Myocardin: New Therapeutic Agent in Vascular Disease?

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There is a general consensus that perturbations to the vessel wall provoke a population of mature vascular smooth muscle cells (VSMCs) to either dedifferentiate into proliferating/migrating cells or transdifferentiate into new cell types.\(^1\) Such phenotypic plasticity has stimulated much research on the signaling pathways and transcriptional mediators, coordinating the expression of a growing number of genes (both coding and noncoding) that constitute a unique transcriptome for the differentiated state of VSMCs. The majority of such genes harbor 1 or more 10-bp codes known as CArG boxes to which the serum response factor (SRF) binds.\(^2\) More than 60 proteins interact with SRF to drive cofactor-dependent programs of gene expression. One prominent SRF cofactor is myocardin (MYOCD), which was first described as a cardiac muscle–enriched transcription factor that powerfully stimulates CArG-dependent promoters driving expression of cardiac-specific genes and contributes to normal cardiac muscle gene expression in vivo.\(^3\) Soon after its discovery in 2001, MYOCD was shown to similarly enhance activity of VSMC-restricted promoters in a CArG-dependent manner and induce expression of several of the corresponding endogenous transcripts in a cell line that does not express VSMC-restricted genes. Furthermore, Myocd mRNA was discovered to be abundantly expressed in aortic SMC, only to be reduced when such cells are placed in culture, suggesting MYOCD is important in maintaining the normal VSMC contractile phenotype.\(^4\) Subsequent studies confirmed and extended many of these findings, thus validating the role of MYOCD as a key component of a molecular switch for VSMC differentiation.\(^5\) Parallel studies have defined a new regulatory control system for VSMC phenotype involving the action of microRNAs.\(^6\) Although we have gained much insight into the biology of MYOCD in various experimental settings, there has been essentially no investigation into its functional role in vascular disease. Now, in this issue of Arteriosclerosis, Thrombosis and Vascular Biology, Talasila et al use MYOCD gain-of function and loss-of-function studies in mice and propose this SRF cofactor antagonizes neointimal formation and VSMC migration after acute vascular injury, in part, through the action of MYOCD-induced microRNAs.

In general, levels of Myocd mRNA are inversely correlated with cellular growth states\(^7\) and intimal expansion.\(^8\) Consistent with these findings, Talasila et al used adenoviral-mediated gene transfer of MYOCD to wire-injured carotid arteries and showed attenuated neointimal formation and VSMC proliferation. Conversely, heterozygous Myocd null mice exhibit exaggerated neointimal formation after carotid artery ligation injury as well as increases in the number of proliferating cells in the neointima staining positive for a commonly used marker of VSMC (ACTA2). The latter results along with a recently described phenotype in bladder SMC\(^9\) provide the first documentation of Myocd haploinsufficiency. Interestingly, gain-of function in MYOCD resulted in reduced ACTA2 positive cells that did not costain with BrdU, whereas loss-of-function in MYOCD elicited an increase in such cells, suggesting MYOCD has an in vivo function related to the inhibition of VSMC migration. Because ACTA2 is not the most specific VSMC marker and the effects of MYOCD on other locally derived progenitor cells cannot be excluded, the proposed migratory phenotype with gain-of function/loss-of-function in MYOCD should be further explored and validated with lineage tracing using a tamoxifen-inducible Myh11-Cre\(^{ERT2}\) driver mouse.\(^1\)

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A potent stimulus for VSMC migration is platelet-derived growth factor-β (PDGF-BB).\(^1\) Accordingly, Talasila et al used in vitro migration assays to show that PDGF-BB–induced VSMC migration could be blocked with MYOCD overexpression. Importantly, MYOCD also inhibited expression of the PDGF-BB receptor (PDGFRB). Such inhibition occurred at both the mRNA and protein levels. Although inhibition of PDGFRB expression could proceed through a number of mechanisms (eg, direct transcriptional repression), Talasila et al considered the post-transcriptional silencing effects of microRNAs. In fact, a previous report had already demonstrated that miR9 could target PDGFRB in cardiomyocytes.\(^1\) Using a directed qRT-PCR screen for MYOCD-induced microRNAs, Talasila et al found elevations in miR143/145 and showed these microRNAs exert regulatory control of VSMC migration in a manner that is independent of PDGFRB repression. The latter results are congruent with those of previous studies showing miR143/145 targeting other mediators of cell migration\(^1\) and suggest there could be additional MYOCD-induced microRNAs that target PDGFRB. Indeed, in addition to miR143/145, Talasila et al found that MYOCD stimulated expression of miR24 and miR29a, both of which are predicted to target the PDGFRB 3′ untranslated region. Although the previous repressor of PDGFRB, miR9,\(^1\) was detected in VSMCs, it was not induced with MYOCD overexpression. Because many target genes of MYOCD contain
functional CArG boxes, Talasila et al surveyed the genomic landscape around miR24 and miR29a for these important regulatory codes. miR24 and miR29a harbor CArG-like boxes; however, Talasila et al were unable to demonstrate binding of either MYOCOD or SRF using a ChIP assay, indicating MYOCOD induction of these microRNAs occurs in an unconventional manner.

To formally demonstrate a link between MYOCOD induction of miR24 and miR29a, PDGFRB expression, and VSMC migration, Talasila et al first used standard luciferase reporter experiments and qRT-PCR studies with antimiRs to validate the repressive action of miR24 and miR29a on PDGFRB expression. Next, they rescued MYOCOD inhibition of PDGFRB expression and VSMC migration using antimiRs to both miR24 and miR29a. Importantly, overexpression of each microRNA was sufficient to inhibit VSMC migration in vitro. In vivo, vascular injury resulted in attenuated expression of miR29a consistent with reduced levels of MYOCOD. Furthermore, there was a decrease in expression of miR24 and miR29a, as well as miR143/145, and an increase in PDGFRB in the injured carotid artery of heterozygous Myocd null mice. Collectively, these results would suggest that the inhibition of VSMC migration by MYOCOD occurs, in part, through the induction of miR24 and miR29a and their repressive action on the PDGFRB transcript. It is likely that other events are occurring with MYOCD overexpression such as the induction of long noncoding RNAs that may directly or indirectly modulate PDGFRB expression levels.

The results of Talasila et al are the first to document a potentially efficacious role for MYOCOD in vascular injury responses. The question will be whether MYOCOD can be harnessed and safely used as a new therapeutic modality for human vascular diseases. Several outstanding questions and challenges exist. First, we need additional tools to study MYOCOD protein expression in a reliably consistent manner. Second, the role of MYOCOD in other vascular disease models should be thoroughly assessed including atherosclerosis, hypertension, and transplant arteriopathy. Third, we need to fully define the transcriptome after MYOCOD overexpression, including all long noncoding RNAs which already outnumber protein-coding genes and display increasingly diverse functions in the cell. Fourth, because MYOCOD is sufficient to orchestrate biochemical, structural, and physiological attributes of VSMCs, strategic targeting of cells (whether through gene therapy or small molecule agonists of MYOCOD) will be critical so as to minimize the reprogramming of other cell types that may be important in maintaining vascular homeostasis (eg, endothelial cells). Despite these limitations, the data from Talasila et al provide an important foundation for future work on MYOCOD in the setting of vascular disease.

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Disclosures

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