Myocardin—Not Quite MyoD
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Smooth muscle cells (SMCs) have evolved to subserve a variety of diverse functions in higher vertebrates, including modulation of arterial tone, regulation of airway resistance, and control of gastrointestinal motility. The diverse functional capacities of SMCs are ultimately determined by the expression of genes encoding SMC-restricted contractile and cytoskeletal proteins, intracellular enzymes, cell surface ligands, and receptors. Several features distinguish the SMC lineage from the skeletal (fast and slow) and cardiac muscle cell lineages. In contrast to skeletal and cardiac muscle cells, SMCs fail to undergo terminal differentiation and permanently exit the cell cycle. In addition, SMCs retain the capacity to reversibly modulate their phenotype during postnatal development in response to a variety of extracellular stimuli including vessel wall injury. As such, the molecular programs underlying SMC differentiation must differ fundamentally from those programs governing skeletal and cardiac myocyte differentiation.

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Relatively little is currently understood about the molecular mechanisms that regulate SMC lineage specification, differentiation, and modulation of SMC phenotype. This is because, in part, of the complex embryological origins of the SMC lineage, which are derived both from mesodermally-derived lateral mesenchyme as well as ectodermally-derived neural crest. In addition, in contrast to skeletal and cardiac lineages, relatively few definitive markers of the SMC lineage have been identified, and expression of these markers may be undetectable when SMCs modulate their phenotype from contractile to synthetic (for review see Parmacek1 and Miano9). SRF is a 508-aa protein that is a member of the ancient MADS box family of transcription factors.9 The conserved MADS box domain of SRF mediates both DNA-binding activity and heterodimerization with other transcription factors. SRF was originally cloned and characterized because in serum-stimulated cells it binds to, and transactivates, the growth responsive c-fos promoter.9 Subsequently, it was shown that SRF plays an important role in activating transcription of some skeletal- and cardiac-specific genes including α-cardiac actin and α-skeletal actin.10,11 SRF binds the consensus nucleotide sequence (CC(A/T)6 GG), which has been variably designated as an SRE or CArG box, as a homodimer or heterodimeric protein complex.12 Transcriptional activity of SRF is regulated by multiple mechanisms including: (1) direct binding of other (lineage-restricted) transcription factors,13–17 (2) posttranslational modifications of SRF,18 (3) posttranslational modifications of ternary complex factors that bind to SRF,19,20 and (4) alternative-splicing.21,22 Functionally important CArG boxes have been identified in the promoters and/or transcriptional enhancers controlling expression of the SM-MyHC, SM-α-actin, SM22α, calponin-h1, and telokin genes.7,8 Mutations that abolish binding of SRF to these transcriptional regulatory elements abolished transcriptional activity of these elements in transgenic mice. Moreover, a multimerized copy of the SM22α CArG box restricts transgene expression to arterial SMCs in transgenic mice.23

Because of its ability to transduce extracellular, cytoskeletal, and intracellular signals and its capacity to activate genes encoding SMC contractile markers, it was postulated that SRF serves as a “nuclear-sensor” integrating multiple extracellular and intracellular signals and regulating SMC phenotype.2 However, until the discovery of myocardin it remained unclear how SRF, which is expressed ubiquitously, could regulate a subset of SMC-restricted genes and thereby SMC differentiation and phenotype. In skeletal muscle cells, the related MADS box transcription factor MEF2 binds to DNA and heterodimerizes with the skeletal muscle–specific transcription factor MyoD to synergistically activate skeletal muscle–specific transcriptional regulatory elements.24 Similarly in the heart, SRF heterodimerizes with the cardiac...
SM-expression of endogenous SMC markers including SMyHC, SM-α-actin, SM22α, calponin-h1, telokin, and desmin. Myocardin (and MRTF-A) activates transcription by heterodimerizing to the MADS box domain of SRF which in turn binds directly to specific CArG motifs located in transcriptional regulatory regions controlling expression of these SMC genes. In contrast, a subset of genes expressed in SMCs is controlled in a myocardin (and SRF)-independent fashion. These genes include FRNK, ACLP, Smoothelin-B, and HRC. Candidate transcription factors regulating expression of these genes include MEF2, GATA6, Ets-1, and members of the kruppel-like family of transcription factors (KLFs).

Using an in silico cloning strategy, Olson and colleagues cloned myocardin and demonstrated that myocardin is a remarkably potent transcriptional activator of SRF-dependent genes including SM22α. In addition, they reported that myocardin gene expression is restricted to the heart and SMC-containing tissues. Shortly thereafter our group and others reported that forced expression of myocardin activates transcription of multiple genes encoding SMC contractile markers including SMα-MyHC, SM-α-actin, SM22α, and calponin-h1. In addition, we reported that knockdown of myocardin gene expression in SMCs, by either siRNA or over-expression of a dominant-negative myocardin mutant, suppresses transcription of genes encoding SMC contractile markers. Remarkably, our group and others found that forced expression of myocardin in undifferentiated embryonic stem (ES) cells and several other cell types activates expression of endogenous SMC markers including SMα-MyHC, SM-α-actin, calponin-h1, and SM22α. Consistent with these findings, Olson and colleagues reported that myocardin-deficient mice die at embryonic day (E)10.5 and show no evidence of vascular SMC differentiation. These compelling data led some to conclude that myocardin is a “master regulator” of the SMC gene expression.

The concept of a master-regulatory gene originated when Lassar and Weintraub cloned and characterized the skeletal muscle–specific basic helix-loop-helix (bHLH) transcription factor MyoD. Remarkably, Lassar and Weintraub demonstrated that forced expression of MyoD causes C3H10T1/2 fibroblasts to adopt a skeletal muscle cell fate. Subsequently, it was shown that MyoD is a member of a larger family of skeletal muscle–specific bHLH transcription factors, each member of which possesses the capacity to drive cells to a skeletal muscle cell fate (for review see Olson31). It is noteworthy that MyoD family members are expressed exclusively in skeletal muscle cells and the myotomal component of the somites whereas myocardin is expressed in both cardiac myocytes and SMCs. Moreover, at the level of sensitivity provided by in situ hybridization, myocardin is not detectable in all vascular SMCs during embryonic development. It is also noteworthy that forced expression of MyoD leads to expression of the full repertoire of skeletal muscle genes, and these cells exhibit the electrophysiological and mechanical properties of differentiated skeletal muscle.

The data presented by Yoshida and colleagues in this issue of *Arteriosclerosis, Thrombosis, and Vascular Biology* suggests that the conclusion that myocardin is a master regulator of the SMC gene expression requires qualification or may be premature. As shown in the Figure, Yoshida observed that forced expression of myocardin activates a subset of SRF-dependent SMC contractile genes. However, expression of other genes expressed in SMCs, including smoothelin-B, aortic carboxypeptidase-like protein (ACLP), and focal adhesion kinase-related nonkinase (FRNK), which reportedly are not CArG box-dependent, was not observed. This observation is consistent with a recent report showing that expression of the histone rich calcium-binding protein (HRC) in smooth, cardiac, and skeletal muscle is controlled by a MEF2-dependent myocardin-independent promoter. Moreover, forced expression of myocardin also induced expression of cardiac- and skeletal muscle–specific genes including atrial natriuretic factor, cardiac α-actin, and skeletal α-actin. Expression of this subset of cardiac and skeletal muscle–specific genes is also dependent on SRF. Thus it appears that myocardin activates SRF-dependent SMC contractile genes (as well as SRF-dependent cardiac- and skeletal muscle–dependent genes), but alone is not sufficient to activate the full repertoire of genes expressed in SMCs. Together, the existing experimental evidence suggests a model wherein myocardin is required for SMC differentiation and promotes the contractile SMC phenotype, but alone is not sufficient to convert undifferentiated cells to the SMC lineage.

These data suggest strongly that SMC differentiation in vivo requires one or more signals, in addition to myocardin, that was/were not provided under the experimental conditions used. In the vasculature strong candidates include endothelial cell–derived growth factor(s). Alternatively, the precise concentration of myocardin or the myocardin to SRF ratio may be a critical determinant of SMC differentiation in vivo. Finally, the role of the two recently described myocardin-related transcription factors must also be considered. Nevertheless, the discovery of myocardin, and two myocardin-related transcription factors, has opened up exciting new avenues of investigation that promise to provide
important insights into the molecular program underlying SMC differentiation and the modulation of SMC phenotype.

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References

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