

ATVB in Focus

MicroRNAs – From Basic Mechanisms to Clinical Application in Cardiovascular Medicine

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MicroRNA-126, -145, and -155 A Therapeutic Triad in Atherosclerosis?

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Abstract—Atherosclerosis is a condition caused by lipid-induced inflammation of the vessel wall orchestrated by a complex interplay of various cell types, such as endothelial cells, smooth muscle cells, and macrophages. MicroRNAs (miRNAs) have emerged as key regulators of gene expression typically by repressing the target mRNA, which determines cell fate and function under homeostatic and disease conditions. Here, we outline the effects of miRNA-145, -126, and -155 in atherosclerosis in vivo. Downregulation of miR-145, which controls differentiation of smooth muscle cells, promotes lesion formation, whereas the endothelial cell-specific miRNA-126 signals the need for endothelial repair through its transfer from apoptotic endothelial cells in microvesicles. Elevated miR-155 levels are characteristic of proinflammatory macrophages and atherosclerotic lesions. However, the effects of miR-155 seem to be different in early and advanced atherosclerosis. The discovery of the role of these miRNAs in atherosclerosis sheds light on the current concepts of atherogenesis and may provide novel treatment options for cardiovascular diseases. (*Arterioscler Thromb Vasc Biol.* 2013;33:449-454.)

Key Words: endothelial cells ■ Kruppel factor ■ microRNAs ■ smooth muscle cells

Morphological changes in arteries during formation of atherosclerotic lesion include substantial changes in the phenotype and function of vascular wall cells, such as endothelial cells (ECs) and smooth muscle cells (SMCs), and the recruitment of leukocytes.¹ Dysfunctional ECs, which are characterized by impaired endothelium-dependent vasodilatation, increased permeability and expression of chemokines and adhesion molecules (which triggers enhanced recruitment of monocytes), and decreased endothelial regeneration, play a central role in atherosclerosis.² SMCs in the medial layer of the arterial wall are specialized to maintain and regulate vascular tone by a highly differentiated contractile apparatus. However, lesional cells that express SMC markers have a completely different phenotype characterized by enhanced synthetic capabilities, increased proliferation, and inflammatory activation.³⁻⁵ Macrophages differentiate in the vessel wall from recruited monocytes and are the most prominent cell type in atherosclerotic lesions of different stages, where they ingest modified lipoproteins and accumulate cholesterol in lipid droplets. Although macrophage cell types that are activated in different ways, such as proinflammatory M1-type and alternatively activated M2-type, have been found in atherosclerotic lesions, the function of different lesional macrophage phenotypes is still unclear.⁶

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Cell fate decisions and the development of cellular phenotypes are controlled by a regulatory system of small (≈ 22 nt) noncoding microRNAs (miRNAs), which usually inhibit but may rarely increase gene expression at the posttranscriptional level.^{7,8} miRNAs bind via partial base pairing of the 5' seed sequence (≈ 7 nt) to a recognition element in the 3' UTR of the mRNA target, which guides the loading of this mRNA to an RNA-protein complex, the miRNA-induced silencing complex. In the miRNA-induced silencing complex, the mRNA target is degraded or its protein translation is inhibited. Due to partially complementary binding between the miRNA seed and the mRNA, a specific miRNA can theoretically target a large number of mRNAs (that can be part of a shared pathway), which confers great versatility to miRNA-mediated regulation of gene expression. In addition, mRNAs sharing the same target site for a specific miRNA can compete for binding to this miRNA and thereby mutually influence the expression of each other.^{7,9} This may result in the same miRNA mediating different effects in

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distinct cell types due to expression of a diverse set of mRNA targets. Furthermore, expression of 2 functional miRNAs with distinct seed sequences from 1 stem-loop precursor adds additional complexity. Although the effect of miRNAs on the protein expression of the target mRNA is usually subtle, these proteins are commonly regulatory, including transcription factors, which are part of a feed-forward loop, a mutually negative feedback loop, or a positive feedback loop.¹⁰ Thus, small changes in the protein level can have large physiological effects and miRNAs can thereby confer robustness to biological processes, such as cell fate switches, by suppressing aberrant transcripts.¹⁰ However, repression by some miRNAs is strong when the level of the target mRNA is low, but weak when the level is high, meaning miRNAs can act as a switch or a fine-tuner depending on mRNA expression levels.¹¹ Moreover, the protein level of a target mRNA can be much lower when it is cotargeted to multiple miRNAs than when it is targeted to a single miRNA.¹⁰ Regulation of epigenetic modifications, such as DNA methylation or histone acetylation, by miRNAs, which directly or indirectly target components of the epigenetic machinery, can induce global changes in the gene expression pattern, including the expression of miRNAs.¹² All these mechanisms may need to be considered to interpret the effects of miRNAs in atherosclerosis.

On the basis of in vitro studies, it has been recently reviewed that diverse miRNAs may play a role in atherosclerosis and vascular remodeling by establishing a cell type-specific regulatory network.¹³ Considering the complexity of the regulatory mechanisms of miRNAs, in this article we will focus on miRNAs that have been demonstrated to play a role in animal models of atherosclerosis.

Loss of miR-143/145 in SMCs Promotes Atherosclerosis

miR-143 and miR-145 are 2 highly conserved miRNAs encoded by a bicistronic gene cluster, which are abundantly expressed in SMCs. Both play a crucial role in SMC differentiation, although the sequence homology between miR-143 and -145 is low.^{8,14,15} The expression of miR-143/145

is upregulated by transcription factors that play a central role in SMC differentiation, such as serum response factor and the coactivators, myocardin and myocardin-related transcription factors.^{8,15} Not only are miRs-143/145 highly expressed in SMCs, but transfer of miR-145 also triggers reprogramming of various non-SMC cell types into SMC-like cells characterized by increased expression of contractile proteins (Table).^{8,15} This effect on the SMC phenotype may be because of 2 positive feedback loops in which miR-145 directly stimulates the translation of myocardin or suppresses the transcriptional repressors of myocardin, Kruppel-like factor 5 (KLF5), and KLF4.^{16,17} In addition, many other targets of miR-145, such as Slit-Robo GTPase-activating protein 1 (Srgap1), Srgap2, Adducin-3, Slingshot 2 (Ssh2) phosphatase, and calmodulin kinase II δ , and of miR-143, such as ETS-like gene 1, have been identified by 3'UTR reporter assays, which may also affect the SMC phenotype (Figure 1A).^{8,15} In line with these in vitro results, miR-143/145-deficient mice have a thinner medial layer in arteries and decreased blood pressure, indicating severely disturbed SMC homeostasis.^{14,15,18} SMCs in miR-143/145^{-/-} mice are partially dedifferentiated, indicated by the reduced expression of SMC-specific contractile proteins and actin stress fibers.^{14,15,18}

In animal models of vascular diseases, such as vascular injury, atherosclerosis, and aneurysm formation, miRs-143/145 are downregulated, although this has not been confirmed in human atherosclerosis.^{8,19-21} Supplementation of these miRNAs decreases neointima formation in injured arteries, suggesting a crucial role of miRNA-mediated maintenance of a differentiated SMC phenotype (Table).^{8,16,18,21} However, miR-143^{-/-} and miR-145^{-/-} mice, which lack miR-143/145 already when lesion formation is induced, develop markedly less neointima after carotid ligation. This is in contrast to the effect of replacement of the downregulated miR-143/145 expression levels during lesion formation. The genetic deficiency of miR-143/145 may lead to an adaptive response in the gene expression pattern in SMCs that limits their response to vascular injury.¹⁵ However, the acutely disturbed SMC homeostasis by the disease-induced suppression of miR-143/145 seems to

Table. Effects of miR-143/145, miR-126, and miR-155 in Atherosclerosis

miRNA	Human Atherosclerosis	Animal Models		In Vitro
		Model	Effects	
miRs-143/145	Reduced circulating levels in patients with CAD ³³	Adenoviral overexpression in rat carotid arteries after balloon injury and in Apoe ^{-/-} mice on a HFD Gene knockout	Reduced neointima formation and atherosclerosis ^{18,21} 1) Reduced blood pressure and neointima formation ¹⁵ ; 2) spontaneous atherosclerotic lesions in aged mice ¹⁴	miR-145: promotes SMC differentiation ^{8,15} ; miR-143: inhibits SMC proliferation ⁸
miR-126	1) Reduced circulating levels in CAD patients ³³ ; 2) reduced expression of miR-126* in atherosclerotic lesions ²⁰	Local treatment with premiR-126	Reduced atherosclerosis ³⁰	1) Promotes CXCL12 expression ³⁰ ; 2) inhibits leukocyte adhesion to ECs ³²
miR-155	1) Upregulated in atherosclerotic lesions ^{20,36} ; 2) reduced circulating levels in CAD patients ³³	Gene knockout in BM cells	1) Increased atherosclerosis in LDL-R ^{-/-} mice ⁴² ; 2) reduced disturbed flow-induced lesions in Apoe ^{-/-} mice ³⁶	1) Promotes proinflammatory activation of macrophages ^{36,40,41} ; 2) inhibits proinflammatory activation of ECs ⁵²

CAD indicates coronary artery disease; ECs, endothelial cells; HFD, high fat diet; and SMCs, smooth muscle cells.

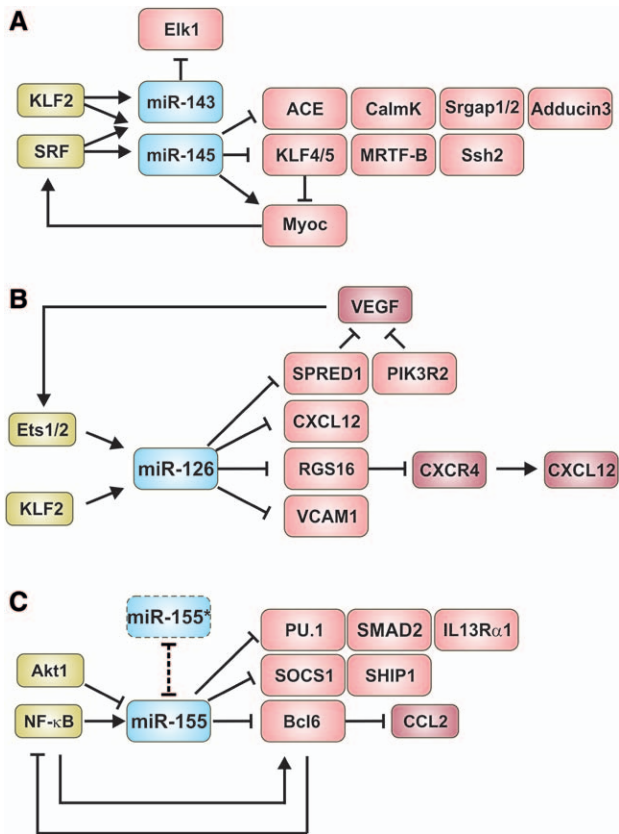


Figure 1. Regulatory networks of miRNAs in atherosclerosis. **A**, miR-145, assisted by the coexpressed miR-143, is part of a feed-forward loop including Kruppel-like factor (KLF)4/5, myocardin (Myoc), and serum response factor (SRF) that is crucial for the development of a smooth muscle cell (SMC) phenotype. Down-regulation of miR-145 promotes atherosclerosis, presumably by impairing SMC differentiation. **B**, Endothelial cell-specific miR-126 suppresses RGS16, an inhibitor of CXCR4 signaling, which upregulates CXCL12 expression and mediates protection against atherosclerosis. In addition, targeting of vascular cell adhesion molecule-1 by miR-126 may limit atherogenic leukocyte recruitment. Enhanced activation of vascular endothelial growth factor (VEGF) signaling by suppression of SPRED-1 and PIK3R2 supports angiogenesis. **C**, In macrophages, miR-155 is upregulated after inflammatory stimulation and facilitates polarization into M1-type macrophages by targeting antiinflammatory transcription factors, such as SOCS1 and Bcl6. Suppression of Bcl6 by miR-155 enhances atherogenesis presumably through increased expression of CCL2. The role of the passenger strand, miR-155*, which is also upregulated in inflammatory macrophages, remains to be determined. ACE indicates angiotensin converting enzyme.

exacerbate vascular repair. Furthermore, aged miR-143/145^{-/-} mice develop spontaneous atherosclerotic lesions in the femoral artery even in the absence of hypercholesterolemia, which is associated with increased expression of the miR-143/145 target angiotensin I converting enzyme (Table).¹⁴ Conversely, overexpression of miR-145 in SMCs reduces atherosclerosis in Apoe^{-/-} mice and increases the lesional SMC and collagen content.²¹ Surprisingly, shear stress upregulates miR-143/145 in ECs and triggers the release of EC-derived microvesicles that are highly enriched in miR-143/145, both via KLF2.²² These EC microvesicles transfer miR-143/145 to SMCs and treatment with microvesicles derived from KLF2-overexpressing ECs reduces atherosclerosis in a miR-143/145-dependent manner (Figure 2).²²

Despite the identification of several mRNA targets of miR-145 in vitro, the detailed mechanisms by which the effects of miR-145 in atherosclerosis are mediated remain unclear. It may be hypothesized from in vivo studies that promotion of a SMC phenotype by miR-145 is atheroprotective.²³

miR-126 Plays an Antiatherogenic Role by Enhancing Endothelial Repair

The endothelial counterpart of miR-143/145, miR-126, is the most abundantly found miRNA during EC differentiation and in adult ECs.²⁴ In contrast to many other miRNAs, the passenger strand of premiR-126 is not completely degraded, meaning the guide strand, miR-126, and miR-126* are both highly expressed.²⁴ The *miR-126* gene is coexpressed with its host gene *Egfl7*, which plays a role in angiogenesis.²⁵ KLF2 transcriptionally regulates miR-126 expression during development, whereas KLF2 induced by shear stress in adult ECs does not increase miR-126 levels.^{22,26} The absence of miR-126 during development increases vascular permeability and leakage, which is mainly due to upregulation of the miR-126 targets SPRED-1 and PIK3R2, which are both inhibitors of vascular endothelial growth factor signaling (Figure 1B).^{24,27} Accordingly, angiogenesis after myocardial infarction is severely impaired in miR-126^{-/-} mice.²⁷ The proangiogenic activity of CD34⁺ mononuclear cells is mainly mediated by the secretion of miR-126 in microvesicles and exosomes, which may play a role in angiogenesis after myocardial infarction (Figure 2).²⁸ However, miR-126 overexpression can reduce atherosclerosis, indicating an alternative target of miR-126 considering the proatherogenic role of vascular endothelial growth factor.²⁹⁻³¹ In this respect, miR-126 mediates upregulation of CXCL12 by apoptotic bodies derived from ECs.³⁰ Targeting of RGS16, an inhibitor of G-protein coupled receptor signaling, by miR-126 promotes a positive feedback loop in which increased activation of CXCR4 in the absence of RGS16 induces CXCL12 expression (Figure 1B).³⁰ Accordingly, the atheroprotective effect of apoptotic bodies derived from ECs is mediated by increased lesional CXCL12 expression, which induces the recruitment of progenitor cells to the endothelial lining (Table).³⁰ Interestingly, miR-126 can also directly target CXCL12 in 3'UTR reporter assays, which seems, at least in atherogenesis, not to be biologically relevant.³⁰ Although miR-126 is not regulated by inflammatory stimulation, it modestly suppresses endothelial vascular cell adhesion molecule-1 expression and leukocyte adhesion by activated ECs, which may contribute to its atheroprotective role (Figure 2).³² Expression of miR-126 is not significantly altered in human atherosclerotic lesions, whereas miR-126* seems to be downregulated in lesions of the carotid artery (Table).²⁰ However, circulating levels of miR-126 are substantially reduced in patients with coronary artery disease or insulin resistance/diabetes mellitus, which is probably due to defective packaging of miR-126 into endothelial microvesicles.^{33,34}

Thus, increased EC apoptosis during atherosclerosis triggers endothelial regeneration because of the release of miR-126 in apoptotic bodies, which highlights the importance of endothelial injury in atherogenesis.

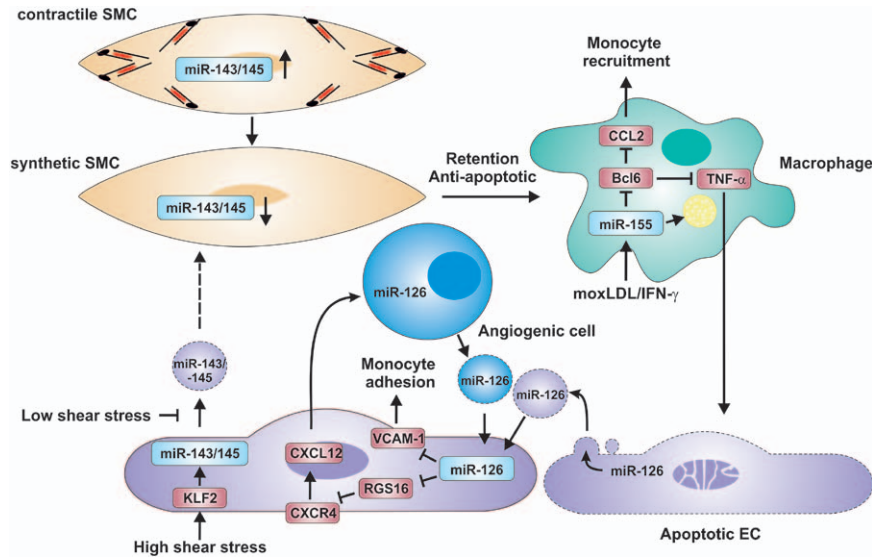


Figure 2. miRNA-mediated interplay of different cell types in atherosclerosis. Apoptotic endothelial cells (ECs) release apoptotic bodies enriched in miR-126, which is transferred to ECs and induces upregulation of CXCL12 via inhibition of RGS16. This indirect induction of CXCL12 reduces atherosclerosis by recruiting proangiogenic cells. In addition, angiogenic peripheral blood mononuclear cells release miR-126-containing microvesicles, which may further enhance the CXCL12-mediated recruitment of angiogenic cells. High shear stress induces the secretion of miR-143/-145-enriched microvesicles from ECs, which protects against atherosclerosis through transfer to smooth muscle cells (SMCs). Downregulation of miR-143/-145 in SMCs results in dedifferentiation of SMCs and promotes lesion formation. miR-155 is upregulated in inflammatory macrophages of atherosclerotic lesions and enhances atherogenesis by facilitating cytokine expression. KLF indicates Kruppel-like factor; and TNF, tumor necrosis factor.

Opposite Effects of miR-155 on Atherogenic Macrophage Function

Macrophages respond to various inflammatory stimuli by differential regulation of a small set of miRNAs.³⁵ The most characteristic feature of the miRNA expression signature in lipopolysaccharide-stimulated macrophages is the upregulation of miR-155 and its passenger strand miR-155*, presumably through binding of nuclear factor- κ B to the promoter of the miR-155 host gene *BIC*.^{35–39} Moreover, Akt1 (a serine-threonine protein kinase) inhibits macrophage polarization into a proinflammatory phenotype by damping the upregulation of miR-155.^{40,41} miR-155 is upregulated in atherosclerotic lesions of humans and mice, and miR-155 expression is localized to lesional macrophages and SMCs (Table).^{20,36} Results of in vivo studies investigating the effect of miR-155 on atherosclerosis are conflicting. Although LDL-R^{-/-} mice harboring miR-155^{-/-} bone marrow (BM) cells on a high-cholesterol diet for 10 weeks develop slightly more atherosclerosis, lesions induced by acutely disturbed flow and hypercholesterolemia are markedly reduced in Apoe^{-/-} mice harboring miR-155^{-/-} BM cells (Table).^{36,42} In contrast to LDL-R^{-/-} mice harboring miR-155^{-/-} BM cells, the lesional macrophage content is substantially reduced in disturbed flow-induced lesions of Apoe^{-/-} mice in the absence of miR-155 expression in BM cells. In general, LDL-R^{-/-} mice develop smaller and less advanced lesions than Apoe^{-/-} mice on a high-cholesterol diet.⁴³ Moreover, acutely disturbed flow in the carotid artery of hypercholesterolemic Apoe^{-/-} mice induces advanced atherosclerotic lesions and arterial stenosis.³⁶ Therefore, miR-155 may have opposite effects on lesion formation depending on the stage of atherosclerosis. miR-155 promotes disturbed flow-induced atherosclerosis by enhancing the inflammatory macrophage response, such as the expression of CCL2.³⁶

Although miR-155 targets several mRNAs in macrophages, such as SOCS1, SHIP1, IL13R α 1, and SMAD2, suppression of Bcl6, which is a nuclear factor- κ B antagonist, mediates the proinflammatory effects of miR-155 (Figure 1C).^{36,44–46} Accordingly, silencing of Bcl6 in atherosclerotic lesions prevents the beneficial effect of genetic miR-155 deficiency in BM cells, clearly demonstrating the functional role of the miR-155 target Bcl6 in atherosclerosis (Figure 2).³⁶ These findings emphasize the need to validate experimentally the functional role of even verified miRNA targets in separate disease models. Although the atheroprotective mechanisms of miR-155 in hematopoietic cells are unclear, increased lesional neutrophil accumulation and a reduction of regulatory T cells in circulation, 2 conditions which are associated with enhanced development of atherosclerosis,^{47,48} in LDL-R^{-/-} mice harboring miR-155^{-/-} BM cells suggests that miR-155 may affect these leukocyte types during atherosclerosis.⁴² Notably, miR-155 can promote the differentiation of regulatory T cells by targeting SOCS1.⁴⁹ Considering the upregulation of miR-155* in activated macrophages, a role of miR-155* in early atherosclerosis that opposes the effects of its sister strand, miR-155, as has been observed in dendritic cells,⁵⁰ may be hypothesized.

In ECs, miR-155 is upregulated by high shear stress and targets the angiotensin II type 1 receptor and Ets-1, which reduces the proinflammatory activity of angiotensin II.^{51–53} Moreover, suppression of angiotensin II type 1 receptor by miR-155 in SMCs may also ameliorate the effects of angiotensin II on vascular remodeling.⁵³ However, expression of miR-155 in vascular cells, such as SMCs, seems not to be involved in atherosclerosis, because lesion formation is not significantly altered in miR-155^{-/-}/Apoe^{-/-} mice harboring miR-155^{+/+} BM cells.³⁶

These results support the concept of a proatherogenic role of proinflammatory M1-type macrophages in advanced atherosclerosis driven by miR-155. Although the mechanism by which the beneficial effects of miR-155 are mediated remains to be identified, it may be due to its effects on leukocyte types other than macrophages.

miR-126, -145, and -155: The Clinical Perspective

Although the important roles of miR-126, -145, and -155 in atherosclerosis are clearly established, the implications in the clinical practice of the detection and treatment of cardiovascular diseases remain unclear. Patients with coronary artery disease have reduced levels of miR-126, -145, and -155 in the circulation.³³ Moreover, decreased levels of miR-126 in patients with diabetes mellitus or insulin resistance have been reported.³⁴ These studies indicate that circulating levels of miR-126, -145, and -155 may indicate the presence or absence of atherosclerosis or endothelial dysfunction and thus may play a role as novel biomarkers.⁵⁴ Although technically demanding, circulating miRNAs can be quantified with a sensitivity and specificity. However, technical standardization regarding the preparation of the samples and data analysis would improve the evaluation of these miRNAs in the prediction of cardiovascular risk.⁵⁵

A therapeutic strategy based on the functional role of miR-126, -145, and -155 in atherosclerosis would need to increase the levels of miR-126 and miR-145, and to inhibit miR-155 in macrophages at least in advanced stages of atherosclerosis. Complementarity-based inhibition of miRNAs by synthetic anti-miRs is a promising therapeutic tool due to the enhanced stability and sustained effects after intravenous injection in the arterial wall.⁵⁶ As a representative of this potentially new class of drugs, miravirsin, an anti-miR against miR-122, has been successfully tested in a Phase 2a study in patients with hepatitis C virus infection.^{57,58} However, the long-term treatment effects and safety of anti-miR-based therapeutics remains unclear. In contrast, the replacement of downregulated miRNAs or the increase of miRNA expression is therapeutically much more challenging and faces basically the same limitations as siRNA administration, such as the rapid degradation and limited duration of the biological effect. Therefore, miRNAs mimics need to be packaged in liposomes or nanoparticles for therapeutic delivery.⁵⁹ Alternatively, the endogenous packaging of miR-126 in apoptotic bodies and of miR-143/145 in shear stress-induced microvesicles may provide a template for bioengineered vesicles for the therapeutic delivery of miRNAs.^{22,30} Accordingly, treatment with KLF2-induced endothelial microvesicles containing miR-143/145 effectively reduced atherosclerosis.²²

Summary

Although many miRNAs regulate atherosclerosis-related processes *in vitro*, only the roles of miR-145, -126, and -155 in atherosclerosis have been studied so far. Downregulation of miR-145 in SMCs promotes lesion formation, whereas upregulation of miR-155 in macrophages ameliorates advanced atherosclerosis by suppressing Bcl6. By contrast, release of miR-126, which is not differentially expressed in

atherosclerotic lesions, from apoptotic ECs targets RGS16 and thus reduces lesion formation, presumably by enhanced CXCL12-mediated endothelial regeneration.

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Disclosures

None.

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