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microRNA regulation of smooth muscle gene expression and phenotype

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Abstract

Purpose of review—In this review, we summarize recent advances regarding microRNA (miRNA) functions in the regulation of vascular smooth muscle cell (VSMC) differentiation and phenotypic modulation.

Recent findings—Multiple miRNAs are found to be responsible for VSMC differentiation and proliferation under physiological or pathological condition. A single miRNA downregulates multiple targets, whereas a single gene is regulated by multiple miRNAs to modulate a specific aspect of VSMC phenotype.

Summary—The phenotype of VSMCs is dynamically regulated in response to environmental stimuli. Deregulation of phenotype switching is associated with vascular diseases. Several miRNAs have been found to be highly expressed in the vasculature, to modulate VSMC phenotype, and to be dysregulated in vascular diseases. By regulating mRNA and/or protein levels post-transcriptionally, miRNAs provide a delicate regulation in the complex molecular networks that regulate the vascular system. Understanding the functions of miRNAs in the regulation of VSMC differentiation and phenotype switching provides new insights to the mechanisms of vascular development, function, and dysfunction.

Keywords

microRNAs; vascular smooth muscle cells; contractile phenotype; synthetic phenotype

Introduction

Vascular smooth muscle cells (VSMCs) are highly differentiated cells that form the medial layer of the vessel wall and control blood pressure by contracting and relaxing the vessels. VSMCs, unlike other terminally differentiated muscle cells, retain the unique ability to switch between differentiated (or “contractile”) and dedifferentiated (or “synthetic”) phenotypes in response to physiological and pathological cues, such as vascular injury, mechanical stretch, or growth factor stimulation[1]. Contractile VSMCs have an elongated, spindle-shaped morphology and demonstrate a very low rate of proliferation, appropriate contractility to contractile cues, and expression of SMC-specific contractile genes, such as

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smooth muscle α -actin (α -SMA), smooth muscle myosin heavy chain (SM-MHC), SM22 α , and calponin. In contrast, synthetic VSMCs have a less elongated morphology and demonstrate increased proliferation, migration, enhanced production of extracellular matrix proteins, such as collagens and matrix metalloproteinases, and reduced expression of contractile genes[2]. This phenotypic switch is a key process for VSMCs to promote injury repair; however, it may also lead to progression of certain vascular diseases, such as atherosclerosis, pulmonary artery hypertension (PAH), or restenosis after angioplasty.

Growth factor signaling pathways are involved in the phenotypic modulation of VSMCs[3–5]. Transforming growth factor- β (TGF- β) signaling is critical for normal vascular development and homeostasis. TGF- β superfamily of growth factors, such as TGF- β and bone morphogenetic proteins (BMPs), has been demonstrated to promote the contractile phenotype by upregulating the expression of contractile genes and inhibiting VSMC growth and migration[3,5]. Conversely, the platelet derived growth factor (PDGF) signaling pathway promotes the synthetic phenotype by suppressing contractile gene expression and stimulating VSMC proliferation and migration[4].

Several transcription factors have been implicated in VSMC differentiation by controlling the expression of SMC-specific contractile genes[1]. Serum response factor (SRF) binds to the sequence element known as the CArG box [CC(AT)₆GG], located in the promoters of contractile genes. In association with SRF, myocardin and myocardin-related transcription factors (MRTFs) activate the transcription of contractile genes and promote SMC differentiation. In contrast, a member of the Krüppel-like zinc finger family (KLF), KLF4, is a potent repressor of contractile genes and functions through multiple mechanisms, including remodeling the chromatin of CArG box-containing promoters, sequestering SRF, and reducing myocardin expression. Recently, miRNAs have been proposed to play a role in VSMC differentiation and proliferation by modulating the expression of these transcription factors [6**,7**].

miRNA

miRNAs are a class of small (21–25 nucleotide (nt)) non-coding RNAs that are evolutionarily conserved in metazoans. There are more than 1000 miRNAs encoded by the human genome. Most animal miRNAs share a common biogenesis pathway (Fig. 1). miRNAs are transcribed by RNA polymerase II as long primary transcripts called pri-miRNAs, which can encode either single or multiple miRNAs. Pri-miRNAs fold into hairpin structures containing imperfectly base-paired stem regions, which are processed by an RNase III enzyme Droscha into 60–100 nt hairpins known as precursor miRNAs or pre-miRNAs[8]. The pre-miRNAs are exported from the nucleus to the cytoplasm by exportin 5, where they are cleaved by an RNase III enzyme Dicer to yield miRNA-miRNA* duplexes. The mature miRNA is incorporated into the RNA-induced silencing complex (RISC), which recognizes specific targets and induces post-transcriptional gene silencing[9]. While the miRNA* strand is often degraded, occasionally, both miRNA and miRNA* can give rise to functional miRNAs.

miRNA and vascular smooth muscle cells

Vascular smooth muscle-specific knockout of Dicer provides compelling evidence that miRNAs are essential for VSMC development, differentiation, and contractile function[10**,11**,12**]. The deletion of Dicer using SM22Cre-Dicer^{flox} results in embryonic lethality at embryonic day 16.5. Several vascular phenotypes were observed, including outward hypotrophic remodeling of the aorta, defective organization of the elastic lamellae of the aorta, loss of contractile function in the umbilical artery as well as reduced expression of SMC-specific genes[10**]. miRNAs generally exert their effects through relatively subtle modulation of a group of targets, and thus they are often considered to function as fine-tuners of gene expression [13]. However, there are also settings in which miRNAs function as “on-off” switches. For example, the cardiac-specific miRNA, miR-208, orchestrates the silencing of a cohort of transcriptional repressors and downregulates the expression of slow muscle fiber genes[14] (Fig. 2). In this review, we summarize recent advances in the function of several miRNAs that are implicated in VSMC phenotype regulation (Fig. 2 and 3).

miRNAs which are implicated in the synthetic phenotype

miR-24

miR-24 is known to be a downstream regulator responsible for PDGF-induced VSMC synthetic phenotype [15*]. Upon PDGF stimulation, the expression of miR-24 is elevated, and miR-24 targets Tribbles-like protein 3 (Trb3) which promotes the degradation of the Smad ubiquitin ligase Smurf1. Therefore, downregulation of Trb3 by miR-24 increases Smurf1, leading to decreased Smad1 expression and reduced BMP signaling, resulting in the induction of the synthetic phenotype, including the repression of contractile gene expression and induction of VSMC proliferation. These data suggest that miR-24 is a point of antagonistic signaling convergence for PDGF and BMP to regulate VSMC phenotype.

miR-26a

miR-26a is downregulated during abdominal aortic aneurysms formation, in which enhanced switching from the contractile to the synthetic phenotype occurs [16*]. miR-26a promotes VSMC proliferation while inhibiting cellular differentiation. Inhibition of miR-26a accelerated VSMC differentiation by increasing contractile gene expression and reducing proliferation and migration. Interestingly, miR-26a targets Smad-1 and Smad-4, members of the TGF- β superfamily signaling cascade, and alters TGF- β pathway signaling. Knock-down of miR-26a increased the expression of Smad-1 and Smad-4, leading to enhanced BMP-Smad signaling, thus suggesting that miR-26a may alter VSMC phenotype, in part, via an inhibitory effect on the signaling pathways downstream of the TGF- β /BMP superfamily of growth factors.

miR-31

miR-31 is an abundant miRNA in VSMCs, and its expression was significantly increased in proliferative VSMCs and in vascular walls with neointimal growth [17*]. Lui et al. demonstrated that miR-31 is able to promote VSMC proliferation via downregulation of the

large tumor suppressor homolog 2 (LATS2), which is known to inhibit tumor cell growth by causing cell cycle arrest. Conversely, knock-down of miR-31 inhibited both serum and PDGF-induced VSMC proliferation. The role of miR-31 in VSMC proliferation was also confirmed in vivo in balloon-injured arteries. In addition, miR-31 is upregulated by mitogen-activated protein kinase/extracellular regulated kinase (MAPK/ERK) in proliferative VSMCs. MAPK/ERK is a critical pathway responsible for extracellular growth stimuli-mediated gene regulation and cell proliferation. Therefore, MAPK/ERK/miR-31/LATS2 may be a novel signaling pathway in VSMC growth.

miR-146a

miR-146a promotes VSMC proliferation in vitro and vascular neointimal hyperplasia in vivo [18**]. Transfection of antisense miR-146a oligonucleotide into balloon-injured rat carotid arteries significantly decreased neointimal hyperplasia, suggesting a pro-proliferative effect of miR-146a on VSMCs. Expression of miR-146a is elevated in proliferative VSMCs in a KLF4- and KLF5-dependent manner. Interestingly, overexpression of miR-146a in VSMCs significantly decreased KLF4 expression and increased the expression of PCNA, a marker for proliferation. Thus, miR-146a and KLF4 form a feedback loop and mutually regulate each other's expression and modulate VSMC proliferation.

miR-204

PAH is characterized by enhanced proliferation and reduced apoptosis of pulmonary artery smooth muscle cells (PASMCs) [19]. miR-204 expression in PASMCs is down-regulated in both human and rodent PAH, and the downregulation of miR-204 is associated with enhanced PDGF expression, which results in VSMC proliferation [20*]. On the other hand, overexpression of miR-204 in PAH-PASMCs reversed the pro-proliferative and anti-apoptotic phenotype of PAH-PASMCs. Furthermore, delivery of synthetic miR-204 to the lungs of animals with PAH significantly reduced disease severity, suggesting that miR-204 can be therapeutically targeted, leading to a decrease of proliferation, vascular remodeling, and PA blood pressure.

miR-208

It is demonstrated that insulin differentially regulates miRNAs in VSMCs and that miR-208 participates in insulin-mediated VSMC proliferation [21*]. Insulin has been reported to induce proliferation of VSMCs by promoting the progression of the cell cycle from the G1 to the S phase. miR-208 expression was increased in response to insulin, and inhibition of miR-208 reduced the stimulatory effect of insulin on VSMC proliferation. Conversely, overexpression of miR-208 promoted VSMC proliferation and further increased the insulin-mediated VSMC proliferation. p21 has been identified as a target of miR-208. p21 is a key member of the CDK-inhibitory protein family and has an inhibitory effect on cell proliferation. Altogether, these findings suggest that miR-208-mediated regulatory pathways, via downregulation of p21, may play a critical role in hyperinsulinemia-induced VSMC proliferation.

miR-221

miR-221 appears to be an important regulator of VSMC proliferation and neointimal hyperplasia [22]. miR-221 is transcriptionally induced by PDGF signaling and mediates VSMCs phenotypic switching [23]. Overexpression of miR-221 increased VSMC proliferation and migration and reduced the expression of VSMC differentiation markers. Conversely, inhibition of miR-221 prevented PDGF-induced proliferation and migration and increased the expression of VSMC differentiation markers. In addition, knock-down of miR-221 inhibited VSMC proliferation and intimal thickening in rat carotid artery after vascular injury[22]. It was shown that miR-221 represses the expression of p27^{Kip1} and p57^{Kip2}, both of which are negative regulators of VSMC proliferation in vivo. miR-221 also downregulates c-Kit, which is a positive regulator of myocardin expression, thus leading to inhibition of contractile gene expression. These findings suggest that miR-221-dependent downregulation of p27^{Kip1} and p57^{Kip2} promotes cell proliferation in VSMCs, and downregulation of c-Kit and Myocd induces VSMCs phenotypic switching from a contractile state to a synthetic state. Interestingly, downregulation of p27^{Kip1} or c-Kit does not recapitulate the PDGF- or miR-221-mediated migration effect, suggesting that characteristics of VSMC phenotype are not coupled, but instead can be regulated by distinct mechanisms through regulation of multiple targets.

let-7

let-7d is significantly reduced in VSMCs of the spontaneously hypertensive rat, which has a higher proliferation rate compared to the relatively healthy wild-type, Wistar Kyoto rat, thus indicating that let-7d is implicated in the modulation of VSMC proliferation [24*]. Overexpression of let-7d inhibits proliferation of VSMCs by preventing the progression of the cell cycle. let-7d reduces VSMC proliferation via downregulation of K-Ras, an important regulator of cell cycle progression and proliferation [24*].

miRNAs which promote the contractile phenotype

miR-1/133

Both miR-1/133 genes are under the transcriptional control of several myogenic regulatory factors, including SRF and myocyte enhancer factor 2. Recent studies highlight that miR-1, which has been studied in the context of cardiomyocyte differentiation, also plays a role in VSMC differentiation [25*,26*,27]. Indeed, expression of endogenous miR-1 increases significantly during the differentiation of mESC to VSMC. Overexpression of miR-1 inhibits SMC proliferation through downregulation of an oncogenic serine/threonine kinase Pim-1, which promotes SMC proliferation and neointimal hyperplasia[26]. In contrast, knock-down of miR-1 partially reverses the inhibitory effects of myocardin on SMC proliferation and results in downregulation of SMC-specific markers, such as α -SMA and SM-MHC, and decreased ESC-derived SMC population. miR-133 is robustly expressed in VSMCs and regulates VSMC phenotype by inhibiting VSMC proliferation and migration [28*]. PDGF is known to increase the expression and activity of Sp-1, which in turn increases the activity of KLF4, thus allowing proliferation of VSMC. Overexpression of miR-133 reduces VSMC proliferation and migration by repressing Sp-1 expression. miR-133 also represses SMC-specific contractile gene expression in the heart by directly

targeting several smooth muscle mRNAs as well as SRF, a key regulator of smooth muscle gene expression. On the other hand, knock-down of miR-133 increases VSMC proliferation and migration. Moreover, miR-133 is downregulated in SMCs stimulated with PDGF and in proliferating VSMCs of carotid arteries in response to balloon injury in rats. Altogether, these data indicate a crucial role of miR-133 in regulating VSMC phenotype and pathological vascular remodeling in vivo.

miR-21

miR-21 mediates the effects of TGF- β /BMP signaling on VSMC differentiation [29]. In response to TGF- β or BMP stimulation, the R-Smad proteins associate with pri-miR-21 in a complex with Drosha to promote pri-miR-21 to pre-miR-21 processing and elevated miR-21 expression. miR-21 increases contractile gene expression by repressing programmed cell death protein 4 (PDCD4), thus promoting differentiation of VSMCs[29]. Horita et al. demonstrated that the expression of miR-21 is also regulated by Fos related antigen (FRA)-1, which is a direct target of miR-143 [30*]. Induction of miR-143 by SRF represses FRA-1 that upregulates miR-21, which is known to repress its target, phosphatase and tensin homolog (PTEN). The cross talk between the miR-143-FRA-1 and miR-21-PTEN axes contributes to VSMC phenotype by regulating cell proliferation. In addition, miR-21 is able to promote the contractile VSMC phenotype by inhibiting cell migration and promoting contractility [31**]. More recently, targets of miR-21 have been isolated by using a biochemical pull-down method. Pulmonary artery SMCs were transfected with biotinylated miR-21 (bio-miR-21), and mRNAs associated with bio-miR-21 were isolated following affinity purification. Multiple members of the DOCK family, which are guanine nucleotide exchange factors (GEFs) for Rac and/or Cdc42, are identified to be targets of miR-21. Downregulation of DOCK 4, 5, and 7 by miR-21 inhibits Rac1 activity and VSMC migration [31**]. Interestingly, DOCK 4, 5, and 7 were induced by PDGF signaling, and knock-down of DOCK 4, 5, or 7 reduced PDGF-mediated cell migration, thus indicating that DOCK family proteins are critical for PDGF-mediated VSMC migration. Furthermore, downregulation of DOCK4 and 5 promotes VSMC contractility through upregulation of contractile genes, such as α -SMA and CNN [31**]. Further investigation of the identified 21 novel targets in addition to the DOCK family may provide insights into understanding the influence of miR-21 on promoting the VSMC contractile phenotype.

miR-100

miR-100 is expressed in endothelial cells and VSMCs and attenuates the proliferation of endothelial cells and migration of VSMCs [32*]. Inhibition of miR-100 promotes the migration of VSMCs, and miR-100 is downregulated during adaptive neovascularization in a mouse model of vascular occlusion. Mammalian target of rapamycin (mTOR) has been validated as a miR-100 target, and miR-100 is able to repress mTOR-dependent cellular processes.

miR-143/145

miR-143/145 are expressed specifically in VSMCs [33]. The critical role of miR-143/145 in VSMC development is demonstrated in miR-143/145 gene targeted mice. miR-143/145 expression is transcriptionally regulated through the CArG box by SRF and myocardin/

MRTF-A [33,34]. miR-143/145 promote the contractile phenotype of VSMCs by regulating the myocardin- or MRTF-A-dependent expression of SMC differentiation markers and cytoskeletal dynamics and inhibiting the proliferation of VSMCs. miR-143/145 also repress multiple targets, including Krüppel-like factor 4 (KLF4), Krüppel-like factor 5 (KLF5), E twenty-six (ETS)-like transcription factor 1 (ELK1), Versican, several actin remodeling proteins, and angiotensin-converting enzyme, all of which are antagonistic to VSMC differentiation[35**,36*]. Furthermore, overexpression of miR-145, but not miR-143, is sufficient to differentiate multipotent neural crest stem cells into SMCs in vitro[33]. miR-143/145-deficient VSMCs, on the other hand, fail to demonstrate a contractile phenotype in response to vasopressive stimuli and remain in the synthetic state[37,38]. Knock-out mice lacking either miR-145 or both miR-143 and miR-145 exhibit significantly reduced numbers of contractile VSMCs and remarkably increased numbers of proliferative VSMCs in the aorta and the femoral artery[34,37,38]. VSMCs in miR-143/145-null arteries demonstrate synthetic morphology and significant downregulation of VSMC-specific contractile markers[37]. Altogether, these data indicate that miR-143/145 are critical for the regulation of the development and homeostasis of VSMCs.

Other miRNAs that modulate VSMC phenotype

miR-10a

miR-10a was found to be a regulator of SMC differentiation from ESCs [39*]. miR-10a is increased during retinoic acid (RA)-induced differentiation of mouse ESCs into SMCs. Inhibition of miR-10a reduces the expression of SMC markers and the efficiency of RA-induced SMC differentiation. Huang et al. demonstrated that histone deacetylase 4 (HDAC4) is a novel target of miR-10a and mediates miR-10a function during SMC differentiation from ESCs [39*]. Interestingly, RA triggers the nuclear translocation of NF- κ B, which then binds to the miR-10a promoter and enhances miR-10a expression transcriptionally. Therefore, increased miR-10a expression by RA can lead to SMC differentiation through repression of HDAC4, which is a negative regulator of SMC differentiation.

miR-125b

Vascular calcification is one of the most common diseases of the aging population and is associated with significant morbidity and mortality. VSMCs play a role in vessel calcification via transdifferentiation toward an osteoblast-like state [40*]. miR-125b is known to be involved in osteoblast differentiation by regulating cell proliferation [41]. Goettsch et al. provide further evidence that miR-125b regulates the osteogenic transdifferentiation of VSMCs, from proliferation to differentiation, in the process of vascular calcification [42*]. miR-125b was decreased during the osteogenic transdifferentiation of human coronary artery SMCs (HCASMCs), suggesting that miR-125b is involved in the proliferation of HCASMCs. Inhibition of miR-125b in calcified HCASMCs promoted osteogenic transdifferentiation. Goettsch et al. also demonstrated that miR-125b modulates the expression of SP7, suggesting that miR-125b regulates the transdifferentiation of VSMCs by targeting the transcription factor SP7.

Conclusion

Recent studies have revealed that miRNAs play critical roles in the modulation of VSMC phenotype and contribute to various pathological processes. In this review, many miRNAs show overlapping functions in controlling VSMC phenotype, thus highlighting the presence of redundant miRNA-dependent pathways for the maintenance of a contractile or synthetic VSMC phenotype (Fig. 2 and 3). These miRNAs may be counterbalanced by other miRNAs on phenotypic switching of VSMC (Fig. 2). Therefore, identification of additional miRNAs and analysis of the functions of their targets may provide new insights into the mechanisms of regulation of VSMC phenotype and vascular diseases [43*]. Furthermore, an understanding of the complex interactions between miRNAs and signaling pathways involved in vascular diseases may offer potential miRNA-based therapeutic applications.

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Conflicts of interest:

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Key points

- VSMCs undergo a unique process known as phenotype switching between the contractile and synthetic state.
- miRNAs are important mediators for the modulation of VSMC phenotype by repressing their targets that function as molecular switches for VSMC differentiation.
- Understanding the functions of miRNAs in the regulation of VSMC phenotype switch provides new insights to the mechanisms of vascular development and diseases.

Figure 1. Schematic diagram of miRNA biogenesis

miRNAs are transcribed by RNA polymerase II (RNA Pol II) as pri-miRNAs. The Drosha complex processes the pri-miRNAs into hairpin-structured pre-miRNAs. The pre-miRNAs are exported from the nucleus to the cytoplasm and are cleaved by the Dicer complex into miRNA-miRNA* duplexes. The mature miRNA is incorporated into the RISC complex and mediates post-transcriptional repression of target mRNA by translational repression and/or deadenylation and degradation.

Figure 2. Schematic diagram of VSMC phenotype modulation

In response to extracellular signals, the expression of a cohort of miRNAs is modulated. Each miRNA is implicated in contractile or synthetic phenotype regulation, and thus VSMCs can switch between phenotypes.

Figure 3. A network of miRNAs and their targets implicated in VSMC phenotype modulation
 Characteristics of VSMC, such as migration, proliferation, and differentiation, are regulated by miRNAs through repression of their targets. Validated targets of each miRNA involved in VSMC phenotype modulation are indicated.