Cardiovascular diseases, such as ischemic heart disease and stroke, represent the number one cause of death worldwide. In most cases, atherosclerosis and hypertension constitute the underlying causes for cardiovascular disease because it leads to arterial occlusion and impaired cardiac function, respectively. Therapeutic compensation of atherosclerotic artery occlusion by bypass grafting, angioplasty, or stenting bears the risk of intimal hyperplasia and subsequent restenosis. In general, a prerequisite for any pathological and physiological vascular remodeling process is the phenotypic switch of vascular smooth muscle cells (VSMCs). Even in adult blood vessels, VSMCs retain a remarkable plasticity that is essential for any changes in the vessel wall architecture. Contractile VSMCs representing the majority of VSMCs in healthy vessels guarantee maintenance of vascular tone and thereby the blood vessel diameter. As outlined in the Figure, a plethora of humoral factors, such as platelet-derived growth factor-BB and biomechanical stimuli, especially chronic changes in the blood vessel diameter, is thought to trigger the phenotypic switch of VSMCs from a quiescent, contractive differentiated state to a synthetic, proliferative, and dedifferentiated state.

Differentiation and phenotypic switching of VSMCs are achieved by changes in gene expression programs that are governed by epigenetic and transcriptional control mechanisms. For instance, serum response factor controls SMC differentiation and phenotype. Serum response factor activity is regulated by cofactors, such as myocardin and myocardin-like transcription factors, that form stable ternary complexes with serum response factor binding to a sequence known as CArG box. Myocardin promotes the expression of many VSMC marker genes, including calponin (CNN1), smooth muscle α-actin (SMA, ACTA2), SM-22α (TAGLN), and smooth muscle myosin heavy chain (MYH11). By contrast, the E-twenty-six (ETS) domain-containing protein-1 is thought to compete with myocardin for binding to serum response factor thereby counteracting its activity. Likewise, Krüppel-like factor 4 (KLF4) represses expression of myocardin and VSMC marker genes. During hypertension-induced arterial remodeling, myocardin activity is impaired through extracellular receptor kinase-dependent nuclear export and subsequent degradation underlining the relevance of this transcriptional coactivator as a keeper of VSMC quiescence.

Activator protein-1 is another key transcriptional regulator of the SMC phenotype mediating, for example, biomechanical, stress responses, and proinflammatory gene expression, in VSMCs and endothelial cells (ECs). The activator protein-1 subunit JUNB is critically involved in angiogenic processes as well as in the control of vascular homeostasis, by governing the migration and contraction capacity of VSMCs through transcriptional activation of the myosin light chain 2/ MYL9—a crucial element of the contractile apparatus. Recently, an additional mode of control was delineated, namely the negative regulation of gene expression by microRNAs (miRNA) that are small noncoding RNAs. miRNAs are generated from primary RNA precursors that are subjected to cleavage steps executed by the endoribonucleases Drosha and Dicer. miRNAs act on the post-transcriptional level and are considered to orchestrate the fine-tuning of transcriptional networks. Several miRNAs were shown to modulate VSMC function and plasticity. Notable players for VSMC differentiation and maintenance of the contractile phenotype are miR-1, miR-21, miR-221/miR-222, and most importantly the miRNA-cluster miR-143/miR-145. On vascular injury and subsequent progression to the synthetic phenotype, the miR-143/miR-145 cluster is downregulated, whereas miR-21, miR-146a, and miR-222/miR-223 are robustly induced. Importantly, overexpression of miR-143/miR-145 is sufficient to promote the VSMC contractile state.

In the current issue of Circulation Research, Li et al report on a novel determinant of the VSMC phenotype, namely miR-663. This miRNA was previously associated with tumor-suppressive and oncogenic features, respectively, dependent on the cancer type. In human umbilical venous ECs exposed to oscillatory shear stress, miR-663 was identified as the most upregulated miRNA and found to be implicated in the proinflammatory response of ECs. The same study showed that miR-663 targets the transcription factors KLF4, CCAAT-enhancer-binding protein β, and activating transcription factor 3 under oscillatory shear stress conditions. Now, Li et al provide evidence that miR-663 is a novel modulator of the human VSMC phenotypic switch by targeting JUNB. According to their findings summarized in the Figure, miR-663 overexpression inhibits platelet-derived growth factor-BB–induced JUNB and MYL9 expression and subsequent VSMC migration, as well as neo-intima formation. In line with these observations, previous

miR-663 and the miRaculous Vascular Smooth Muscle Phenotypic Switch

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work by Licht et al\textsuperscript{17} shows that the JUNB-MYL9 axis is required for cell migration. Moreover, Li et al\textsuperscript{23} provide evidence that miR-663 overexpression inhibits neointima formation and is associated with a decrease in JUNB abundance and VSMC proliferation. However, loss of JUNB did not affect proliferation of medial SMCs during hypertension-induced remodeling of the arterial wall,\textsuperscript{17} suggesting that JUNB may not control all aspects of the phenotypic switch. Amazingly, the experimental setting used by Li et al\textsuperscript{23} worked because the miR-663–binding site within the \textit{JUNB} 3′-untranslated region is highly conserved from human to rodents although miR-663 is only expressed in primates. Unfortunately, miR-663 function cannot be studied by targeted mutagenesis in mice further to unravel its function in VSMC differentiation and proliferation and to resolve the conflicting data. Moreover, it remains to be addressed whether this regulation applies to rodents at all or whether another miRNA may replace miR-663 function in rodents.

With respect to putative clinical applications of miR-663, for example, for preventing restenosis or intimal hyperplasia, the results of Li et al\textsuperscript{23} raise several questions. First, what is the baseline expression level of miR-663 in human VSMCs and how relevant is it for maintaining their phenotype? In fact, a complex pattern of miRNAs seem to cope with that task as can be deduced from experiments, indicating that the SMC-specific Dicer knockout causes a more severe phenotype than the ablation of the miR-143/miR-145 cluster in VSMCs.\textsuperscript{27} In this context, expression of miRNAs miR-143/miR-145 specifically supports the contractile phenotype, for instance, by interfering with KLF4 expression in response to transforming growth factor β and bone morphogenic protein 4 signaling.\textsuperscript{28} Likewise, the observations made by Li et al\textsuperscript{23} suggest that miR-663 only controls individual aspects of the SMC phenotype thereby promoting the expression of VSMC marker genes by an as yet undetermined mechanism. Through its target \textit{JUNB}, miR-663 additionally regulates the transcriptional activity/specificity of activator protein-1. Yet, activator protein-1 may contribute to both the contractile phenotype by promoting the expression of \textit{MYL9} and the synthetic phenotype by regulating the expression of MMP-9. Despite the lack of additional mechanistic data, at first glance miR-663 seems to control relevant regulatory elements of the VSMC phenotype with \textit{JUNB} as a target that goes beyond the usual suspects.

Second, could therapeutic enhancement of miR-663 expression serve as a cure for vascular diseases? With respect to miR-145, Lovren et al\textsuperscript{29} demonstrated that its overexpression in ApoE−/− mice resulted in a reduction of KLF4, increase in myocardin, and subsequent promotion of the contractile VSMC phenotype. Consequently, an experimental therapy of ApoE-deficient mice by forced miR-145 expression reduced atherosclerosis.\textsuperscript{29} However, even if specific overexpression of miR-663 in VSMCs would be available, this comes with an obstacle: On the one hand, such a therapy would presumably inhibit proliferation of VSMCs and stimulate their expression.

**Figure. The effect of microRNA (miR)-633 on vascular homeostasis and remodeling.** Major stimuli, transcription factors, and miRs that are involved in the differentiation and phenotypic switch of vascular smooth muscle cells (VSMCs) are depicted. miR-143/miR-145 promotes the contractile state by inhibiting suppressors of myocardin/serum response factor (SRF) activity (eg, ETS domain-containing protein-1 [Elk-1] and Krüppel-like factor [KLF] 4/5). Interestingly, myocardin activity seems to be regulated by an intrinsic loop because it stabilizes the expression of miR-145, whereas miR-143 seems to inhibit myocardin expression. miR-633 is a novel player with a dual effect on the VSMC phenotype not only by attenuating proliferation, migration, and proteolytic activity, but also by limiting their contractile capacity most likely by targeting JUNB, myosin regulatory light peptide 9 (MYL9), and matrix metalloproteinases (MMPs). Platelet-derived growth factor-BB (PDGF-BB) inhibits both miR-143/miR-145 and miR-663 expression and thereby counteracts the maintenance of the contractile VSMC phenotype. SMA, ACTA2 indicates smooth muscle α actin; and TGFβ, transforming growth factor β.
of differentiation markers. On the other hand, diminishing the expression of JUNB and thus MYL9 would eventually render the SMCs unable to exert contractile responses and limit their regular function in maintaining blood pressure. This can be deduced from the study of Licht et al. showing that ablation of Junb results in a severely diminished norepinephrine-induced constriction and impaired pressure-induced contractile responses. From a physiological point of view, therapies based on forced miR-663 expression for the treatment of arterial diseases may, therefore, act as a double-edged sword as they would limit SMC proliferation and, therefore, neointima formation but concomitantly compromise the contractile capacity of VSMCs.

Third, which side effects may be caused by miR-663 overexpression in ECs as long as specific targeting of VSMCs is not available? Several stimuli have been delineated, which trigger the expression of miR-663 in ECs. For instance, activating transcription factor 4–dependent VEGF expression in ECs exposed to oxidized phospholipids—because it occurs at atherosclerosis-prone sites—is regulated through miR-663. In line with this, the work by Ni et al. shows expression of miR-663 robustly upregulates in human ECs exposed to oscillatory shear stress, targets KLF4, and promotes proinflammatory responses. Although laminar flow is atheroprotective, oscillatory shear stress is present especially in branches and curvatures of large arteries and promotes EC dysfunction, inflammation, and subsequently atherosclerotic plaque formation. Considering this, a misrouted miR-663–based therapy would presumably support rather than inhibit progression of inflammation, and subsequently atherosclerotic plaque formation. For instance, activation of cardiac gene expression by myocardin, a transcriptional cofactor for serum response factor. Cell. 2001;105:851–862. Hence, efforts to determine the role of miR-663 in SMCs, it will be required to evaluate expression levels of miR-663 in ECs and VSMCs under physiological/pathophysiologial flow conditions before the effect of a miR-663–based therapy on other cell types can realistically be estimated. In particular, it will be important to unravel how miR-663 expression is regulated and whether miR-663 takes part in a negative regulator loop often observed for miRNAs controlling the expression of transcription factors.

Collectively, the data shown by Li et al give another glimpse of what can be expected from future miRNA-based therapies, especially if the targets of individual miRNAs can be identified to limit side, or off-target effects. Their work nicely demonstrates the complexity of miRNA functions because they may control the expression of both transcription factors and specific genes as shown in the Figure. Here, miR-663 has been proven as a novel and important regulatory element of the VSMC phenotype exerting a dual effect by limiting the contractile/migratory capacity through suppression of JUNB and promoting VSMC marker gene expression.

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