate of proliferation, migration, and extracellular matrix secretion.3 Although it is tempting to conceptualize the VSMC as existing in only 1 of 2 dichotomous states (ie, either a synthetic or contractile state), it is important to note that these changes occur on a spectrum and that in addition to re-entering the cell-cycle, the dedifferentiated VSMC can also activate programmed cell death pathways and elaborate proinflammatory cytokines.6 Furthermore, the activated VSMC can go to date as to assume behaviors typically ascribed only to other cell types, including phagocytosis and effecotrosis,9 ossification,10 reverse cholesterol transport,9,11 and even foam cell formation.12,13 Indeed, one of the more exciting recent findings made possible by the use of elegant in vivo lineage tracing systems suggests that the VSMC can assume a macrophage-like phenotype in the developing atherosclerotic plaque, thus challenging the paradigm that lesional foam cells arise predominantly from bone marrow-derived monocytes.5,14-16

The mechanisms that control VSMC plasticity are complex and poorly understood. What is known is that the VSMC can respond to both direct and indirect stimuli, including mechanical injury, ligand–receptor interactions, and signaling downstream of extracellular matrix-sensing factors, such as integrins and focal adhesion complexes.2,3,5 To date, several master regulators have been identified, which mediate expression of the classical VSMC contractile genes (Figure), including serum response factor (SRF) and myocardin (MYOC).17 It is becoming clear, however, that these factors are necessary but not sufficient for complete control of VSMC physiology, and that additional
cofactors and unrelated signaling pathways must be integrated to determine the ultimate fate of the VSMC in the vascular lesion. Although not reviewed here because of space constraints, strong evidence is now available that factors, such as Kruppel-like factor 4 (KLF4),19 platelet-derived growth factor (PDGF-BB),20,21 the Notch pathway,2 and transforming growth factor (TGF)-β22–25 have equally potent effects on the differentiation status and survival of the VSMC.2 Elucidation of these signaling pathways may allow for the development of translational therapies that can modify VSMC plasticity, and therefore, potentially provide for methods to reduce myointimal proliferation during restenosis, prevent dropout of medial cells during aneurysmal degeneration, or retard foam cell formation during atherosclerosis.6,26–28 Conversely, it is possible that we may ultimately want to activate certain aspects of dedifferentiation, so as to promote stabilization of the SMC cap overlying the vulnerable plaque as a method to prevent lesion ulceration and myocardial infarction.29

Epigenetic Regulation of the VSMC

Beyond the classical pathways mentioned above, recent studies have identified several additional layers of regulation that provide for the significant plasticity retained by the adult VSMC. These include a variety of epigenetic factors that alter the accessibility of contractile genes via chromatin remodeling30 and the exciting class of long noncoding RNAs, which silence SMC-related pathways via antisense interactions.31 The best studied of these epigenetic factors, however, are microRNAs. MicroRNAs are a class of short (∼22 nucleotide) single-stranded RNAs that generally function to inhibit gene expression by binding and sterically inhibiting the translation of target mRNAs (reviewed in Wei et al32). These factors are of particular interest to the vascular biology community because they tend to target entire regulatory networks (rather than individual genes separately), and serve as appealing candidates, which could serve to fine tune the complex differentiation and homeostasis pathways described above.33,34 In the summary provided below, we will first review the studies describing a role for microRNAs in SMC physiology in vitro, then discuss the studies that detail in vivo manipulation of candidate microRNAs in vascular disease models, and conclude with an overview of the potential for leveraging microRNAs as a diagnostic and therapeutic in the effort to address conditions dominated by VSMC dysfunction and the response to vessel injury.

MicroRNAs in the Regulation of VSMCs, In Vitro

As the understanding of microRNA biology grows, so too does the list of microRNAs that control VSMC behavior. miR-29 is emerging as a multifaceted regulator of VSMC pathology, targeting genes involved in elasticity, calcification and cell migration. Loss of miR-29 expression occurs in calcified VSMCs in vitro, and in highly calcified rat arteries in vivo, which corresponded to an increase in ADAMTS-7—a gene previously associated with CAD by genome-wide association studies.35 ADAMTS-7 is directly targeted by miR-29, and loss of miR-29 expression in calcified VSMCs increases ADAMTS-7 expression and promotes calcification in vitro.36 Although miR-29 has previously been shown to regulate osteoclast differentiation and function, these data are the first to implicate miR-29 in VSMC calcification in vivo, and is now among the growing list of microRNAs implicated in both intra- and extracellular modulation of vascular calcification.37 Estrogen and other steroid hormones control multiple aspects of VSMC biology, including proliferation...
and migration, with multiple underlying mechanisms. Surprisingly, Zhao et al found that estrogen exerts its vascular protection in part via a microRNA, miR-203, which is transcriptionally upregulated in VSMCs via ERα stimulation. Estrogen treatment of VSMCs in vitro derepresses the expression of miR-203, which then acts to repress its target genes p63 and Ab11, inhibiting VSMC proliferation. This miR-203 pathway could ultimately serve to protect the vessel wall from intimal lesion development when estrogen is present, and could thus be considered as a therapeutic miRNA. Although the role for miR-203 has yet to be confirmed in vivo, these studies highlight how microRNAs are now being associated with well-established molecular pathways that are likely underlying many aspects of vascular disease.

Vascular complications in diabetics lead to serious adverse clinical events, and Reddy et al recently implicated microRNAs in the diabetic VSMC. They found that the miR-200 family was increased in VSMCs and aortas from diabetic mice compared with their nondiabetic counterparts. Direct 3′UTR binding of miR-200 to Zeb1, a multifaceted transcription factor, leads to downregulation of Zeb1 expression and a concomitant upregulation of the proinflammatory genes COX-2 and MCP-1. This increased proinflammatory gene expression resulted in increased monocyte binding to VSMCs under diabetic conditions, which was enhanced by overexpression of miR-200 and blocked by inhibitors of miR-200. These studies elegantly demonstrate how dysregulation of microRNA expression during disease may have important consequences in the vessel wall that promote inflammatory disease progression.

**MicroRNAs in the Regulation of VSMCs, In Vivo**

Several experimental in vivo studies have evaluated the role, contribution, and translational therapeutic potential of microRNAs and their modulation in cardiovascular disease. Different models, such as carotid ligation, balloon or wire injury, as well as hindlimb ischemia, have provided evidence for the crucial role of several microRNAs in VSMC plasticity, survival, and dedifferentiation.

Inhibition of miR-21 reduces neointima formation after vascular injury via upregulation of the proapoptotic proteins BCL2 (B-cell CLL/lymphoma 2) and PTEN (phosphatase and tensin homolog). On the basis of the same mechanism, overexpression of miR-21 induces proliferation of VSMCs in the aortic wall, again mainly through inhibition of PTEN, and thus protects against accelerated murine aortic aneurysm progression and rupture. In addition, miR-21 has been shown to contribute crucially to TGF-β-regulated endothelial-to-mesenchymal transition. A well-studied cluster of vascular microRNAs are the VSMC-enriched miRs-143/-145, which are known to be regulated transcriptionally by serum response factor and myocardin. miR-143/-145-deficient mice revealed that a VSMC phenotype-switching processes of the miR-143/-145 cluster include angiotensin-converting enzyme and Kruppellike factor4/5. On a similar note, miR-145 has recently been shown to be important in restoring the contractile VSMC phenotype and coronary collateral growth in metabolic syndrome.

Additional microRNAs involved in the response to vascular injury by regulating VSMC proliferation and thus myocardial hyperplasia are miRs-24 and -29a, both being inducible again via myocardin. Interestingly, miR-24 has furthermore been shown to regulate the activation of vascular disease-associated macrophages and atherosclerosis retardation. miR-29, however, has been identified as an important regulator of extracellular matrix remodeling and the fibrotic response in cardiovascular disease, with its inhibition being able to—among other mechanisms—effectively restore elastin levels in fibroblasts and SMCs.

Another microRNA with an effect on VSMC biology and vascular remodeling is miR-26a. Its downregulation accelerates VSMC differentiation, whereas inhibiting proliferation and migration by influencing TGF-β-pathway signaling. In more detail, the authors were able to show that inhibition of miRNA-26a enhances the expression of SMAD-1 and SMAD-4. Interestingly, miR-26a is furthermore repressed in murine models of abdominal aortic aneurysm formation wherein phenotypic switching of VSMCs (from contractile to synthetic) occurs. In addition to miR-26a, miR-712 (and its human homologue miR-205) have been shown recently to be involved in murine abdominal aortic aneurysm development, by targeting and repressing tissue inhibitors of metalloproteinases-3 and RECK in angiotensin II-induced mouse aneurysms, thus stimulating vascular inflammation and MMP activity.

Both miRs-221 and -222 have been shown to be implicated in VSMC differentiation. Overexpression of miR-221 reduced differentiation and increased proliferation and migration of VSMCs. Reduction of miR-221 increases expression of VSMC differentiation markers and blocks the effects of platelet-derived growth factor on proliferation and migration. miR-221 targets c-Kit and p27Kip1 mRNA expression.

**Conclusion and Translational Considerations**

With the discovery that microRNAs are crucial regulators of cardiovascular disease, it is only a logical consequence that their therapeutic and biomarker potential should extensively be explored. One major drawback of microRNA-based therapeutics at this point are off-target effects in organ systems in which microRNA modulators assimilate to a much greater extent than the targeted vasculature, such as liver, kidney, lung, and spleen. Forced overexpression of the VSMC-enriched miR-21, for example, represses expression of important tumor suppressor genes (Pten, Bcl2, and Spry1) in these organs. Thus, the implementation of local or cell-type–specific delivery mechanisms, using drug eluted angioplasty balloons and stents are highly desirable in making the translation of microRNA modulators more feasible in humans. Excitingly, the understanding of the genetic risks associated with vascular disease has expanded to include the observation that polymorphisms in microRNA genes or microRNA-binding
sites in critical protein-coding genes can contribute to pathogenesis of vascular disease.\(^{56}\) Recently, Jeon et al.\(^{7}\) found that single nucleotide variation in the miRs-146a, -149, -196a-2, and -499 were significantly associated with stroke prevalence in a Korean population, and the authors surmise this may be because of decreased expression of inflammatory mediators, such as TNF\(\alpha\) and CRP. Although the field of microRNAs as biomarkers is continuing to evolve, there is little doubt that their use for the prediction and diagnosis of vascular disease will be significant. Overall, these recent studies and others have underscored the importance of microRNAs in VSMCs, and are beginning to inform us of the epigenetic mechanisms of that regulate the functionality and plasticity of VSMCs in both health and disease.

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