miR-143 and miR-145
Molecular Keys to Switch the Phenotype of Vascular Smooth Muscle Cells

Ashraf Yusuf Rangrez, PhD; Ziad A. Massy, MD, PhD; Valérie Metzinger-Le Meuth, PhD; Laurent Metzinger, PharmD, PhD

Vascular smooth muscle cells (VSMCs) are able to perform both contractile and synthetic functions, which are associated with changes in morphology, proliferation, and migration rates and are characterized by the specific expression of different marker proteins. Under normal physiological conditions, VSMC rarely proliferate in adult tissues, but undergo major phenotypic changes from the contractile to the synthetic in response to environmental cues, a phenomenon known as switching, or phenotypic modulation.1,2 Phenotypic switching is accompanied by production of abundant cytokines, extracellular matrix, and an increased rate of proliferation and migration. Therefore, the transition of VSMCs from a differentiated phenotype to a dedifferentiated state plays a critical role in the pathogenesis of cardiovascular diseases such as hypertension, vascular injury, and arteriosclerosis.2,3 However, the molecular mechanisms involved in phenotypic switching remain elusive.

The last decade has witnessed an exciting discovery that led to a revolution in our understanding of the extensive regulatory gene expression networks modulated by small, untranslated RNAs, microRNAs (miRNAs).4 miRNAs comprise a novel class of endogenous, small RNAs of 20 to 25 nucleotides. Although the mature miRNA is very small, it is derived from a transcripational product of a few hundred to a few thousand nucleotides. This process of maturation is known as miRNA biogenesis, extensively reviewed by Kim.5 Biogenesis of miR-143 and miR-145 is pictorially presented in Figure 1. Functionally, miRNAs are noncoding RNAs that negatively regulate gene expression. In the current, generally accepted model, they act mostly by inducing an inhibition of translation of their target mRNAs, and, in a minority of cases, via their degradation.6,7 Very recently, however, Bartel’s team challenged this view by showing that, in a vast majority of cases, mammalian microRNAs act by destabilizing their target mRNAs and decreasing their levels.8 They function as posttranscriptional regulators of mRNA expression by binding to the 3′UTR and repressing translation of the target gene.6,7 Note, however, that a few studies have described that some miRNAs bind the coding region or 5′UTR of respective target mRNAs.9–14 One single miRNA is able to regulate the expression of multiple genes because it is able to bind to its mRNA targets as either a perfect or imperfect complement.6,7 Thus, 1 miRNA can regulate the expression of multiple target genes. Similarly, 1 mRNA can be regulated by several miRNAs. It is speculated that the human genome may encode >1000 miRNAs15 that are abundant in many human cell types. Thus, the process of regulation of mRNA expression by miRNAs is complex, and explains that these small RNAs may target about 30% to 60% of the mammalian genes.16

Several miRNAs, including miR-21, miR-221, miR-222, miR-143, and miR-145, have a demonstrated role in VSMC differentiation. miR-21 negatively regulates programmed cell death 4 (PDCD4),17 promoting VSMC differentiation, whereas upregulation of miR-221 and miR-222 promotes VSMC proliferation by targeting the negative regulators of the cell cycle, p27 and p57.18,19 Several recent studies have demonstrated the critical role of miR-143 and miR-145 in VSMC phenotype switching.1,20–26 In this review, we specifically focus on the current, state-of-the-art information on miR-143 and miR-145 (henceforth these 2 miRNAs together will be designated as miR-143/145) and their involvement in phenotypic modulation of VSMCs and cardiovascular diseases. Further information about other miRNAs associated with VSMCs can be found elsewhere.27–33

miR-143/145 Are Transcribed as a Cluster That Is Regulated by Cardiac Transcriptional Factors
miR-143 and miR-145 encoding genes are highly conserved (Figure 2), and lie in close proximity with each other on murine chromosome 18 (~1.4 kilobases [kb]) and human chromosome 5 (~1.7 kb).12,34 Concurrent genomic organization suggests that miR-143/145 are cotranscribed from the same gene (Figure 1). This fact was indeed validated by RT-PCR, when primers from stem loop sequences of the two miRNAs were found to be transcribed as a bicistronic

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From INSERM-ERI 12 (EA4292) (A.Y.R., Z.A.M., V.M.-L., L.M.), Amiens, France; Faculty of Pharmacy and Medicine (A.Y.R., Z.A.M., L.M.), University of Picardy Jules Verne, Amiens, France; the Divisions of Pharmacology and Nephrology (Z.A.M.), Amiens University Hospital, Amiens, France; and Université Paris 13 (V.M.-L.), UFR SMBH, Bobigny, France.
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Correspondence to Laurent Metzinger, PhD, INSERM-ERI 12, Faculty of Pharmacy and Medicine, University of Picardy Jules Verne, 1 Rue des Louvets, F-80037, Amiens, France. E-mail laurent.metzinger@u-picardie.fr
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Transcriptional regulatory studies of miR-143/145 revealed that an upstream region of \( \approx 0.9 \) kb was sufficient for miR-143/145 cardiac and smooth muscle expression. This region consists of highly conserved \( cis \) elements representing potential binding sites for transcriptional factors, such as serum response factor (SRF) and Nkx2–5 (cardiac NK-2 transcription factor). Cordes et al showed that both SRF and Nkx2–5 could independently activate the expression of miR-143/145. Myocardin is a potent transcriptional coactivator of SRF and is considered a component of a molecular switch for smooth muscle differentiation. Therefore, SRF, in combination with myocardin, synergistically and robustly activates miR-143/145 expression, whereas Nkx2–5 together with SRF and myocardin have additive effects. In agreement with these results, Xin et al also demonstrated that both SRF and myocardin upregulate the expression of miR-143/145 in VSMCs.

**miR-143/145 Are Highly Expressed in VSMCs**

Many recent studies have shown that miR-143/145 are highly expressed in VSMCs. In a screen for miRNA expression in different tissues, Elia et al found that miR-143 has higher expression in heart than in other organs. The expression studies of miR-143/145 in various mouse tissues by northern blotting establish that miR-143 is expressed in lung, skeletal muscle, heart, and skin and is most abundant in aorta and fat, where miR-145 is also at its highest expression level. In agreement with this, in situ hybridization of cross sections of the adult mouse heart revealed a very strong expression for miR-143/145 in the walls of the aorta and coronary vessels. Boettger et al performed a series of microarray hybridization experiments using various mouse tissues from different developmental stages and found that the expression of miR-143/145 is proportional to the number of VSMCs in all organs studied. One more study validated the abundance of miR-145 in rat carotid arteries and its selective expression in VSMCs and not in endothelial cells.

Further expression studies at different developmental stages in transgenic mice discovered that miR-143/145 is initially expressed in the developing embryonic heart at E8.5 to E9.5. During fetal stages (E16.5), the expression of the miR-143 gene gets confined to SMCs of various organs such as aorta and coronary vessels.
as the aorta, smaller blood vessels, esophagus, lung, small intestine, colon, bladder, and umbilical cord. In adult animals, miR-143 expression is present in all VSMCs throughout the body, including the aorta, heart, and coronary arteries.1,34

In Vitro Studies Postulated the Role of miR-143/145 in VSMC Fate
Independent pioneering studies by Cheng et al20 and Cordes et al21 described the involvement of miR-143/145 in determining the fate of VSMCs. The former group (Cheng et al) found that overexpression of miR-145 increased the expression of VSMC differentiation marker genes, such as smooth muscle α-actin (SM α-actin), calponin, and SM-myosin heavy chain (SM-MHC). Accordingly, levels of these marker genes were decreased in VSMCs treated with a miR-145 inhibitor in cultured VSMCs. In addition to regulating VSMC differentiation markers, miR-145 alone was able to maintain the differentiated spindle-like shape and inhibited VSMC proliferation. The regulatory effect of miR-145 on VSMC phenotype was further verified by the latter group, when they demonstrated a strong regulatory effect of miR-145 over miR-143 on VSMC differentiation.21 They also proved that miR-145 was sufficient for transforming multipotent neural crest stem cells into VSMCs. It is to be noted that multipotent neural crest stem cells normally populate the aortic smooth muscle tissue, which is also the site of miR-145 expression. Additionally, both groups showed that myocardin is the regulator of miR-143/145 expression which is also a known master regulator of smooth muscle phenotype. In an independent experiment, Cordes et al21 demonstrated that miR-145 activity is required for myocardin-dependent conversion of fibroblasts into VSMCs and that miR-145 strongly potentiates the effects of myocardin. These in vitro findings suggested a crucial role of miR-143/145 in VSMC phenotype determination and were further verified and validated by in vivo studies described in later sections.

Knockout Studies in Mouse Provided Further Insights Into the Molecular and Mechanistic Roles of miR-143/145
Phenotypic Observations in miR-143/145 Knockout Mice
Three independent groups1,22,34 have generated mouse models of miR-143/145 knockout (KO) and shown that the expression of miR-143/145 cluster is essential for VSMCs to acquire the contractile phenotype. The first group replaced the miR-143/145-coding genomic region with a lacZ reporter, deleting the sequences coding for the mature miR-143 and miR-145 and a 1.3 kb fragment located between the 2 genes.1 Elia et al22 generated a KO mouse model in which the exon specifying miR-143 was replaced by lacZ reporter, and they noticed that the expression of miR-145 was concomitantly decreased by loss of miR-143. This observation further concurred the cotranscriptional hypothesis for miR-143/145, discussed in earlier section of the review. Finally, the third group34 generated 3 separate deletion mutant mice lines for miR-143, miR-145, and miR-143/145 by introducing loxP sites for Cre-mediated recombination in the regions flanking the premiR coding regions of each miRNA through homologous recombination. All 3 groups found that the homozygous miR-143/145 KO mice were viable, fertile, and did not display gross macroscopic alterations, indicating that these 2 miRs are dispensable for development.1,22,34 Homozygous KO mice for either miRNA were also viable, indicating neither miR-143 nor miR-145 is essential for cardiovascular development in vivo.34 Developmental studies revealed that miR-143 locus was active during early stages of heart development from E8.5 onward but disappeared from cardiomyocytes at E16.5 and became exclusively confined to SMCs of the cardiovascular system, the gastrointestinal tract, bladder, uterus, and lung.1 Interestingly, the expression of miR-143/145 was similar to that of other smooth muscle genes such as SM α-actin and SM22α.1,22,37,38

Structural Changes in miR-143/145 KO Mice
Histological and electron microscopic studies revealed that the structure of the aorta of homozygous miR-143/145 KO mice is different from their wild-type counterpart,22 with a severe reduction in the number of contractile VSMCs and an increase in synthetic VSMCs in the aorta and femoral artery.1 However, the aorta showed less severe phenotype when compared with a femoral artery. Both groups have reported that there were no differences in the number of proliferating or apoptotic VSMCs between control and KO groups.1,22

A striking observation by Xin et al34 was that the smooth muscle layers of the aorta and other arteries from miR-145 and miR-143/145 KO mice were noticeably thinner than those of wild-type or miR-143 KO mice, indicating the prominent and distinct role of miR-145 compared with miR-143. Further electron microscopic investigations revealed that the above observation was due to decreased actin-based stress fibers in miR-145 and miR-143/145 KO mice, suggesting that these miRNAs modulate actin dynamics and cytoskeletal assembly. Ultrastructural analysis of miR-143, miR-145, and miR-143/145 KO aorta also disclosed that the VSMCs had an increased and dilated rough endoplasmic reticulum which is typical for synthetically active VSMCs.22,34

Functional Comparison Between miR-143/145 KO and Control Mice
Cell culture studies of VSMCs isolated from wild-type and miR-143/145 KO mice aorta showed that the VSMCs from wild-type mice were larger and migrated more than those of KO mice.22,34 This fact was also confirmed by morphometric measurements, which indicated that the VSMCs of miR-143/145 KO mice were smaller when compared with control animals.1 Functional analysis of the vascular tone revealed that miR-143/145 KO mice had statistically significant arterial hypotension in comparison with wild-type under steady-state conditions.34 Similar effects, for example, reduced systolic and diastolic blood pressures, were seen in miR-143/145 KO compared with wild-type mice under anesthesia.1 Heart weight index and ventricular mass measurements suggested that the arterial hypotension was associated with reductions in cardiac and left ventricular mass.34 Similarly, miR-143/145 KO mice exhibited reduced systolic blood pressure in response to angiotensin II (Ang II, a vasopressor agent) stimulation. In an in vitro experiment using artery
explants, there was a nearly complete loss and significant decrease of the ability of the explants to contract when treated with Ang II and phenylephrine, respectively.1

Taken together, structural and functional observations, including reduced size and accumulation of synthetic VSMCs, increased proliferation, protein synthesis, and migration and blunted hypertension response to receptor-mediated signals, suggest that the miR-143/145 cluster is required for maintaining VSMC phenotype, for normal contractility of arteries, and for controlling blood pressure.

miR-143/145 Act Through Regulating the Regulators Such as Myocardin and SRF

We discussed earlier that the master regulator of smooth muscle contractile phenotype, SRF, in combination with myocardin, regulates the miR-143/145 expression. However, one of the most striking observations revealed by Cordes et al21 was that miR-143/145 cooperatively target a network of transcriptions factors such as myocardin, Krüppel-like factor 4 (Klf4), Klf5, and so forth, to promote differentiation and repress proliferation of VSMCs (Figure 3D). These results
miR-143 and miR-145 Regulation of VSMC Proliferation and Differentiation

Cordes et al. proposed the model (adapted in Figure 3) in which they showed that miR-143/145 are positively regulated by SRF and myocardin and function to repress multiple factors that normally promote the less differentiated, more proliferative smooth muscle phenotype (Figure 3, part D and central part). They suggested that miR-145 promotes VSMC differentiation in part by increasing myocardin expression and functioning in feed-forward reinforcement of its own expression by the SRF-myocardin complex. miR-145 represses Klf4, which otherwise interacts with SRF and also represses myocardin, and calmodulin kinase II-δ (CamKII-δ), which was shown to be involved in multiple events including neointimal proliferation.11,42 miR-143 represses Elk-1 (Ets Like gene 1), which competes with myocardin to bind SRF and exhibits an inhibitory effect on smooth muscle differentiation.43 Klf4 plays a key role in regulating VSMC phenotype because it antagonizes proliferation, facilitates migration, and downregulates VSMC differentiation marker genes.44

In another study, versican was identified as a new target for miR-143.45 Versican is a chondroitin sulfate proteoglycan of the extracellular matrix, produced by synthetic VSMCs, and promotes VSMC migration and proliferation. Wang et al. also demonstrated that myocardin coordinates VSMC differentiation by inducing transcription of miR-143, which in turn attenuates the expression of versican. Therefore, combined and regulatory effects of miR-143/145 and SRF-myocardin are necessary to decide the proliferative or differentiated phenotype of VSMCs.

miR-143/145 Regulation of Actin Remodeling

Another interesting mechanism of action of miR-143/145 was suggested by Xin et al., based on the prediction and validation of multiple targets regulating actin dynamics (Figure 3A). They identified a disproportionate number of the same targets involved in actin dynamics, cytoskeletal function, and phenotypic switching of VSMCs for both miR-143 and miR-145. Among these are the actin-dependent SRF coactivator myocardin-related transcription factor-B (MRTF-B) and Adducin-3 (ADD3), which caps the barbed ends of actin filaments and acts as a bridge between the membrane and actin cytoskeleton.50,51 Rho kinase-dependent phosphorylation of ADD3 results in enhanced F-actin binding and cell motility.52 Sling-shot 2 (Ssh2) phosphatase, another target of both miR-143 and miR-145, promotes cell motility and enhances F-actin reorganization by dephosphorylating and activating cofilin, an actin depolymerizing factor.53 miR-145 selectively targets the zinc finger proteins Klf4 and Klf5, which repress SRF activity and can either inhibit or promote VSMC differentiation or proliferation, depending on the context.54–56 Slit-Robo GTPase-activating protein 1 (Srgap1) and Srgap2, also targeted by miR-145, modulate Slit-Robo-dependent repulsive cues and cell migration by inactivating the small GTPase, Cdc42 and inhibiting actin polymerization.57 The preferential targeting of these modulators of cytoskeletal function by miR-145 may account, at least in part, for the stronger phenotype (as discussed earlier), resulting from miR-145 versus miR-143 deletion.54 The cycle of actin remodeling starts with MRTFs sequestered in the cytoplasm by monomeric actin. On release from actin, MRTFs translocates to the nucleus and interacts with SRF to activate the transcription of genes encoding actin and other cytoskeletal components, as well as miR-143 and miR-145. These miRNAs repress the expression of a collection of regulators of actin dynamics and MRTF/ SRF activity, thereby creating a complex set of feedback loops to modulate cytoskeletal assembly and dynamics.

Table. Function-Based Known Targets of miR-143 and miR-145 Involved in VSMC Fate Determination

<table>
<thead>
<tr>
<th>Function</th>
<th>Target</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proliferation and differentiation</td>
<td>Ets Like gene 1 (Elk1)</td>
<td>21</td>
</tr>
<tr>
<td>Podosome formation and migration</td>
<td>Versican</td>
<td>45</td>
</tr>
<tr>
<td>Protein kinase C-ε</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Platelet derived growth factor receptor-α</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Proliferation and differentiation</td>
<td>Myocardin</td>
<td>20, 21</td>
</tr>
<tr>
<td>Krüppel-like factor 4 (Klf4)</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Calmodulin kinase II-δ</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Actin remodeling</td>
<td>Klf5</td>
<td>34</td>
</tr>
<tr>
<td>Krüppel-like factor 5 (Klf5)</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>Slit-Robo GTPase-activating protein 1</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>Slit-Robo GTPase-activating protein 2</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>Myocardin</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>Contractility</td>
<td>Klf5</td>
<td>63</td>
</tr>
<tr>
<td>Fascin</td>
<td>24</td>
<td></td>
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<tr>
<td>Adducin-3</td>
<td>34</td>
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</tr>
<tr>
<td>miR-143/145 Actin remodeling</td>
<td>Myocardin related transcription factor-B</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>Sling-shot 2</td>
<td>34</td>
</tr>
<tr>
<td>Contractility</td>
<td>Tropomyosin-4</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Angiotensin-converting enzyme</td>
<td>1</td>
</tr>
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</table>

put forth a yet undescribed concept against the generally accepted view that miRNAs inhibit protein translation and/or degrade target mRNA. This shows that miRNAs can self-regulate their expression by altering their transcriptional regulators via feed-forward, feed-backward, or double-negative feedback mechanisms.21,39 Several targets (Table) and mechanisms based on respective targets have been proposed and validated. We will discuss these mechanisms below in detail. Additionally, we also used dedicated software to detect the overall predicted targets of miR-143/145 (online-only Supplement Table 1). This analysis shows that miR-143/145 have a wide range of targets, such as transcription and translation factors, receptors, phosphatases, kinases, growth factors, RNA binding proteins, and so forth. These targets are involved in different cellular processes apart from VSMC plasticity and represent an example how 1 miRNA can regulate different related or unrelated cellular processes.
The mechanism proposed by Xin et al. has further been strengthened by a recent report in which authors have generated VSMC-specific deletion of Dicer in mice. They found that inactivation of Dicer in VSMC results in impaired actin cytoskeleton and defects in VSM-specific gene expression. Cytoskeletal defects caused by Dicer deletion could be partially rescued by miR-145 confirming its involvement in the control of actin dynamics.

miR-143/145 Regulation of Contractility
Boetger et al. have identified tropomyosin 4 (TPM-4) and angiotensin-converting enzyme (ACE) as major miR-143/145 targets (Figure 3B). TPM-4 is a structural protein that is specifically upregulated in synthetic VSMCs, whereas ACE converts circulating Ang I into its active form, Ang II. Ang II is a potent agonist for the contraction of VSMCs and also a major regulator of the contractile phenotype of VSMCs.

An interesting observation was the identification of additional changes in the transcript and/or protein expression levels of molecules known to influence the VSMC phenotype. These changes would be the consequences of secondary events. In principle, many elements of signaling cascades that govern contraction and migration of VSMCs were changed, including a downregulation of angiotensin receptor 1 (AT-1) and of molecules that control the trafficking of plasma membrane receptors such as Caveolin-2 (Cav-2) and Cav-3. On the other hand, regulator of G-protein signaling (RGS)-interacting molecule GNB5, as well as components of the Rho-signaling cascade (Rac1, Rnd2/3, Cdc42ep3, Argef17) and molecules that direct calcium handling and signaling (SERCA, caldesmon-1, Camk2g) of VSMCs, were upregulated. The effect on the Rho signaling cascade was of particular significance because it affects nuclear translocation and/or activation of SRF. Collectively, modulation of ACE and consequently Ang II by miR-143/145 explains, at least in part, the shift from the contractile to the synthetic phenotype in miR-143/145 KO mice.

Recently, Liu et al. have documented that Klf5 is involved in Ang II--induced VSMC proliferation through transactivating cyclin D1 expression. Ang II induced expression and activation of Klf5 via the ERK and p38 MAPK pathways triggered by AT-1. Klf5 is one of the targets of miR-145, a known repressor of myocardin expression and a negative regulator of VSMC differentiation. This cascade of events promotes migration of VSMCs and podosome formation.

miR-143/145 Regulation of Podosome Formation and Migration
A very recent study has proposed a novel mechanism demonstrating the role of miR-143/145 in VSMC migration and podosome formation. Podosomes are important morphological actin-rich membrane protrusions involved in the migration of several cell types including VSMCs. Src tyrosine kinase activity promotes podosome formation and the PKC induction of podosomes is a result of Src activation. Platelet-derived growth factor (PDGF) is a known regulator of VSMC differentiation and migration by stimulating the PDGF-receptor (PDGF-R), which in turn activates Src, Protein kinase C (PKC)-ε, and downstream signal transduction pathways. Quintavalle et al. have shown that miR-143/145 inhibits podosome formation, whereas Src and PDGF reduce expression of miR-143/145. Moreover, they deduced the underlying pathway demonstrating that in response to PDGF, Src downregulates miR-143/145 expression through p53 inhibition. These findings also support previous observations indicating that PDGF can reduce miR-145 expression, and p53 increases miR-143/145 levels in cancer cells.

Klf5 is a target of miR-145, a known repressor of myocardin expression and a negative regulator of VSMC differentiation. Klf5 is one of the targets of miR-145, which further complements and illustrates the partial mechanism by which Ang II negatively regulates the contractile phenotype of VSMCs.
control group. This result strongly suggests that the expression of miR-143/145 can be downregulated in human vascular diseases. Further strengthening this observation, a recent study reported a significant reduction of miR-145 in the blood of patients with coronary artery disease (CAD) when compared with healthy individuals. They concluded that the circulating levels of several vascular miRNAs are significantly downregulated in patients with CAD. However, whether downregulation of miR-143/145 is the primary cause of aortic aneurism and/or CAD or it is a secondary event due to the disease remains to be established. Notwithstanding, quantifying the expression of miRNAs such as miR-143/145 could be a useful tool for the diagnosis and evaluation of CAD and other vascular diseases, as will be discussed in further details below.

Therapeutic Potential and Future Perspectives
All the discussion thus far leads to the conclusion that miR-143/145 is an important molecular key required to switch the VSMC phenotype. Initial studies have demonstrated the importance of these small RNAs in determining the fate of VSMCs, whereas recent studies substantiated their association with human vascular diseases such as aortic aneurism, atherosclerosis, restenosis, and CAD. VSMCs have the ability to undergo profound phenotypic changes in response to changes in their extracellular environment, as occurs in vascular diseases such as atherosclerosis, restenosis, aortic aneurism, and CAD. These changes often include dedifferentiation, enhanced migration, proliferation, and apoptosis of VSMCs. Several recent studies have established a negative correlation between the expression of miR-143/145 and the dedifferentiation of VSMCs. Therefore, downregulation of miR-143/145 induced by pathological stimuli in the vascular diseases cited above facilitates VSMCs dedifferentiation. This fact indicates that modulating the expression of miR-143/145 can potentially be used for the treatment of vascular diseases. One should, however, keep in mind that modulating the phenotype of VSMCs by using miRNA could prove to be beneficial in some vascular disease states, in which phenotype switching may be helpful, such as aneurysm of the abdominal aorta, and harmful in others, such as postangioplasty restenosis.

In these lines, Cheng et al have shown that the restoration of the downregulated miR-145 is sufficient to inhibit the neointimal lesion formation in rat carotid arteries after angioplasty. Similar results were obtained where balloon-injury–induced neointimal formation was significantly reduced when the carotid artery was locally perfused with adenovirus expressing either miR-143 or miR-145. Several other therapeutic approaches and modes of delivery are available for miRNA-based treatment. They include chemically modified mimics of miRNAs, vector-based delivery of miRNAs, antisense oligonucleotides, miR-masks, miR-sponges, and so forth, and are summarized in recent reviews. Importantly, one has to be cautious when considering miRNA based therapy due to the involvement of a single miRNA in more than 1 physiological process, because 1 miRNA can control multiple related or unrelated targets. However, as stated, this aspect may also be advantageous because targeting multiple genes with a single mimetic could modulate complex processes such as neovascularization, atherosclerosis, and similar vascular or other physiological processes.

Summary
Until recently, miRNAs, which are cytoplasmic components, have been quantified in healthy/pathological tissues, a procedure that usually involves a surgical act. A recent clinical breakthrough occurred when miRNAs were detected in circulating body fluids together with other types of noncoding RNAs, DNAs, and miRNAs. They are detected in serum and EDTA-treated plasma, are very stable, can stand repetitive freeze-thaw cycles and are protected from RNases. Moreover, a growing number of studies have shown that the levels of these circulating miRNAs vary significantly in pathological conditions such as various cancers, myocardial infarction, diabetes, and so forth. An interesting article by Gupta et al provides the current knowledge about circulating miRNAs during CAD, myocardial infarction, and heart failure. Recently, Fichtlscherer et al performed miRNA profiles using RNA isolated from the blood of healthy volunteers and patients with stable CAD. They found that the expression of several miRNAs was significantly altered in patients. In the same study, they validated their results by quantitative PCR in 2 independent cohorts of patients and showed that the expression of miR-145, among other miRNAs, was significantly reduced in patients with CAD compared with healthy control patients. Thus, quantifying total blood levels of miR 143/145 and other experimentally validated miRNA candidates may provide a specific signature that could be an independent predictor of diagnosis, prognosis and/or etiology of CADs, keeping in mind that this concept will need comprehensive investigations to validate the theory.

As a consequence, apart from being considered as potential therapeutic targets and the feasibility of these strategies become routinely available, we believe that the relationship between circulating miR-143/145 levels and CAD may at present serve as novel markers for diagnosis and prognosis of CAD and other vascular diseases.

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