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Can microRNAs control vascular smooth muscle phenotypic modulation and the response to injury?

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Albinsson S, Sessa WC. Can microRNAs control vascular smooth muscle phenotypic modulation and the response to injury? Physiol Genomics 43: 529–533, 2011. First published September 14, 2010; doi:10.1152/physiolgenomics.00146.2010.—Vascular smooth muscle cell (VSMC) migration and proliferation are critical events in vascular proliferative diseases. Recent studies have established microRNAs (miRNAs) as important mediators for the modulation of VSMC phenotype by targeting transcription factors and the cytoskeleton, which act as molecular switches for VSMC differentiation. The importance of miRNAs for VSMC development, differentiation, and function is evident by the fact that loss of the miRNA processing enzyme Dicer in VSMCs results in embryonic lethality due to severe vascular abnormalities. Similar abnormalities are observed in adult miR-143/145 knockout mice, indicating that these miRNAs are important for VSMC differentiation and function. However, since miR-143/145 knockout is not embryonically lethal, additional miRNA must be required during embryonic development of VSMCs. In addition, specific miRNAs such as miR-145, miR-21, and miR-221 have been found to regulate neointimal hyperplasia following vascular injury, which provides interesting possibilities for future therapeutic targets against vascular disease. Herein, we summarize recent advances regarding the role of miRNAs in VSMC phenotype modulation and response to injury.

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VASCULAR CELL ACTIVATION and remodeling are critical events in the pathogenesis of atherosclerosis, vein graft adaptation, transplant arteriopathy, and restenosis after percutaneous transluminal coronary angioplasty. The nature of the initial cause of vascular activation is often multifactorial but may result from oxidative injury, mechanical damage, ischemia reperfusion, or inflammation (16). Following endothelial cell dysfunction, vascular smooth muscle cells (VSMCs), which are normally quiescent and programmed for contraction, migrate from the media into the lumen of the vessel in response to the local inflammation. These phenotypically modulated VSMCs can proliferate and synthesize cytokines, resulting in a progression of the vascular lesion and thickening and stiffness of the arterial wall (30). This is just one example of the remarkable capacity of VSMCs to adapt to environmental cues by phenotypic modulation, which is likely a key mechanism to allow repair of vascular injury but may also lead to progression of vascular disease (28). The mechanisms of VSMC phenotypic modulation are under intense investigation and incompletely understood. Several transcription factors, including serum response factor (SRF), myocardin, myocardin related transcription factors (MRTFs), and members of the Krüppel-like zinc finger family (KLF), have been suggested to act as molecular switches regulating VSMC differentiation (19, 22). Recently, miRNAs were proposed to play a role in VSMC differentiation and proliferation by modulating the expression of several of these transcription factors (5, 9, 11, 38). In addition, miRNAs are involved in the regulation of VSMC development and the vascular response to injury, which makes these molecules interesting as potential new therapeutic targets (1, 32).
REGULATION OF SMOOTH MUSCLE PHENOTYPE BY miR-145

Many groups have attempted to identify miRNAs that regulate VSMC differentiation, and in 2009 a breakthrough was achieved with the finding that miR-145 exerts potent actions in VSMCs. MiR-145 is a highly conserved miRNA, which is expressed in a gene cluster together with miR-143, and was initially found to be consistently downregulated in diverse forms of cancer including colorectal (23) and breast cancer (15). In 2009, miR-145 was shown to be highly upregulated during differentiation of human embryonic stem cells and inhibited stem cell self renewal by targeting the pluripotency transcription factors, which are involved in VSMC differentiation, including KLF4, myocardin, and CamKIIβ. Interestingly, miR-145 suppressed the levels of KLF4 and CamKIIβ while myocardin levels were increased, which is consistent with miR-145 promoting a contractile phenotype of VSMCs. In addition, miR-145 was shown to potentiate the function of myocardin and was necessary for myocardin-induced reprogramming of adult fibroblasts into VSMCs (9). Cheng et al. (5) also found that expression of myocardin was increased by miR-145; however, they suggest that this effect is mediated by an indirect effect via translational inhibition of KLF5. As shown in Table 1 additional targets of miR-145 have now been discovered, and it is likely that these targets are also involved in the effects of miR-145 on VSMCs.

In Dicer KO VSMCs, we found a remarkable loss of actin filament structures (1). This effect was rescued by overexpression of miR-145, which also rescued the reduced SMC marker expression in Dicer KO VSMCs. The ratio of filamentous actin (F-actin) versus monomeric G-actin is an important regulator of VSMC phenotype as monomeric actin binds and inhibits contractile function. It is likely that the reduced expression of KLF5 and CamKIIβ in Dicer KO VSMCs contributed to this effect. Interestingly, miR-145 suppressed the levels of KLF4 and CamKIIβ while myocardin levels were increased, which is consistent with miR-145 promoting a contractile phenotype of VSMCs. In addition, miR-145 was shown to potentiate the function of myocardin and was necessary for myocardin-induced reprogramming of adult fibroblasts into VSMCs (9). Cheng et al. (5) also found that expression of myocardin was increased by miR-145; however, they suggest that this effect is mediated by an indirect effect via translational inhibition of KLF5. As shown in Table 1 additional targets of miR-145 have now been discovered, and it is likely that these targets are also involved in the effects of miR-145 on VSMCs.

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Although the mechanisms of how miR-145 induces VSMC differentiation are not well understood, there are several putative targets of miR-145 that have been identified (Table 1 and Fig. 1). Cordes et al. (9) found that miR-145 targeted multiple transcription factors, which are involved in VSMC differentiation, including KLF4, myocardin, and CamKIIβ. Interestingly, miR-145 suppressed the levels of KLF4 and CamKIIβ while myocardin levels were increased, which is consistent with miR-145 promoting a contractile phenotype of VSMCs. In addition, miR-145 was shown to potentiate the function of myocardin and was necessary for myocardin-induced reprogramming of adult fibroblasts into VSMCs (9). Cheng et al. (5) also found that expression of myocardin was increased by miR-145; however, they suggest that this effect is mediated by an indirect effect via translational inhibition of KLF5. As shown in Table 1 additional targets of miR-145 have now been discovered, and it is likely that these targets are also involved in the effects of miR-145 on VSMCs.

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nuclear translocation of the SRF cofactor MRTF (24). Actin dynamics are regulated by multiple mechanism including rho/rho-kinase activation, actin capping proteins and branching proteins (27). Xin et al. (38) found several of these factors to be targets of miR-143/145 (Table 1), and we suggest that this may be an additional mechanism by which miR-145 regulates VSMC phenotype (Fig. 1). In fact, when actin polymerization is prevented by Latrunculin B, overexpression of miR-145 in Dicer KO VSMCs did not rescue the loss of SMC markers (1). Interestingly, regulation of actin organization by additional miRNAs has also been demonstrated in zebrafish cardiac and skeletal muscle, indicating that this effect may not be specific only for miR-145 in VSMCs (12, 25).

Further studies on the miR-143/145 cluster were done in mutant mice where these miRNAs were deleted individually or in combination (3, 13, 38). Unlike SM-Dicer KO, loss of miR-143/145 is not embryonic lethal suggesting that other miRNAs are also involved in VSMC development and that miR-143/145 are not essential for VSMC differentiation in vivo. However, several abnormalities were found in miR143/145 KO mice including a reduction in SMC marker expression and contractile function (3, 13), reduced medial thickness (3, 13, 38), increased rough endoplasmic reticulum (13, 38), and loss of actin stress fibers and dense bodies (3, 38). All of these observations are consistent with the phenotype we observed in SM-Dicer KO embryos (1). Blood pressure in miR-143/145 KO mice was also reduced due to the vascular abnormalities (3, 13, 38).

ADDITIONAL miRNAs INVOLVED IN SMOOTH MUSCLE PHENOTYPE REGULATION

Regulation of VSMC phenotype is indeed a complex process, and it would certainly be surprising if a large number of miRNAs were not involved its regulation. So far, miR-21 and miR-221 have received most attention, aside from miR-143/145, but new VSMC miRNAs are continuously being discovered. MiR-21 was in fact the first miRNA demonstrated to be involved in VSMC differentiation and proliferation (17). Ji et al. (17) found that miR-21 promotes proliferation and inhibits apoptosis of VSMCs by downregulating phosphatase and tensin homolog (PTEN) and upregulating B-cell lymphoma 2 (Fig. 1). Proliferation and differentiation are sometimes considered to be mutually exclusive events, which is a misconception since these events are regulated by separate mechanisms and can occur independently of each other (28). Accordingly, miR-21 was later shown to also promote differentiation of VSMCs in response to transforming growth factor-β and bone morphogenetic protein stimulation via a decrease in programmed cell death protein 4 expression (Fig. 1) (10).

MiR-221 was the second miRNA implicated in the regulation of VSMC phenotype as it was found to be upregulated following stimulation by platelet-derived growth factor (PDGF) (11, 21). In addition, inhibition of miR-221 prevented PDGF induced proliferation, while overexpression of miR-221 increased basal proliferation and reduced the expression of VSMC markers. The proliferative effect of miR-221 was shown to be mediated via downregulation of its targets p27Kip1 and p57Kip2, both of which are negative regulators of VSMC proliferation in vivo (Fig. 1) (11, 21). Inhibition of VSMC marker expression by miR-221 is caused by inhibition of c-Kit, which is a positive regulator of myocardin expression (11).

Effects of additional miRNAs in VSMCs are continuously being identified. Recently, miR-10a was found to contribute to retinoic acid-induced SMC differentiation by inhibition of its target histone deacetylase 4 (14). Furthermore, miR-1 has been suggested to reduce VSMC contractility by repressing the expression of VSMC contractile markers and impairing the actin cytoskeleton (18). However, the effects of miR-10a and miR-1 are yet to be confirmed in VSMCs in vivo.

ROLE OF miRNAs IN THE RESPONSE TO VASCULAR INJURY AND POTENTIAL THERAPEUTIC STRATEGIES

VSMCs present in proliferative vascular diseases are characterized by an increased rate of proliferation and loss of contractile markers (28). By preventing phenotypic modulation, neointimal progression may be controlled, which is an important therapeutic strategy that can delay vascular disease. MiRNAs are possible targets for therapeutic intervention since their expression can be modified using genetic approaches.

To study neointima formation in vivo several experimental models of vascular injury have been developed, including carotid ligation, wire-induced injury, and balloon injury (40). Wire and balloon injury causes endothelium denudation due to physical trauma and relatively rapid neointimal hyperplasia in 1–2 wk (40). Carotid ligation differs from the above-mentioned models as it is not associated with endothelial denudation and neointimal lesions develop more slowly, in ~2–4 wk. Alternate mechanisms may be responsible for the neointimal formation depending on which model that is used, which is important to consider when interpreting results from different studies.

The role of miRNAs in neointimal hyperplasia after balloon injury of rat carotid arteries was investigated in a series of papers by Zhang and coworkers (5, 17, 21). Several miRNAs were up- or downregulated in neointimal lesions. Specifically, upregulation of miR-21 and miR-221/222 and downregulation of miR-145 were observed. Interestingly, the profile of these miRNAs in neointimal lesions correlates with what has been previously described in various cancers, suggesting that the role of miRNAs for cell proliferation is similar in several cell types (4, 6, 15, 23). Local oligonucleotide delivery of miRNA inhibitors targeting miR-21, and miR-221/222 to balloon-injured carotid arteries, was found to decrease neointima formation significantly (17, 21). Similar results were also observed after adenovirus-mediated delivery of miR-145 or miR-143 to vascular lesions (5, 13). These findings indicate that a beneficial therapeutic effect can be achieved by restoring the miRNA profile in vascular lesions to resemble that of the normal tissue.

In theory, neointimal hyperplasia after balloon or wire injury would be increased in miR-143/143 KO mice, but this has not yet been tested using these methods. Surprisingly, however, neointima formation following carotid ligation, which is also associated with a decreased miR-143/145 expression (9), was nearly abolished in miR-143/145 KO mice (38). It appears that although miR-145 has an antiproliferative effect when overexpressed, miR-145 is also required for the acute VSMC response to injury. Reduced migration due to perturbed actin dynamics in miR-143/145 KO VSMCs was suggested to be one possible mechanism behind the decreased neointimal for-
miR-145 mimic would be beneficial for the prevention of neointimal formation (Fig. 1) (38). However, it was recently reported that migration in response to PDGF is actually increased in VSMCs isolated from miR-143/145 KO aorta (13). Formation of podosomes, which are actin-rich protrusions important for VSMC migration, was found to be increased in miR-143/145 KO cells due to upregulation of key podosome regulators, PDGF-Ra, PKCe, and fascin (Fig. 1) (29). In a separate study, miR-143 was shown to prevent VSMC migration in response to PDGF by attenuated expression of the extracellular matrix protein, versican (Fig. 1) (37). In support of an increased migratory capacity of miR-143/145 KO VSMCs, Boettger et al. (3) found, in the femoral arteries of 18 mo old miR-143/145 KO mice, spontaneous neointimal lesions that were completely absent in WT mice. The role of miR-145 in neointima formation may thus be dependent on the cause of the vascular lesion, but the majority of studies indicate that administration of a miR-145 mimic would be beneficial for the prevention of neointimal hyperplasia.

CONCLUSION

The importance of VSMC phenotypic modulation in vascular response to injury has been well established, and a role for miRNAs in the regulation of VSMC phenotype is now beginning to emerge. The role of miR-145 is especially intriguing since it is relatively specific for VSMCs and thus attractive as a potential therapeutic target. However, multiple miRNAs are likely to be involved in VSMC development and differentiation. This is evident by the embryonic phenotype of SM-Dicer KO mice, which is absent in miR-143/145 KO mice. Future studies are warranted to identify additional miRNAs involved in VSMC phenotype modulation and clarify their interactions in vascular disease.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES


