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Implications of MicroRNAs in the Vascular Homeostasis and Remodeling

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<http://dx.doi.org/10.5772/60740>

Abstract

Vascular remodeling or arterial remodeling is a process of adaptive alteration of vascular wall architecture and leads to the endothelial cell (EC) dysfunction and synthetic or contractile phenotypic change of VSMCs, and the infiltration of monocytes and Macrophages that promotes vascular diseases including atherosclerosis. Recent findings have demonstrated that microRNAs (miRNAs) are involved in regulating gene expression at posttranscriptional level and disease pathogenesis. A change of miRNA expression profiles plays key roles in the gene expressions and the regulation of cellular functions. In this chapter, we summarize the vascular remodeling-related miRNAs and their functions in vascular biology.

Keywords: Vascular remodeling, vascular homeostasis, smooth muscle cells, endothelial cells, macrophage, microRNAs, vascular smooth muscle cell phenotypic switch

1. Introduction

Vascular remodeling (or arterial remodeling) is a process of adaptive alteration of vascular wall architecture and is caused by variety of environmental stimuli such as oxidative stress, vascular injury, and hemodynamic stress [1]. Endothelial cells (ECs) and vascular smooth muscle cells (VSMCs) compose the arteries and play critical roles in vascular remodeling in conjunction with inflammatory cells such as monocytes or macrophages [2]. During vascular

remodeling, the EC dysfunction, synthetic or contractile phenotypic change of VSMCs, and the infiltration of monocytes and macrophages promote vascular diseases including atherosclerosis [3, 4]. Therefore, modulation of VSMC phenotype, maintenance of ECs, and regulation of inflammation in the vessel wall are important in arterial function and homeostasis.

Recent findings have been demonstrated that microRNAs (miRNAs) are involved in regulating gene expressions at posttranscriptional level and disease pathogenesis [5, 6]. Importantly, altered specific set of miRNAs is closely related to cell fate determination, tissue function, and homeostasis [7, 8], indicating that miRNAs plays key roles in the gene expressions and the regulation of cellular functions [9]. Consequently, the change of miRNA expressions can lead to VSMC phenotype switch, EC dysfunction, and the inflammatory response and lipid accumulation of macrophage in vascular pathophysiology [10–12].

In this chapter, we summarize the vascular remodeling-related miRNAs and the function of these miRNAs in vascular biology, and suggest novel therapeutic strategies for the treatment and/or prevention of vascular diseases via controlling the expressions of miRNA regulation.

2. MicroRNAs

MicroRNAs (miRNAs or miRs) are small noncoding RNA molecules approximately 18–25 nucleotides in length that participate in controlling gene expression at the posttranscriptional level [13]. MicroRNAs directly bind to 3 untranslated regions (3UTR) of target mRNAs, leading to translational inhibition and/or mRNA degradation [9]. One miRNA can suppress multiple target protein-coding mRNAs, which also means that a single protein can be regulated by numerous miRNAs [14]. Interestingly, about two-thirds of the coding-mRNA genes are regulated by miRNAs [15]. Since the first discovery of miRNAs in *Caenorhabditis elegans* in 1993, more than 1800 miRNAs in human have been identified and recorded in miRBase 21 (www.mirbase.org) [16].

The biogenesis of miRNAs are initiated by transcription from their genes by RNA polymerase II, which produces primary miRNAs (pri-miRNAs) with hundreds to thousands of nucleotides and single or multiple stem-loop-like structures [17]. After transcription, the pri-miRNAs are cleaved at ~70-nucleotide hairpin-shaped precursor miRNA (pre-miRNA) by RNase-III endonuclease (Drosha) and DiGeorge syndrome Critical Region 8 protein (DGCR8) complex [18]. The pre-miRNAs are transferred from nuclear to cytoplasm through the nuclear export protein exportin-5 [19]. In the cytoplasm, the pre-miRNAs are processed to produce mature miRNA duplex mediated by RNase-III endonuclease, Dicer: the passenger strand and the guide strand (~22 nucleotides long) [20]. The guide strand is loaded to RNA-induced silencing complex (RISC), and the passenger strand is degraded [21]. The miRNA–RISC complex recognizes and binds the miRNA based on the complementary match between the miRNA seed sequence (2–8 nucleotides in the 5' end) and the 3 UTR of the target mRNA, and this results in translational suppression and/or mRNA degradation (Figure 1) [22, 23].

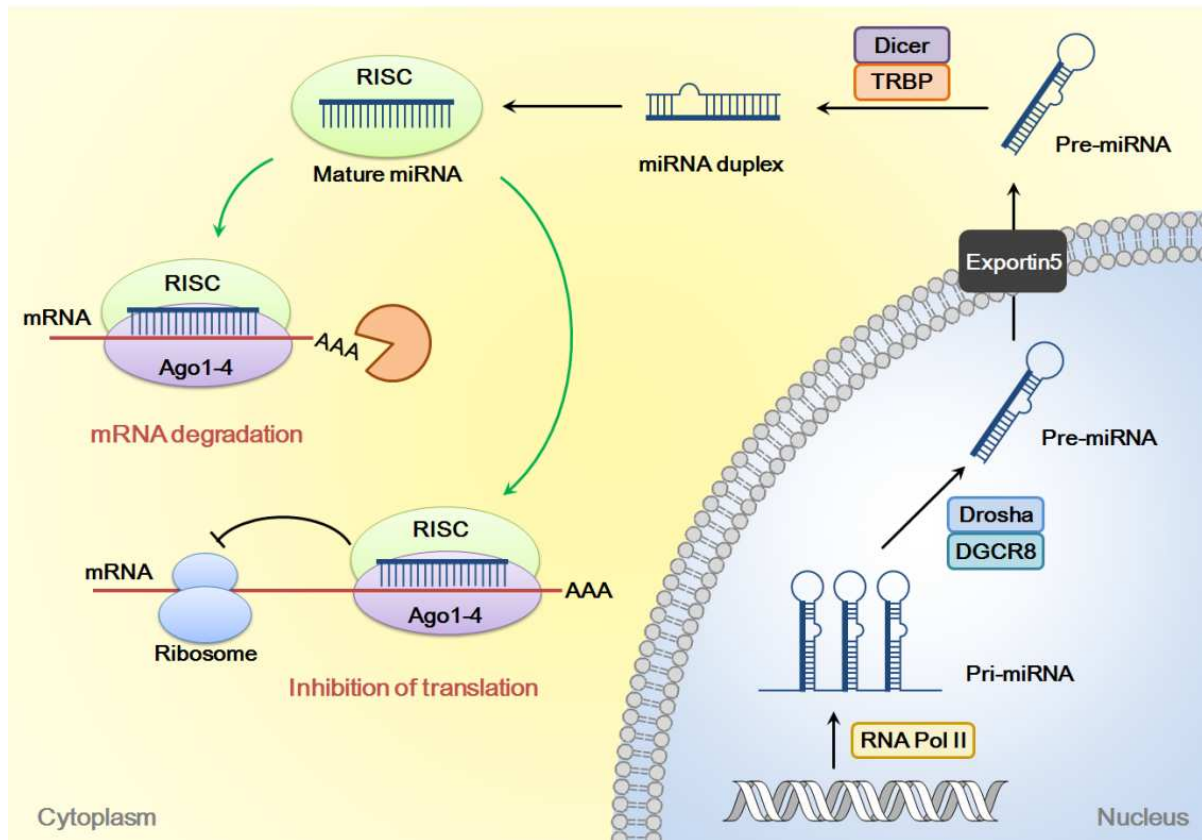


Figure 1. The biogenesis of miRNAs.

Increasing evidence suggests that miRNAs play important roles in a wide range of biological functions and processes, including but not limited to, development, growth, pathophysiology, regeneration, inflammation, stem cell fate regulation, and aging [24–29]. Because the changes of miRNA expression profiles are related to various human diseases, such as cardiovascular diseases, cancer, neuropathy, infection, and metabolic disorders [9, 30–34], the modulation of miRNA expressions consider as a novel therapeutic target and may inspire a new strategy for treatment of these diseases [35–37].

3. Vascular smooth muscle cells

The VSMCs of blood vessels in adult generally show a low proliferation rate and well-maintained homeostasis [38]. However, upon external stimuli, such as cytokines, blood flow rates, shear stress, hormones, injury, and inflammation, provoke the phenotypic switch of VSMCs from contractile to synthetic [2, 39]. The contractile or differentiated VSMCs express contractile markers, smooth muscle myosin heavy chain (SM-MHC), alpha smooth muscle actin (SM α -actin), and calponin; whereas the synthetic or dedifferentiated VSMCs show an increase of cell proliferation, migration, upregulation of extracellular matrix proteins,

such as collagen, elastin, and proteoglycans, and a decrease in the expression of contractile markers [16, 40]. The plasticity of VSMCs plays important roles in the vascular development and pathological/physiological vascular remodeling. The contractile phenotype is required to maintain the normal arterial wall structure and function, while the synthetic phenotype is involved in the development of atherosclerosis, restenosis after angioplasty, and hypertension [1, 39, 41].

The recent studies show that the regulation of VSMC phenotypic change is associated with the alteration of local environment as well as the modulation of gene expressions by miRNAs. Albinsson et al. reported that VSMC-specific deletion of Dicer-induced embryonic lethality at embryonic days 16 to 17 due to the thin and hypotensive vessel walls, impaired contractility, and hemorrhage [42]. In addition, the loss of Dicer in VSMCs of adult mice also showed a dramatic decrease in blood pressure, the impairment of contractile function, morphology, and phenotypic modulation [43]. These results strongly suggest that miRNAs are essential to vascular development and keep a balance of VSMC phenotype (Figure 2).

3.1. miRNAs in the contractile phenotype of VSMCs

3.1.1. miR-143 and miR-145

MiR-143 and miR-145 are the most well-known and enriched miRNAs in VSMCs and are in a bicistronic miRNA cluster on human chromosome 5 [44]. The significant role of miR-143 and miR-145 is to drive VSMC development and differentiation from human embryonic stem cells via targeting the stem cell pluripotency factors OCT4 (octamer-binding transcription factor 4), SOX2 [SRY (sex-determining region Y)-box 2], and KLF4 (Krüppel-like factor 4) [45]. After VSMC development, miR-143 and miR-145 are required for maintaining homeostasis of VSMCs through the suppression of multiple target proteins, such as KLF4, KLF5, E twenty-six (ETS)-like transcription factor 1 (ELK1), versican, several actin remodeling proteins, and angiotensin-converting enzyme. The transcriptional activation of miR-143 and miR-145 also leads to the expression of contractile genes [41, 46–48].

The maintenance of the VSMC contractile phenotype is modulated by several signaling pathways including serum response factor (SRF)/myocardin, transforming growth factor β (TGF- β), bone morphogenetic proteins 4 (BMP4), and Jagged-1 (Jag-1)/Notch signaling, which regulates the expression of miR-143 and miR-145 (Figure 3) [46]. The promoter region of miR-143 and miR-145 represents highly conserved *cis* elements and potential binding sites containing CArG box of SRF and Nkx2.5. In addition, myocardin and myocardin-related transcription factor A (MRTF-A), cofactors of SRF, synergistically and strongly activate the miR-143 and miR-145 transcription [49, 50]. The upregulation of miR-143 and miR-145 by TGF- β and BMP4 mediates the induction of SRF cofactors: TGF- β upregulates myocardin via the activation of parallel pathways of p38MAPK and SMAD dependent [51], whereas BMP4 stimulates the nuclear translocation of MRTF-A [52]. The Jag-1-mediated activation of Notch receptors is also activating miR-143 and miR-145 transcription [46]. Activation of Notch receptor by Jag-1 results in proteolytic cleavage and translocation to the nucleus of the Notch

intracellular domain (NICD). In the nucleus, NICD forms complex with C promoter binding factor 1 (CBF1), which is binding to miR-143 and miR-145 promoter and increases in miR-145 and miR-145 expression [46].

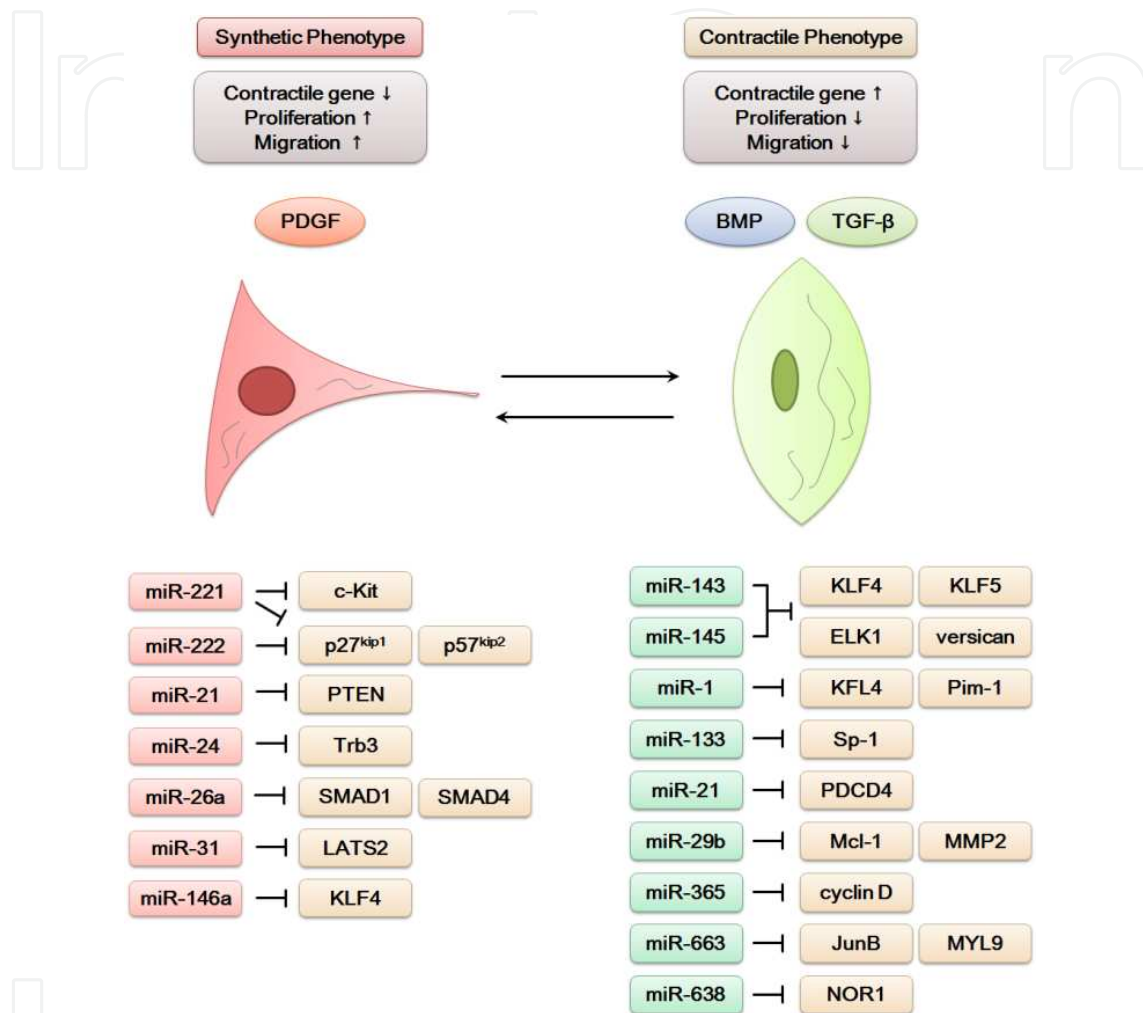


Figure 2. miRNAs in phenotypic switch of VSMCs.

The expression levels of miR-143 and miR-145 significantly decrease in animal vascular diseases models including apolipoprotein E knockout mice (ApoE^{-/-}), carotid artery ligation injury models, carotid balloon injury models, and in aortas from patients with aortic aneurism [53, 54]. MiR-143 and miR-145 knockout murine VSMCs showed a shift from contractile to synthetic state and the development of neointimal lesions [55], whereas the VSMC-specific overexpression of miR-143 and 145 increased the plaque stability and the expression of contractile proteins, and decreased macrophage infiltration, the expression of KLF4 and KLF5, and neointimal lesion formation [54, 56]. Altogether, these data indicate that miR-143 and miR-145 play an important role in the VSMC homeostasis and strengthen the VSMC contractile phenotype.

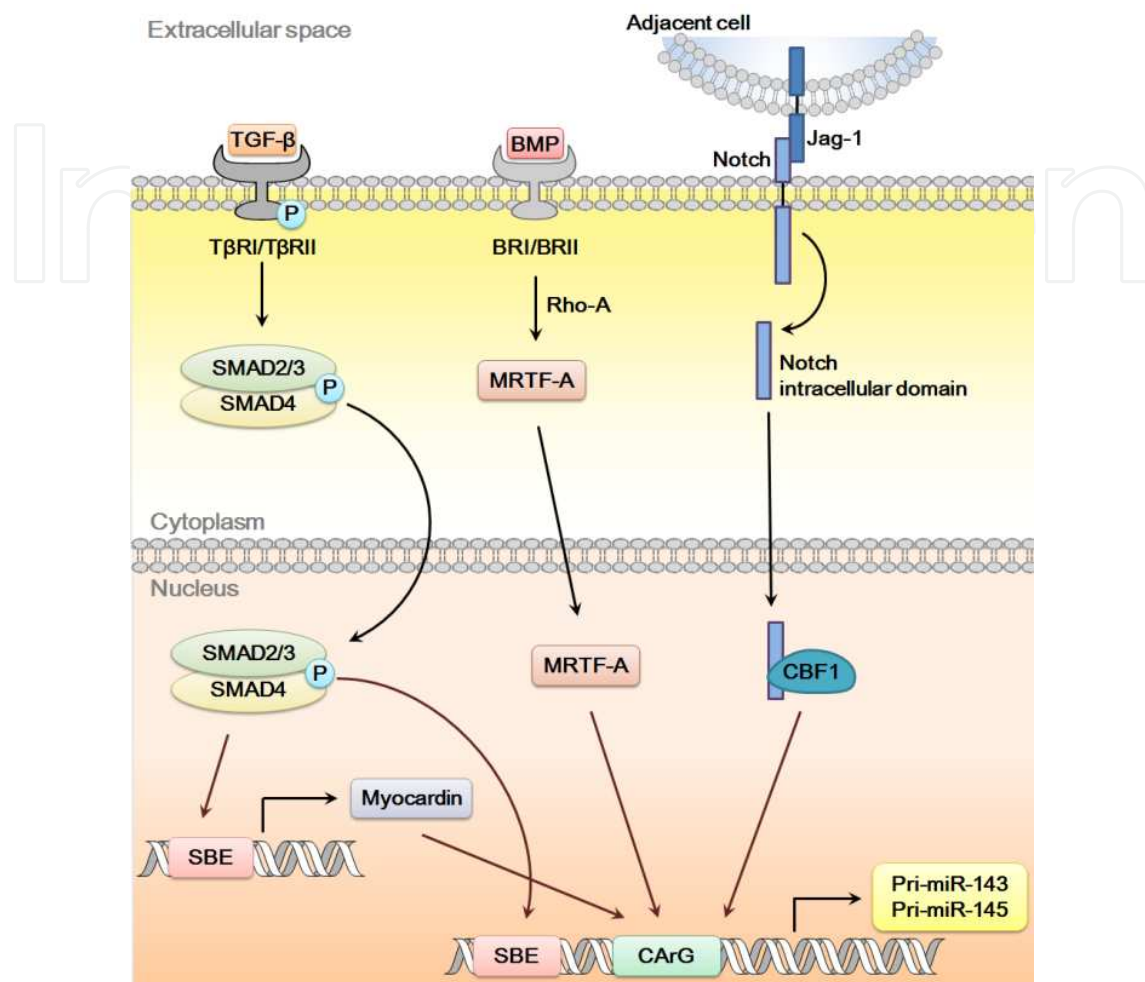


Figure 3. The signaling pathway of miR-143 and miR-145 expression.

3.1.2. miR-1 and miR-133

Similar to miR-143 and miR-145, miR-1 and miR-133 are also in bicistronic miRNA clusters and are induced by myocardin in VSMCs [57]. During differentiation of mouse ESC to VSMCs, the expression level of miR-1 is gradually increased, which results in the upregulation of VSMC-specific contractile proteins by repressing KLF4 [58]. Chen et al. demonstrated that overexpression of myocardin in VSMCs increased miR-1 expression and inhibited VSMC proliferation, which mediated the repression of Pim-1, a serine/threonine kinase [59].

The expression level of miR-133 is downregulated in proliferating VSMCs and after vascular injury. Overexpression of miR-133 reduces VSMC growth, but the downregulation of miR-133 induces VSMC proliferation *in vitro* [57]. Both the transcription factor Sp-1 (specificity protein 1) which regulates VSMC phenotypic switch and the actin-binding protein moesin that modulates VSMC migration are direct targets of miR-133. Accordingly, miR-133 overexpres-

sion in the rat carotid artery reduced neointimal hyperplasia, whereas anti-miR-133 increased VSMC proliferation and neointimal formation after balloon injury [57].

3.1.3. *miR-21*

The expression of miR-21 mediates TGF- β and BMP4 signaling pathway and promotes the contractile phenotype of VSMCs via downregulating programmed cell death 4 (PDCD4), a negative regulator of smooth muscle contractile genes [60]. Another target of miR-21 is the family of the dedicator of cytokinesis (DOCK) proteins, which promote VSMC migration by modulating the activity of small Rac1 GTPase [61]. On the other hand, Horita et al. reported that Fos-related antigen (FRA)-1, a direct target of miR-143, was a positive regulator of increased miR-21 expression [62]. The upregulation of miR-143 by SRF repressed FRA-1 expression, which led to the decrease of VSMC proliferation via the repression of miR-21 and subsequent increase of phosphatase and tensin homolog (PTEN), one of the target proteins of miR-21 [62]. The dual function of miR-21 in promoting both contractile and synthetic phenotype of VSMCs may suggest that diverse targets of miR-21 are involved in different biological processes depending on the cellular environments or context.

3.1.4. *Other miRNAs*

Our group has reported that miR-29b and miR-365 inhibit the proliferation and migration of VSMCs [63, 64]. The interleukin-3 (IL-3) is known to stimulate proliferation and migration in vascular diseases, and it downregulates miR-29b expression. Mir-29b significantly decreases the proliferation and migration of VSMCs through the inhibition of the signaling pathway related to Mcl-1 (myeloid cell leukemia 1) and MMP2 (matrix metalloproteinase 2). Consistent with the declined miR-29b expression in balloon-injured rat carotid arteries, overexpression of miR-29b by local oligonucleotide delivery can inhibit neointimal formation [64]. The proliferation of VSMCs by various stimuli, including platelet-derived growth factor (PDGF)-BB, angiotensin II (Ang II), and serum, led to the downregulation of miR-365 expression levels. The cell-cycle-specific cyclin D1 was found to be a potential target of miR-365; thus exogenous miR-365 overexpression reduced VSMC proliferation and proliferating cell nuclear antigen (PCNA) expression, blocking transition of G1/S [63, 65].

Li et al. reported that miR-663 is related to human VSMC phenotypic switch and the development of neointimal formation [66]. According to this particular study, overexpression of miR-663 upregulated VSMC differentiation marker genes and inhibited PDGF-induced VSMC proliferation and migration via targeting the transcription factor JunB and myosin light chain 9 (MYL9). Overexpression of miR-663 also dramatically attenuated the neointimal lesion formation in mice after carotid artery ligation [66]. In addition, the same group identified that miR-638 also is markedly downregulated by PDGF-stimulated human VSMCs, whereas upregulated in human VSMCs cultured in differentiation medium, a condition that inhibits proliferation [67]. Furthermore, the orphan nuclear receptor NOR1 (neuron-derived orphan receptor 1) was identified as a target of miR-638 and downregulation of NOR1 was implicated in the miR-638-mediated inhibitory effects on cyclin D1 expression, cell growth, and migration of PDGF-induced VSMCs.

3.2. miRNAs in the synthetic phenotype of VSMCs

3.2.1. miR-221 and miR-222

Similar to TGF- β and BMP4 promote VSMC contractile phenotype, PDGF induces VSMC phenotype switch from contractile to synthetic type [41]. Mir-221 and miR-222 are well characterized in VSMC phenotype switch and clustered on the X chromosome, and have same seed sequence [68]. Mir-221 and miR-222 are transcriptionally induced by activating PDGF pathway, which reduces the expression of contractile genes and promotes VSMC proliferation and migration [69]. In addition, the expression of miR-221 and miR-222 are elevated in neointimal lesion from balloon-injured rat carotid arteries [70]. The targets of miR-221 and miR-22 include p27^{Kip1} and p57^{Kip2}, both of which are a negative regulator of VSMC proliferation [70, 71]. Interestingly, miR-221 decreased the expression of c-kit, and this reduced c-kit expression subsequently repressed the expression of a VSMC-specific nuclear coactivator myocardin [69]. As the PDGF-treatment stimulated phenotype change of VSMCs, overexpression of miR-221 and miR-222 accelerated VSMC proliferation and migration, while downregulation of them attenuated VSMC proliferation and neointimal formation in rat carotid artery after angioplasty [70]. These data indicate that miR-221 and miR-222 have a crucial role in regulating VSMC phenotype change, and suggest their therapeutic potential in pathological vascular remodeling.

3.2.2. miR-146a

Recent study has shown that miR-146a is upregulated in rat balloon-injured arteries and serum-induced proliferative VSMCs [72, 73]. KLF4, a negative regulator of VSMC proliferation, was downregulated by miR-146a, and in turn, KLF4 repressed miR-146a expression through binding to CACCC (or GGGTG) elements on the miR-146a promoter. This negative feedback regulatory network between miR-146a and KLF4 regulates transcription of each other, controlling VSMC proliferation *in vitro* and vascular neointimal hyperplasia *in vivo* [72]. Dong et al. demonstrated that miR-146a upregulation is related to the downregulation of critical transcriptional factors such as the protein expression of nuclear factor- κ Bp65 (NF- κ Bp65) and PCNA. In contrast, miR-146a knockdown is linked to the increase in pro-apoptotic protein Bax expression [73]. Therefore, miR-146a is a novel regulator of VSMC proliferation in cardiovascular diseases.

3.2.3. miR-24

Both TGF- β and BMP4 signaling are important in maintaining the VSMC contractile phenotype, whereas PDGF signaling pathways can promote the synthetic phenotype conversion of VSMCs. Although these signaling pathways play key roles in VSMC phenotypic changes, the crosstalk between these pathways is not elucidating until quite recently. Chan et al. reported that despite of BMP4 stimulation, the contractile genes are downregulated by co-treatment with PDGF in human primary pulmonary SMCs (PASMCs) [74]. Furthermore, PDGF stimulation decreases the expression of Tribbles-like protein-3 (Trb3), an important modulator of the BMP and TGF- β signaling pathway. Interestingly, the result of Trb3 promoter-luciferase-

reporter assay showed that the downregulation mechanism of *Trb3* expression by PDGF is associated with the effect of PDGF-induced miR-24, directly targeting 3'UTR of *Trb3* mRNA, rather than PDGF stimulation [74]. The knockout endogenous miR-24 by antisense oligonucleotides restored *Trb3* expression as well as attenuates PDGF-mediated synthetic activity, suggesting that PDGF-induced miR-24 expression which downregulated *Trb3*, and this miR-24-mediated downregulation of *Trb3* is sufficient to induce synthetic phenotypic changes of VSMCs [74].

3.2.4. *miR-26a*

Leeper et al. demonstrated that serum-starved human aortic SMCs upregulates the expression of contractile marker genes, and miR-26a is significantly downregulated during serum-starvation and in murine abdominal aortic aneurysm (AAA) development models [75]. Overexpression of miR-26a promoted VSMC proliferation and migration as well as inhibited apoptosis, whereas downregulation of miR-26a induced contractile gene expression and reduced proliferation and migration. The potential targets of miR-26a include SMAD1 and SMAD4, members of TGF- β and BMP superfamily downstream signaling cascade. Consistently, knockdown of miR-26a led to SMAD1 and *Samd4* upregulation and activates TGF- β and BMP signaling pathway. Thus, miR-26a is expected to actively participate in the regulation of VSMCs as an enhancer of cell growth and migration, and an inhibitor of apoptosis, modulating TGF- β and BMP signaling [75].

3.2.5. *miR-31*

Although miR-31 plays an important role in cancer cell growth and proliferation [76–78], the biological function of miR-31 in VSMCs remains unclear. Recently, Liu et al. found that miR-31 was an abundant miRNA in VSMCs of vascular walls, and was significantly upregulated in proliferative VSMCs and rat carotid arteries with neointimal growth after balloon injury [79]. The serum- or PDGF-induced VSMC proliferation is inhibited by miR-31 inhibitor, 2'OMe-miR-31 (the antisense oligonucleotide for miR-31 that is modified at each nucleotide by an O-methyl moiety at the 2'-ribose position). On the contrary, overexpression of miR-31 using the adenoviruses expressing rat miR-31 promotes VSMC proliferation [79]. The pro-proliferative effect of miR-31 is mediated by the direct inhibition of the large tumor suppressor homolog 2 (*LATS2*) expression, and is accelerated by the activation of mitogen-activated protein kinase/extracellular regulated kinase (MAPK/ERK) [79].

4. Endothelial cells

Vascular endothelial cells (ECs) cover the internal surface of blood vessels. The primary role of ECs is the maintenance of vessel wall permeability and function as a sensor for the altered physical and chemical signals by physiological or pathological processes including thrombosis, inflammation, and vascular wall remodeling [80–82]. The knockdown of *Dicer* in human ECs resulted in the change of several key regulator proteins of EC biology and angiogenesis,

such as TEK (Tie-2), KDR (VEGFR2), adhesion molecules and proteins, cytokine, chemokine, and IL-8. Furthermore, EC-specific knockdown of Dicer activated the endothelial nitric oxide synthase (eNOS) pathway, which reduces proliferation and capillary formation of EC, and the endogenous miRNA expression levels were also significantly altered [83]. These results indicate that altered miRNA expression in ECs affects the maintenance and functions of ECs (Figure 4).

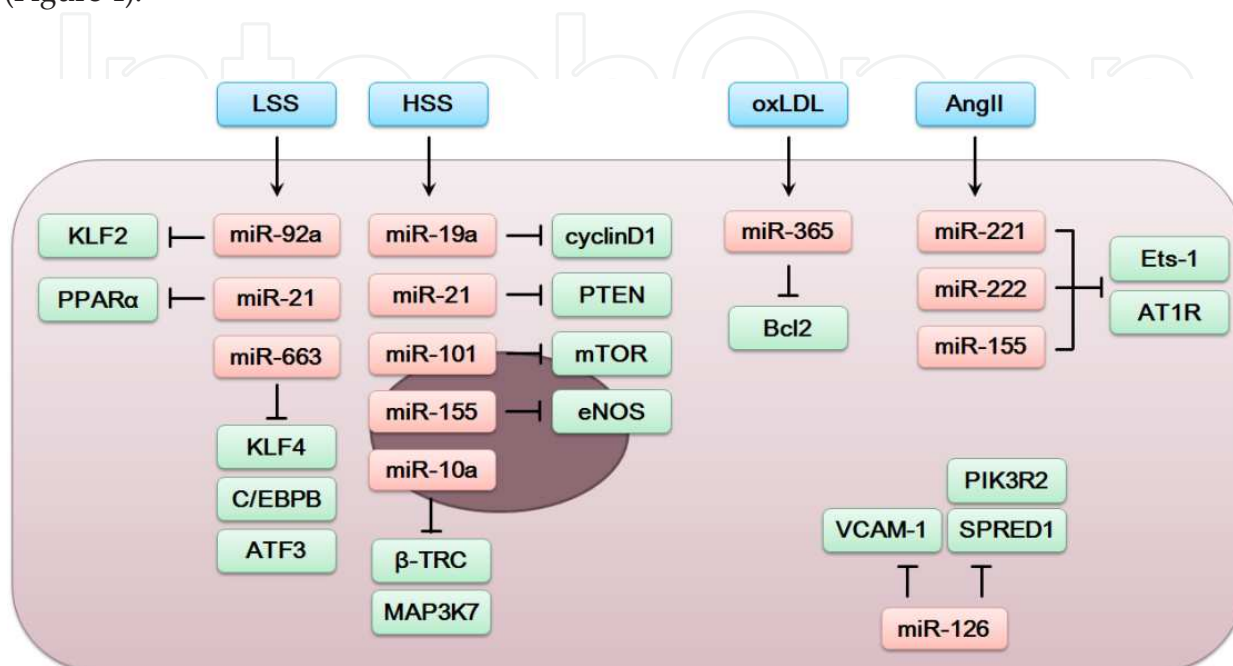


Figure 4. miRNAs in ECs regulating during vascular remodeling.

4.1. miR-126

Adhesion molecules in ECs are directly associated with leukocyte trafficking to the region of the injury and/or inflammation [84]. Generally, resting ECs do not express adhesion molecules, but activated ECs express adhesion molecules such as vascular cell adhesion molecule 1 (VCAM-1), which plays an important role in modulating leukocyte trafficking and inflammation [85]. Based on microarray and Northern analysis, Harris et al. showed that miR-126 is the most frequently expressed in HUVECs as well as other origin ECs from dermal, brain, and vein [86]. *In silico* analysis suggests that VCAM-1 is one of the targets of miR-126. The upregulation of miR-126 by transfection of pre-miR-126 results in inhibiting TNF- α induced VCAM-1 expression, whereas the knockdown of miR-126 by antisense miR-126 oligonucleotide is causing TNF- α induced VCAM-1 upregulation in a dose-dependent manner. In addition, overexpression of miR-126 mediated a decrease of VCAM-1 downregulating leukocyte adherence to ECs. These data suggest that miR-126 regulates the expression of adhesion molecules and plays a crucial role in the control of vascular inflammation [86].

Fish et al. found that EC-specific miRNA miR-126 is also highly enriched in Flk-1 positive vascular progenitors from differentiating mouse embryonic stem cells in the embryonic body [87]. Nevertheless, overexpression of miR-126 using miR-126 mimic does not promote

endothelial differentiation of embryonic stem cells. The loss-of-function of miR-126 by a morpholino antisense to miR-126 shows that the EC migration and the formation/stability of capillary tubes are decreased compared to normal HUVECs. In addition, EC-targeted deletion of miR-126 results in vascular abnormalities such as vascular leakage, hemorrhaging, and embryonic lethality in both zebrafish [87] and mice [88], because of reduced EC growth, sprouting, and adhesion by decrease in angiogenic growth factor signaling. The pro-angiogenic function of miR-126 was due to its ability to repress two negative regulators of angiogenic process, namely sprouty-related EVH1 domain-containing protein 1 (SPRED1) and phosphoinositol-3 kinase regulatory subunit 2 (PIK3R2) [87, 88]. Taken together, miR-126 is considered as new therapeutic implication for modulating vascular formation, function, leakage, and abnormal angiogenesis.

4.2. Endothelial Shear Stress (ESS) and miRNAs

The atherosclerotic lesions form at specific arterial regions such as the vicinity of branch points, the outer wall of bifurcations, and the inner wall of curvatures, where they show the disturbed flow and the change of hemodynamic forces, especially endothelial shear stress (ESS) [89]. ESS has been reported to regulate endothelial gene expression and promotes EC activation and atherosclerotic plaque progression and vascular remodeling [90, 91].

By miRNA microarray analysis using human umbilical vein endothelial cells (HUVECs), Ni et al. identified that the expression of miR-663 was mediated by low ESS (LSS) and LSS-induced miR-663 expression improved monocyte adhesion to ECs, but it had no effect on EC apoptosis. MicroRNA-663 has been reported to regulate several transcription factors related to inflammatory responses, such as KLF4, C/EBPB, and ATF3 [92].

In addition, miR-21 was also upregulated by LSS at transcriptional level in HUVECs through an increased activity and binding of c-Jun, a component of transcription factor activator protein-1 (AP-1), to the promoter region of miR-21 [93]. Mir-21 upregulated the expression of VCAM-1 and monocyte chemoattractant protein-1 (MCP-1) by suppressing the peroxisome proliferators-activated receptor- α (PPAR α), increasing adhesion of monocytes to ECs and pro-inflammatory responses [93].

MicroRNA-92a, a member of miR-17~92 cluster, was upregulated by LSS in HUVECs [94]. The decreased expression of miR-92a was correlated to the upregulation of KLF2 and subsequent KLF2-mediated eNOS and thrombomodulin (TM) expression and NO production, all of which are considered as potent anti-thrombotic, anti-adhesive, and anti-inflammatory properties [94, 95]. Therefore, LSS-induced miR-92a inhibited KLF2-mediated eNOS and TM expression that resulted in impaired EC functions.

In contrast to LSS, high ESS (HSS) appears to be protective against atherosclerosis, but the precise functions still remain unclear [96]. Mir-10a is one of the flow-induced miRNAs in ECs and is known to be downregulated in athero-susceptible regions of the inner aortic arch and aorto-renal branches than other regions [97]. The knockout of miR-10a led to I κ B/NF- κ B-mediated inflammation, which suggested anti-inflammatory role of miR-10a. Interestingly, two key regulators of I κ B α degradation, mitogen-activated kinase kinase 7 (MAP3K7;

TAK1) and β -transducin repeat-containing gene (β -TRC), are putative targets of miR-10a [97]. Therefore, miR-10a contributes to anti-inflammatory endothelial phenotype through the inhibition of pro-inflammatory molecules in athero-susceptible regions.

In addition, Qin et al. found that laminar shear stress induced miR-19a that suppressed cyclin D1 expression, leading to an arrest of cell cycle at G1/S transition [98]. Similar research by Chen et al. showed that miR-101 was also induced by laminar shear stress and cell cycle arrest at the G1/S transition and suppressed endothelial cell proliferation via targeting mTOR [99].

MicroRNA-155 is upregulated by prolonged HSS in HUVECs and it modulated endothelium-dependent vasorelaxation by repressing eNOS. Therefore, the inhibition of miR-155 improved endothelial dysfunction during the development of atherosclerosis [100].

MicroRNA-21 and miR-92a were induced by both LSS and HSS. Compared with LSS-induced miR-21, HSS-induced miR-21 decreased EC apoptosis and activated the NO pathway by targeting PTEN [101]. As mentioned earlier, downregulation of miR-92a led to upregulation of KLF2 and subsequent KLF2-mediated eNOS and TM, which facilitated the maintenance of EC homeostasis and functions against inflammation and pro-atherosclerotic effects [94, 95].

4.3. Angiotensin II (AngII) and miRNAs

Angiotensin II (AngII) has been implicated in the development and progression of cardiovascular diseases such as hypertension, atherosclerosis, and restenosis after vascular injury [102]. AngII-mediated intracellular signaling is activated through its G-protein-coupled AngII Type 1 receptor (AT1R). In both VSMCs and ECs, AngII leads to vascular and endothelial dysfunctions by generating intracellular reactive oxygen species (ROS) and negatively regulating NO signaling pathway, respectively [103, 104]. Zhu et al. found that miR-155, miR-221, and miR-222 were highly expressed in both HUVECs and VSMCs, and a key endothelial transcription factor for inflammation and tube formation Ets-1 was a putative target of miR-155, miR-221, and miR-222 [105]. Interestingly, miR-155 also targeted AT1R, and AngII-stimulated HUVECs upregulates Ets-1 and its downstream genes, including VCAM1, MCP1, and FLT1 [105]. These results indicated that miR-155, miR-221, and miR-222 act as negative regulators to inflammatory response of ECs caused by AngII stimulation.

4.4. Oxidized low-density lipoprotein (oxLDL) and miRNAs

Oxidized low-density lipoprotein (oxLDL) induces EC death and dysfunction through the activation of NF- κ B and AP-1 pathways, and it also activates pro-inflammatory response during the progression of atherosclerotic vascular diseases [2, 106]. Based on microarray and qRT-PCR analysis with oxLDL-stimulated HUVECs, Qin et al. found that four miRNAs are upregulating including miR-365 and miR-142-3p, whereas eleven miRNAs are downregulating including miR-590-5p and miR-33a in microarray, which expression levels are validated with qRT-PCR [107]. Using computational prediction algorithms (TargetScan, microRNA.org, and MicroCosm v5), the gene ontology (GO) database (www.geneontology.org), and KEGG pathway database (www.genome.ad.jp/kegg), the authors predicted and identified the functions of miRNA-targeted genes. As a result, miR-365 has been reported to be involved in

oxLDL-induced apoptosis of ECs by suppressing the anti-apoptotic protein Bcl-2 [107]. Therefore, the inhibition of miR-365 may reduce EC apoptosis and inhibit the pro-atherogenic progression caused by oxLDL.

5. Macrophage

The accumulation and activation of macrophages within the vascular wall are a crucial event in vascular remodeling and implicate the progression of atherogenesis, which is initiated by inflammation, a rise in circulating LDL levels, and the accumulation of oxLDL in macrophages [108, 109]. Inflammation triggers the recruitment and infiltration of monocytes and macrophage in atherosclerotic lesions, continuously accumulating lipids and oxLDL [110]. The macrophages respond to various inflammatory stimuli, which lead to the changes in expression of many genes and several miRNAs, including miR-155, miR-125a, miR-146a, and miR-146b [111, 112]. Although the microRNAs involved in oxLDL accumulation and inflammatory response in macrophages are currently unclear, considering the role of miRNAs in VSMCs and ECs during arterial remodeling, the idea of miRNAs that also contribute the macrophage functions does not seem to be illogical (Figure 5) [2].

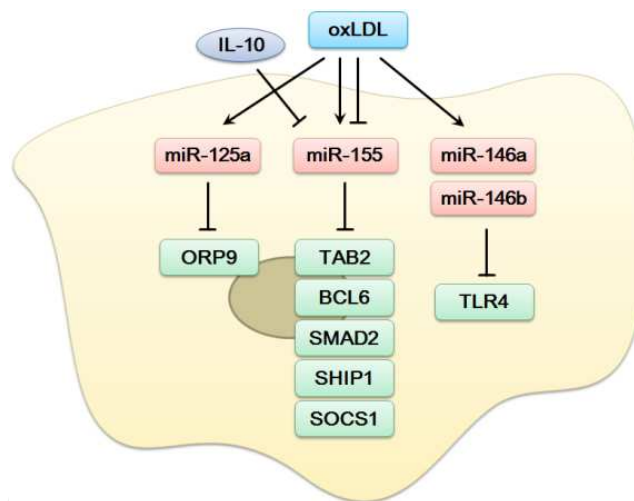


Figure 5. miRNAs in macrophages during vascular remodeling.

5.1. miR-155

The expression of miR-155 increased in human atherosclerotic lesions, but circulating miR-155 is known to be downregulated in patients with coronary artery diseases [113, 114]. During vascular remodeling, miR-155 is expressed in VSMCs, ECs, as well as activated macrophages [115]. The oxLDL and/or ESS promote the recruitment of monocytes into the arterial wall through ECs and then monocytes are differentiated toward macrophages and dendritic cells (DCs) [108]. MiR-155 is the most important miRNA in macrophage-mediated inflammation [116], and its expression is increased by several Toll-like receptor (TLR) ligands via myeloid

differentiation primary response gene (MyD88) and/or TIR-domain-containing adapter-inducing interferon- β (TRIF)-dependent signaling in the inflammatory activation of macrophages [117]. In addition, the lipopolysaccharide (LPS) is also known to induce miR-155 in monocytes and DCs. LPS-induced miR-155 expression is mediated by the single-strand RNA-binding protein KH-type splicing regulatory protein (KSRP) in bone marrow-derived macrophages [118]. In activated primary human monocyte-derived DCs, LPS-induced upregulation of miR-155 inhibited Toll-like receptor/interleukin-1 (TLR/IL-1) inflammatory pathway as well as TAB2 (an adaptor in the TLR/IL-1 signaling cascade), attenuating IL-1 β and inflammatory signals [119]. Furthermore, oxLDL and cytokine IFN- γ induced the expression of miR-155 that modulated the inflammatory response in macrophages by repressing BCL6, a negative regulator of pro-inflammatory NF- κ B signaling. The attenuation of BCL6 was correlated to upregulation of chemokine CCL2, an activator of monocyte recruiting into atherosclerotic plaques [120]. MicroRNA-155 also regulates TGF- β signaling in macrophages by targeting SMAD2. Overexpression of miR-155 inhibited TGF- β induction of SMAD2 phosphorylation, which repressed TGF- β -dependent transcription of cytokines, such as IL-1 β [121].

On the other hand, a potent anti-inflammatory cytokine IL-10 inhibited LPS-induced miR-155 expression via STAT3-dependent manner, which led to upregulation of the target proteins of miR-155 such as Src homology 2 domain-containing inositol-5-phosphatase 1 (SHIP1) and anti-inflammatory genes [122]. Moreover, LPS-activated PI3K/Akt1 pathway inhibited expressions of miR-155 and pro-inflammatory mediators [123]. Paradoxically, a small number of studies reported that miR-155 is also downregulated in oxLDL stimulation and reduces lipid uptake and accumulation in macrophages [120, 124]. Therefore, miR-155 in macrophages maintains the balance between pro- and anti-inflammatory responses, which is influenced by the diseases context or extracellular microenvironments.

5.2. miR-125a

Similar to miR-155, upregulation of miR-125a seems to decrease lipid accumulation by directly targeting oxysterol-binding protein-like 9 (ORP9), but the functional role of miR-125a in oxLDL-stimulated monocytes and macrophage still remains unclear [125]. MicroRNA-125a has been reported to be upregulated through oxLDL in primary human monocytes, and inhibition of miR-125a increased the secretion of IL-6, TNF- α , IL-2, and TGF- β , possibly due to enhanced expression of scavenger receptors (LOX-1, CD68).

5.3. miR-146a and miR-146b

MicroRNA-146a/b is upregulated after stimulation with oxLDL in primary human monocytes [125]. MiR-146a and -b are located on different chromosomes, and differ by two nucleotides. Stimulation of TLR-2, 4, and 5 has been reported to induce miR-146a/b expressions in macrophages through NF- κ B activation [126]. The oxLDL-mediated suppression of miR-146a promoted lipid uptake and cytokine release, most likely due to reduced suppression of TLR-4 (target of miR-146a) in macrophage [124]. Additionally, miR-146a and b are known to upregulated in human atherosclerotic plaques [127].

6. Circulating miRNAs as biomarkers

In addition to the significance of miRNAs regulating gene expression at the intracellular regions, some miRNAs have been discovered in the extracellular body fluids including serum/plasma and bloodstream [128, 129]. Interestingly, the extracellular circulating miRNAs show remarkably stable and resistant against ribonucleases, freezing, boiling, low or high pH, and so on, which signifies that some protective mechanisms can countervail degradation [130–132]. The mature miRNAs are released from intracellular to extracellular environment via the complex with RNA-binding proteins such as Argonaute-2 (Ago2), binding to high-density lipoprotein (HDL), and loading into microvesicle bodies such as microvesicles, exosomes, or apoptotic bodies (Figure 6) [133–136].

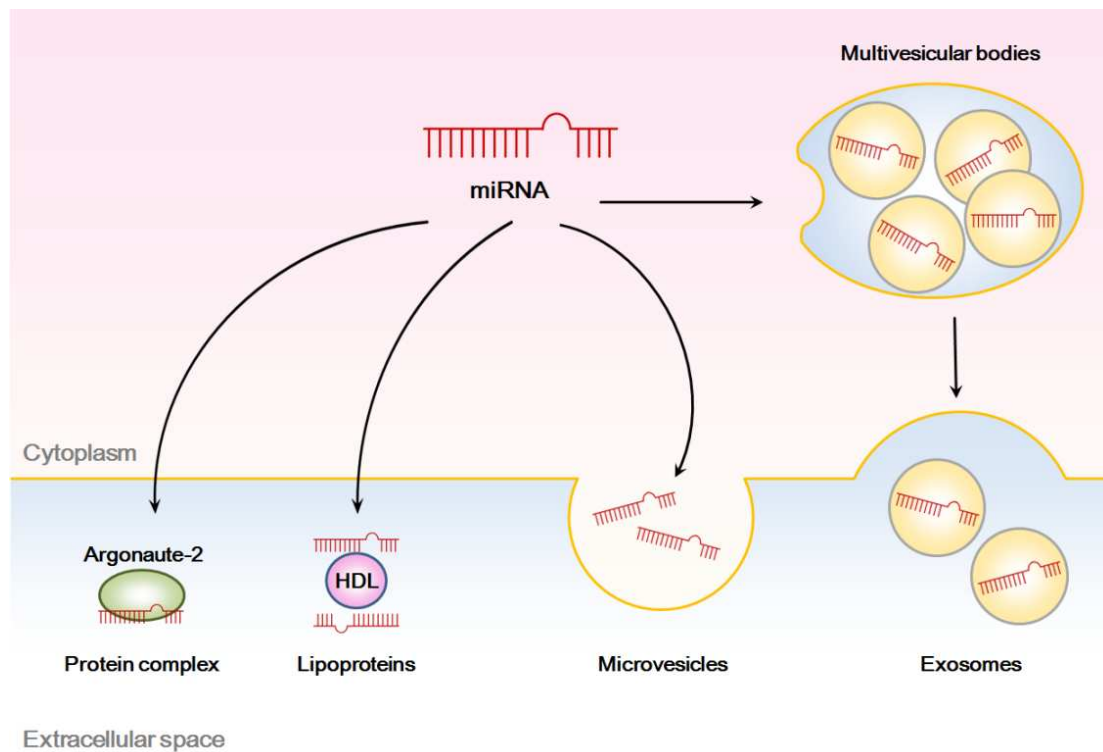


Figure 6. The cellular release mechanisms of miRNAs.

Although the cellular origin and function of the circulating miRNAs remain uncertain, their expression profiles are changed by fluid types and physiology or pathology conditions [137, 138]. The extraordinarily stable and tissue/diseases specific profiles of circulating miRNAs have been considered as promising biomarkers for diagnosis of diseases [139, 140].

Several studies suggest that circulating miRNAs may be taken into the recipient cells and regulate target gene expression [141–143]. In vascular diseases, a few studies showed that circulating miRNAs modulate the function of ECs [144, 145]. Zhang et al. found that the secretion of microvesicles containing miR-150 from human monocytic cells THP-1 is increased in the plasma of patients with atherosclerosis. Secreted monocytic miR-150 is transported into human microvascular endothelial cells (HMEC-1) and then promotes HMEC-1 migration via downregulating the miR-150 target gene *c-Myb* [144]. Likewise, Zerneck et al. showed that

EC-derived apoptotic bodies, containing miR-126, upregulates the production of CXC chemokine CXCL12, which promotes the recruitment of Sca-1⁺ progenitor cells and reduces the atherosclerotic lesion formation in ApoE^{-/-} mice. These results suggest that circulating miR-126 in apoptotic bodies may have protective potential against atherosclerosis [145].

7. Conclusion

Vascular remodeling encompasses a series of complex biological pathways and involves the phenotype change of VSMCs, EC dysfunction, as well as macrophage activation. The alteration of VSMCs, ECs, and macrophage cellular functions is related to the various extracellular stimulus-dependent changes in transcriptional regulation, which is regulated by miRNAs. Thus, the identification of stimuli-dependent vascular remodeling which affects miRNA expression in different vascular cell types is imperative. The augmenting or inhibiting of the expression levels of specific miRNAs may provide opportunity for the development of miRNA-based therapeutic application to treating diverse vascular pathologies. In addition, extracellular circulating miRNAs have been reported to be altered under specific pathologic conditions, implicating their usage as biomarkers for specific diseases including cardiovascular disease. Although there are unsolved issues of the efficiency and safety of using miRNAs in diagnosis and therapy, accumulating evidence indicate that continuous research on the functions and mechanisms of miRNAs and the identification of a network between miRNAs and their targets is highly recommended and the results will expand our understanding of vascular remodeling and diseases.

Acknowledgements

This study was supported by a Korea Science and Engineering Foundation grant funded by the Korean government (MEST) (NRF-2011-0019243, NRF-2011-0019254) and a grant from the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (A120478).

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