



REVIEW ARTICLE

Pathogenic role of microRNAs in atherosclerotic ischemic stroke: Implications for diagnosis and therapy

Qidi Jiang ^{a,b,1}, Yiran Li ^{a,b,1}, Quanli Wu ^d, Li Huang ^c,
Jiasheng Xu ^c, Qingfu Zeng ^{c,*}

^a Department of Biomedicine, Queen Mary University of London, Nanchang, Jiangxi 330006, PR China

^b Department of Clinical Laboratory, The Second Affiliated Hospital of Nanchang University, Nanchang, Jiangxi 330006, PR China

^c Department of Vascular Surgery, The Second Affiliated Hospital of Nanchang University, Nanchang, Jiangxi 330006, PR China

^d School of Public Health, Nanchang University, Nanchang, Jiangxi 330001, PR China

Received 26 March 2020; received in revised form 16 December 2020; accepted 4 January 2021

Available online 12 January 2021

KEYWORDS

Atherosclerotic
ischemic stroke;
ECs;
Macrophages;
miRNA;
Plaque stability;
VSMCs

Abstract Ischemic stroke resulting from atherosclerosis (particularly in the carotid artery) is one of the major subtypes of stroke and has a high incidence of death. Disordered lipid homeostasis, lipid deposition, local macrophage infiltration, smooth muscle cell proliferation, and plaque rupture are the main pathological processes of atherosclerotic ischemic stroke. Hepatocytes, macrophages, endothelial cells and vascular smooth muscle cells are the main cell types participating in these processes. By inhibiting the expression of the target genes in these cells, microRNAs play a key role in regulating lipid disorders and atherosclerotic ischemic stroke. In this article, we listed the microRNAs implicated in the pathology of atherosclerotic ischemic stroke and aimed to explain their pro- or antiatherosclerotic roles. Our article provides an update on the potential diagnostic use of miRNAs for detecting growing plaques and impending clinical events. Finally, we provide a perspective on the therapeutic use of local microRNA delivery and discuss the challenges for this potential therapy.

Copyright © 2021, Chongqing Medical University. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

* Corresponding author. No. 1 Minde Road, Nanchang, Jiangxi 330006, PR China.

E-mail address: qingfuzengedu@163.com (Q. Zeng).

Peer review under responsibility of Chongqing Medical University.

¹ Co-first authors.

Introduction

Stroke is the second leading cause of death worldwide and is a main cause of permanent disability in adults.¹ When the brain is deprived of oxygen and nutrients either because of artery occlusion or rupture, the neurons near the injured area die, usually leading to irreversible damage, such as disability. According to a report by the World Health Organization,² stroke leads to over 5 million deaths and over 40 million disabilities every year. In the United States, ischemic strokes are responsible for approximately 87% of all stroke cases, and the remaining cases are hemorrhagic strokes or result from unknown causes.³ In China, ischemic strokes are responsible to approximately 70% of all stroke cases. Men are significantly more vulnerable to this disease than women.⁴ Cerebral ischemic stroke is an occurrence of neurological injury caused by a lack of cerebral blood flow.⁵ As the brain is the largest consumer of oxygen and glucose in the body, the subsequent appearance of dead neural tissue may occur very quickly, usually in a few seconds.⁶ The death of tissue in different areas of the brain may lead to disability of diverse degrees after stroke or even to death of the sufferers.

Atherosclerosis, hyperlipidemia, or plaque rupture all result in focal ischemia.⁷ Intravascular atheroma is a pathologically deleterious tissue composed of fat, collagen, elastin, macrophages and a thin layer of smooth muscle cells wrapping around the contents. This atheromatous plaque is fragile and easily ruptures or falls off the vascular intima under shear stress caused by the bloodstream and blocks blood flow. Even if the plaque is stably adhered to the intima, it continues to grow (particularly in patients consuming a high-fat diet), and over time, the vessels become narrower and the blood flow decreases. Insufficient oxygen and nutrient delivery are unable to satisfy the huge demand and support the normal function of healthy neural tissues, which leads to the production of reactive oxygen species (ROS). In addition, wastes (e.g., CO₂ and urea) generated by cellular activities are not removed by the plasma in a timely manner; therefore, neuronal cells initiate the inflammatory response and ultimately undergo apoptosis.

The formation of atheromatous plaques involves a series of cellular and vascular events over a long period, usually several years or decades. Although plaques have been studied for many years, the exact mechanism of plaque formation remains unclear. One hypothesis is that some factor induces monocytes to begin to adhere to and enter the endothelium of vessels, leading to focal inflammation. The theory of lipid retention in the atheroprone areas of arteries that leads to inflammation of endothelial cells is also reasonable. Regardless of how atherosclerosis is initiated, this pathological event is mediated by maladaptive inflammation. Monocytes that migrate into the sub-endothelial space differentiate into macrophages and then proliferate and ingest invading oxidized low-density lipoprotein (oxLDL) from the bloodstream. At the same time, they are equipped with increasingly upregulated lipid uptake receptors to absorb lipids; ultimately, these macrophages become foam cells and die. From the intravascular side, these foam cells form a visible fatty streak covered by

a fibrous capsule derived from smooth muscle cells. These smooth muscle cells are originally located in the muscle layer of the vessel wall but migrate to the endothelial surface in response to cytokines secreted by foam cells and damaged endothelial cells. More monocytes are continuously recruited to the lesion, and the plaque continues to grow. Later, calcium, collagen and platelets deposit both under the endothelium and on the surface of the growing plaque. Smooth muscle cells also ingest cholesterol and eventually become foam cells. Together, these cells and structures form a fatty plaque, releasing enzymes that enlarge the artery. Once the expansion of the artery is unable to compensate for the narrowing lumen caused by the atheroma, the blood flow is reduced. However, rupture of the plaque more frequently leads to acute clotting and enlargement of the plaque, which causes a complete obstruction of blood flow and results in ischemia of the brain tissue.^{8–10}

MicroRNAs are 22-nucleotide small noncoding RNAs that exist in diverse animals, plants and viruses. They regulate the expression of target genes through complementary binding to messenger RNAs (mRNAs). MicroRNAs belong to the small noncoding RNA family and are not translated. They are originally double-stranded RNAs that are transcribed from target genes but then are cleaved into two single-stranded RNAs, miRNA-3p and miRNA-5p, by an endonuclease, DICER. Each single-stranded RNA is then incorporated into a ribonucleoprotein called the RNA-induced silencing complex (RISC), in which the RNA serves as a template. Once the miRNA template binds to the complementary binding sites in the 3' untranslated regions (3'UTR) of the free target mRNA, the RISC is activated, and the target mRNA is either degraded or its translation is slowed. By targeting functional genes or genes involved in regulating the expression of functional genes (such as intermediate regulators of signaling pathways), miRNAs confer direct loss-of-function or indirect gain-of-function effects on target genes. In the last 20 years, an increasing number of miRNAs has been shown to be associated with ischemic stroke. These miRNAs are involved in the mechanism of vessel occlusion, in the injury caused by ischemia, and in self-protection and repair after stroke. Among these functions, the role of miRNAs in the formation of atherosclerotic plaques leading to ischemic stroke is a topic of heated discussion. Therefore, in this article, we aimed to thoroughly review the role of microRNAs in the etiology of atherosclerotic ischemic stroke by focusing on 4 specific cell types: liver cells, macrophages, endothelial cells, and vascular smooth muscle cells. We summarized how miRNAs intervene in lipid delivery and deposition, local inflammation, fibrous cap stability, etc., which are all crucial processes involved in atheroma formation.

Various cell types contribute to the etiology of atherosclerosis

Liver cells play an important role in regulating lipoprotein homeostasis. They control both the production and elimination of lipoproteins, including delivering lipids to the tissue by low-density lipoprotein (LDL) and removing lipids

from the tissue by high-density lipoprotein (HDL). The disruption of this homeostasis (such as excessive production or insufficient retrieval of lipids) is one of the main factors inducing hypercholesterolemia, as the number of monocytes is increased 1.5 times and subsequently increases atherosclerosis.¹¹

Macrophages are the main inflammatory cells located in atherosclerotic lesions. Upon the initiation of inflammation, they are attracted to the lesion area and activated. Activated macrophages are either proinflammatory (M1 phenotype) or anti-inflammatory (M2 phenotype), depending on the different signals that initiate two opposite activation programs.¹² The M1 phenotype is induced by proinflammatory cytokines such as lipopolysaccharide (LPS), TNF- α and interferon (IFN)- γ produced by Th1 cells, whereas inducers of the M2 phenotype include IL-4, IL-10, IL-13 and tumor growth factor (TGF)- β produced by Th2 cells.¹³ Predominant M1 macrophages in the lesion are associated with atheroma progression, whereas M2 macrophages mediate the regression of atheroma. Regardless of the M1 or M2 phenotype, the accumulated macrophages (foam cells) are the dominant component of plaques and are associated with plaque stability.

Endothelial cells (ECs) function as a docking site for monocytes and play a pivotal role in promoting inflammation. Under hyperlipidemic conditions, ECs (particularly cells located in the predilection area of vessels that are exposed to disturbed blood flow) are prone to injury caused by oxidized LDL. Once insulted by leukocytes, TNF- α released from leukocytes activates signaling cascades that ultimately alter gene expression in endothelial cells.¹⁴ This change initiates inflammation, activates endothelial cells, increases permeability, and upregulates adhesion molecules on the cell surface, which allow leukocytes to adhere to the endothelium, enter the extracellular matrix under the endothelium and induce an inflammatory response.

Vascular smooth muscle cells (VSMCs) are derived from diverse cell types that contribute to atheroma formation, such as collagen-releasing cells and macrophage-like cells. VSMCs also form foam cells by expressing LDL receptors to endocytose lipids.¹⁵ In addition, VSMCs play an important role in inflammation by expressing adhesion molecules such as VCAM-1 and ICAM-1 that facilitate monocyte adhesion and migration.¹⁶ Throughout the progression of atherosclerosis, the proliferation of VSMCs is beneficial, whereas the apoptosis, senescence and differentiation of VSMCs into macrophage-like cells all contribute to atherosclerosis.¹⁷ In advanced lesions, proliferating VSMCs play an important role in protecting plaques from rupture because VSMCs are the main cell component of the fibrous cap, and a thicker fibrous cap indicates a more stable plaque.

MiRNAs play diverse roles in different cell types by inhibiting the expression of specific targets

MiRNAs regulate lipid homeostasis in liver cells

To date, a number of miRNAs have exhibited an ability to regulate lipid homeostasis in the liver. For example, miR-

27a/b comprehensively targets the genes involved in cholesterol esterification (acyl coenzyme a-cholesterol acyltransferase, ACAT-1), uptake (LDL and CD36), and efflux (ATP binding cassette transporter A1, ABCA1).¹⁸ A miRNA-122 deficiency results in suppressed expression of various genes encoding cholesterol synthesis-related proteins, such as HMGCR.¹⁹ Notably, miR-223 exerts its atheroprotective effect by suppressing foam cell formation, lipid deposition and proinflammatory cytokine generation. It targets cholesterol synthesis genes such as HMGCS1, SMO, and HDL uptake genes such as SRB1. SRB1 not only mediates the uptake of HDL into liver cells but also removes cholesterol from macrophages in the periphery,²⁰ indicating its key role in preventing atheroma formation. In addition to miR-223, other miRNAs, such as miR-96, miR-125a, miR-185, and miR-455, also target SRB1, but *in vivo* evidence is currently insufficient to confirm their roles in increasing plasma HDL levels (Fig. 1).

In addition to SRB1, the LDL receptor also controls plasma cholesterol levels through the endocytosis of cholesterol-rich LDL. The miRNAs targeting LDLR include miR-27a/b, miR-128-1, miR-130b, miR-148a, miR-185 and miR-301b, which are proatherosclerotic miRNAs because they increase plasma LDL levels. The inhibition of miR-128-1 and miR-148a upregulates LDLR in liver cells and reduces plasma lipid levels.^{21,22} The inhibition of miR-185 *in vivo* significantly increases LDLR expression and decreases the plaque area in mice.²³ Some of these miRNAs also have additional targets to inhibit atheroma formation. Pan S et al found that miR-130b also translationally repressed peroxisome proliferator-activated receptor γ (PPAR- γ).²⁴ As a multifunctional nuclear receptor, PPAR- γ is associated with adipocyte differentiation and inflammatory cytokine production from monocytes,²⁵ indicating that miR-130b possesses antiatherosclerotic properties. Pan S et al also found that miR-130b increased the level of miR-378a-3p; however, the latter is implicated in enhanced adipogenesis by antagonizing mitogen-activated protein kinase,²⁶ but the authors did not determine whether this property of miR-378a-3p has implications for atherosclerosis. Notably, miR-27b appears to have a controversial role in atherosclerosis. Although miR-27b inhibits LDLR and ABCA1, as mentioned above, scientists also found that it did not necessarily influence plasma cholesterol levels.²⁷ Further studies are needed to identify the explicit role of miR-27b in atherosclerosis.

Apolipoprotein B (apoB) is a protein that carries insoluble lipids in blood vessels and helps deliver lipids to tissues. It is produced in liver cells, wraps around lipids to form chylomicrons or LDLs and is then released into the bloodstream. Overexpression of miR-30c decreases the release of apoB lipoproteins from liver cells by inhibiting lysophosphatidylglycerol acyltransferase 1 (LPGAT1), which is involved in lipid biosynthesis.²⁸ Moreover, miR-30c also targets microsomal triglyceride transfer protein (MTP) involved in the assembly and secretion of VLDL, ultimately alleviating hypercholesterolemia and atherosclerosis.²⁸ Likewise, miR-122 decreases the production of very low-density lipoprotein by targeting MTP.²⁹ Importantly, miR-548 functions by targeting the apoB protein, HMGCR, and ACSL4, which are all involved in VLDL production.³⁰

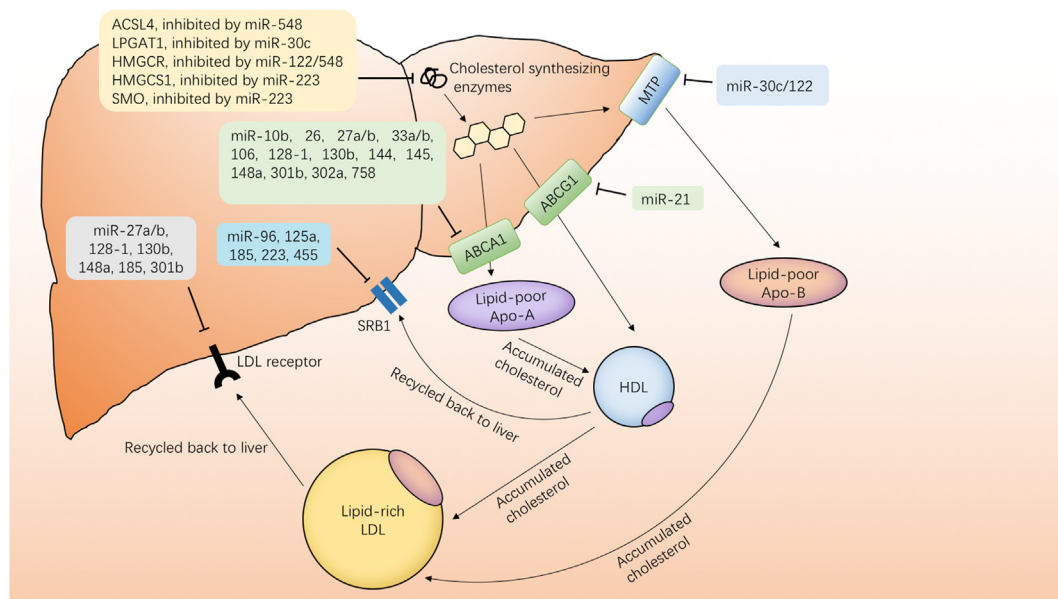


Figure 1 The important miRNAs in lipid metabolism in the liver. Note that the flat arrow indicates the inhibitory effect of miRNAs on the target. The primary mechanism by which miRNAs regulate lipid homeostasis is through targeting lipid transporters and lipid receptors and regulating lipid biosynthesis. Some lipid output processes in this figure are also applicable to macrophages because some transporters (such as ABCA1 and ABCG1) are commonly expressed in macrophages and liver cells. However, the transport of cholesterol from macrophages to the blood prevents hypercholesterolemia and atherosclerosis. Therefore, although the miRNAs targeting these transporters are expressed in both macrophages and liver cells, they exert different functions in inducing atherosclerosis. Abbreviations: ACSL4, acyl CoA synthetase long chain family member 4; LPGAT1, lysophosphatidylglycerol acyl-transferase 1; HMGCR, 3-hydroxy-3-methyl-glutaryl-CoA reductase; SMO, sterol-C4-methyl oxidase; HMGCS1, 3-hydroxy-3-methyl-glutaryl-CoA synthase 1; ABCA1, ATP binding cassette transporter A1; MTP, microsomal triglyceride transfer protein; SRB1, scavenger receptor class B type 1.

MiRNAs regulate macrophage behaviors in atherosclerosis

MiRNAs regulate the recruitment and activation of macrophages. Several miRNAs are involved in regulating the inflammatory response of macrophages to stimuli by enhancing or attenuating signaling pathways. For example, by regulating the intracellular Ca^{2+} -activated PKC-oxLDL-LOX-1 pathway, miR-let-7g attenuates macrophage recruitment and migration in the subendothelial region.³¹ By targeting programmed cell death 4 (PDCD4), which is implicated in the secretion of proinflammatory cytokines important for macrophage activation, such as tumor necrosis factor (TNF)- α and interleukin (IL)-6, miR-16 inhibits macrophage activation; miR-16 is also known to regulate proinflammatory MAPK and NF- κ B signaling.³² MiR-21 is a multifunctional atheroprotective miRNA involved in various aspects of atherosclerosis, such as vascular inflammation, foam cell formation, cell apoptosis and plaque necrosis. A miR-21 deficiency in macrophages increases the level of mitogen-activated protein kinase kinase 3 (MKK3), which activates p38/JNK/ERK signaling. This signaling pathway increases macrophage apoptosis and aggravates inflammation.³³ In addition to miR-21, miR-147 has also been shown to counteract macrophage apoptosis and attenuate inflammation (Fig. 2).³⁴

The NF- κ B signaling pathway is well known to regulate the inflammatory response in macrophages, endothelial

cells and vascular smooth muscle cells. Various proteins, such as TNF- α , IL- α and TLRs, activate NF- κ B signaling.³⁵ Upon ligand binding, PI3K and PKC are activated and then activate NADPH oxidase, which is responsible for producing ROS, an activator of I κ B kinase. Subsequently, activated I κ B, an inhibitor of NF- κ B signaling, is degraded, and the NF- κ B protein is released.³⁶ Activated NF- κ B enters the nucleus and induces the expression of numerous genes. These genes are involved in inducing the production of cytokines such as TNF- α and adhesion molecules such as E-/P-selectin, vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule (ICAM-1).³⁷ Cytokines amplify the inflammatory response, while adhesion molecules mediate chemoattraction between ECs and leukocytes. However, the outcomes of NF- κ B activation in different cells are diverse. For example, in endothelial cells, NF- κ B signaling promotes inflammation, while in macrophages, it alleviates inflammation.³⁷ One of the targets of NF- κ B is miR-146a that is involved in a feedback loop of the NF- κ B pathway in macrophages. During the last few decades, its roles have been extensively exploited and clearly illustrated by numerous scientists. In terms of the regulation of inflammation in macrophages, miR-146 binds to TNF receptor-associated factor 6 (TRAF6) and IL receptor-associated kinase 1 (IRAK1), which are both upstream adaptors of NF- κ B signaling, to reduce NF- κ B signaling and attenuate inflammation.³⁸ A miR-21 deficiency coincides with the activation of NF- κ B, indicating

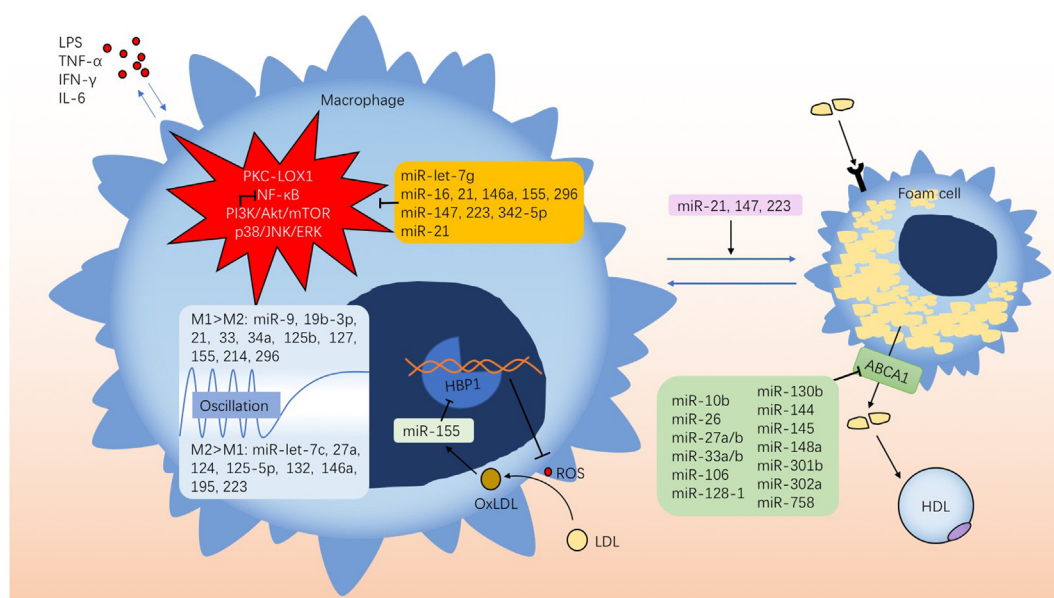


Figure 2 The miRNAs involved in biological processes that occur when macrophages transform into foam cells. Note that the flat arrow indicates the inhibitory effect of miRNAs on the target, while the normal arrow indicates the effect of miRNAs on increasing the expression of the target. The large cell on the left represents a macrophage, and the small cell on the right represents a foam cell. We chose the miRNAs associated with macrophage polarization, inflammation, foam cell transformation and lipid output and present these miRNAs in this figure. Once macrophages are exposed to proinflammatory signals, such as LPS, TNF- α , IFN- γ and IL-6, they experience substantial M1-M2 oscillations. MiR-9, 16b-3p, 21, 33, 34a, 125b, 127, 155, 214, and 296 drive macrophages toward the proinflammatory M1 phenotype, and miR-let-7c, 27a, 124, 125-5p, 132, 146a, 195, and 223 drive macrophages toward the anti-inflammatory M2 phenotype. The M1 macrophages induce inflammation, which is mediated by several signaling pathways, such as the PKC-LOX1, NF- κ B, PI3K/Akt/mTOR, and p38/JNK/ERK signaling pathways. These pathways are also targeted by distinct miRNAs. MiR-let-7g targets PKC-LOX1; miR-16, 21, 146a, 155, and 196 target NF- κ B; miR-147, 223, and 342-5p target PI3K/Akt/mTOR; and miR-21 targets p38/JNK/ERK. Note that PI3K/Akt/mTOR signaling inhibits NF- κ B signaling. At the same time, these activated macrophages produce more inflammatory cytokines and act on ECs, VSMCs, and themselves. This signaling undoubtedly forms a positive feedback loop that aggravates inflammation. ROS are also inflammatory factors, but their upregulation does not propagate inflammation; instead, ROS participate in a negative feedback loop. ROS are responsible for transforming LDL to oxLDL, and the latter upregulates miR-155. MiR-155 suppresses HBP1, a transcriptional repressor, which ultimately inhibits ROS production. Disordered lipid metabolism and inflammation gradually drive macrophages to transform into foam cells. MiR-21, 147, and 223 may facilitate and accelerate this process. ABCA1 is probably the most important transmembrane lipid pump expressed in macrophages. A list of miRNAs that inhibit its expression is shown in the figure. Abbreviations: NF- κ B, nuclear factor- κ light chain enhancer of activated B cells; HBP1, HMG box-transcriptional protein 1.

that miR-21 negatively regulates inflammation in macrophages.³⁹ In contrast, miR-296 targets Numb 1, which inhibits NF- κ B, thereby enhancing inflammation.⁴⁰

Another predominant miRNA that regulates atherosclerosis, miRNA-155, is also a downstream target of NF- κ B signaling. However, the role of miR-155 in atherosclerosis is still unclear. Some researchers found that miR-155 promotes atherosclerosis, while others reported conflicting results. For example, Nazari-Jahantigh M et al argued that miR-155 targets B-cell lymphoma 6 (Bcl6), a transcription factor that suppresses NF- κ B signaling.⁴¹ Therefore, miR-155 appears to promote NF- κ B signaling through a positive feedback mechanism. Additionally, miR-155 knockout in mice decreases the expression of CCL2, which is important in recruiting monocytes.⁴¹ Moreover, miR-155 suppresses the expression of negative regulators of inflammatory mediators, such as suppressor of cytokine signaling (SOCS1, which is also the target of several other miRNAs mentioned later) and Src homology 2 domain-containing inositol-5-phosphatase-1 (SHIP1).^{42,43} This

evidence suggests that miR-155 plays a crucial role in local inflammation. However, in other studies, completely opposite results were obtained. For example, Huang RS et al showed that miR-155 inhibition promoted NF- κ B activation and the release of several proinflammatory cytokines.⁴⁴ Moreover, Li X et al suggested that miR-155 alleviates inflammation by repressing calcium-regulated heat stable protein 1 (CARHSP1) expression.⁴⁵ Obviously, the exact role of miR-155 is still a topic of heated discussion, and more evidence is needed to verify its function.

PI3K/Akt/mTOR signaling is another well-known proinflammatory pathway that has been proven to be a negative regulator of TLR4/NF- κ B signaling.⁴⁶ Likewise, this pathway is targeted by a few miRNAs, such as miR-147 and miR-223.^{47–49} The expression of miR-223 is predominantly upregulated in the lesion area, and this overexpression is associated with decreased foam cell formation, lipid accumulation and proinflammatory cytokine production, indicating that miR-223 reduces atherosclerosis through

many pathways.⁴⁹ The mechanism underlying these functions may be that miR-223 activates the PI3K/AKT pathway, hence attenuating inflammation.⁴⁹ Notably, miR-342-5p is a prominent enhancer of inflammation in macrophages that targets Akt1. Akt1 suppresses miR-155 expression, and the expression of miR-155 is associated with nitric oxide synthase 2 (Nos2) and IL-6 induction (both are proinflammatory cytokines of macrophages). During early atherosclerosis, miR-342-5p is upregulated and targets Akt1, resulting in increased Nos2 and IL-6 production.⁵⁰

Another mechanism by which miRNAs regulate macrophage behavior in the pathogenesis of atherosclerosis is by modulating macrophage polarization. Various miRNAs are implicated in regulating the balance between M1 and M2 macrophages, including miR-let7c, miR-9, miR-19, miR-21, miR-27a, miR-33, miR-34a, miR-124, miR-125a-5p, miR-125b, miR-127, miR-132, miR-146a, miR-155, miR-195, miR-214, miR-223, and miR-296. Among these miRNAs, some have M2-promoting and M1-preventing functions. For example, miR-let7c is believed to maintain M2 traits, and overexpression of miR-let7c in M1 macrophages favors the loss of M1 traits.⁵¹ Additionally, miR-27 promotes the M2 gene expression program and stimulates the secretion of IL-10, a well-known inflammation inhibitor.⁵² By targeting the C/EBP- α -PU.1 pathway, miR-124 favors the M2 phenotype.⁵³ Moreover, miR-125a-5p also favors M2 polarization and inhibits the M1 phenotype, probably by targeting Krueppel-like factor 13 (KLF13).⁵⁴ MiR-195 significantly reduces the production of cytokines needed for M1 polarization and impairs smooth muscle cell migration; therefore, it is an atheroprotective miRNA.⁵⁵ IL-4 stimulates the expression of miR-223, whereas LPS exposure reduces miR-223 expression. A deficiency in miR-223 leads to a phenotype that is more similar to M1 macrophages, probably through targeting Pbx/knotted 1 homeobox (Pknx1), but more evidence is needed to prove the role of Pknx1 in M1 polarization.⁵⁶

Other miRNAs promote the M1 gene expression program. For example, peroxisome proliferator-activated receptor δ (PPAR δ) is suggested to be involved in the M1 proinflammatory program, which coincides with the upregulation of miR-9.⁵⁷ SOCS1 is a target of miR-19b-3p, and the inhibition of SOCS1 leads to the M1 phenotype.⁵⁸ MiR-21 upregulates TNF- α and IL-6 and downregulates IL-10, facilitating M1 polarization.⁵⁹ MiR-33 drives macrophages toward the M1 phenotype by promoting aerobic glycolysis, which provides energy to M1 macrophages. In addition, miR-33 suppresses lipid oxidation, which fuels M2 macrophages.⁶⁰ MiR-34a is upregulated in response to TNF- α and is associated with the M1 phenotype.⁵⁶ MiR-125b targets interferon regulatory factor 4, thus enhancing the response of activated M1 macrophages to IFN- γ .⁶¹ MiR-155 not only promotes the M1 phenotype but also represses the M2 phenotype. By targeting SOCS1, miR-155 increases proinflammatory IFN signaling by releasing the inhibition of its adaptor STAT1 and simultaneously decreasing the level of IL-13 that drives the M2 phenotype.⁶² Similarly, miR-296 targets STAT5A, which is responsible for inducing SOCS-2, a negative regulator of M1, thereby inducing the M1 phenotype.⁴⁰ MiR-214 is a target of NF- κ B that is implicated in increasing TNF- α and IL-6 production, thus

playing an important role in the positive feedback loop that amplifies M1 signals.⁶³

Several miRNAs also regulate macrophage lipid deposition and control the foam cell phenotype transition by targeting genes involved in lipid deposition in macrophages. For example, ABCA1, ABCB11, and ATP8B1 are transmembrane cholesterol pumps expressed on both liver cells and macrophages. They transport cholesterol from the cytosol to apolipoprotein A-I (apoA-I), the main carrier of lipids in the circulatory system. Since ABCA1 is located on both liver cells and macrophages, an ABCA1 deficiency might lead to different atherosclerotic outcomes in different cells; in macrophages, a deficiency in this protein remarkably increases the risk of atherosclerosis, whereas in the liver, this deficiency alleviates hyperlipidemia and atherosclerosis. A growing number of miRNAs have been shown to target ABCA1, including miR-10b, miR-26, miR-27a/b, miR-33a/b, miR-106, miR-128-1, miR-130b, miR-144, miR-143/145, miR-148a, miR-301b, miR-302a and miR-758.^{18,21,37,64–67} A decrease in the level of ABCA1 on the cell membrane of macrophages traps the absorbed lipids in macrophages and eventually drives these macrophages to transform into foam cells, leading to the growth of atherosclerotic lesions. Notably, some miRNAs, such as miR-27a/b, miR-128-1, miR-148a, and miR-223, regulate both lipid uptake and lipid output, as mentioned above, which requires further investigation of the exact pro- or anti-atherosclerotic outcome. In addition to ABCA1, ABCG1 is also a critical transporter involved in lipid efflux in macrophages that is increasingly degraded in cells lacking miR-21.³³

As discussed above, miR-155 itself in fact plays a controversial role in atherosclerosis. One line of evidence supporting the antiatherosclerotic role of miR-155 is that a miR-155 deficiency promotes lipid deposition in macrophages.⁶⁸ This finding may be explained by miR-155-mediated inhibition of HMG-box transcription factor 1 (HBP1), a transcriptional repressor known to repress macrophage inhibitory factor (MIF) and p47^{phox} expression,^{69,70} consequently decreasing oxLDL uptake and ROS production in macrophages.⁷¹ As stated above, further studies are needed to elucidate the role of miR-155 in atherosclerosis. However, not all miRNAs exacerbate lipid deposition. For example, both miR-21 and miR-147 counteract the foam cell phenotypic transition.³⁴ Moreover, miR-223 overexpression is associated with the amelioration of lipid deposition by targeting TLR4.⁴⁹

MiRNAs regulate EC behaviors in atherosclerosis

The primary mechanism by which miRNAs control atherosclerosis progression is to regulate the inflammatory process of ECs. MiR-126 is one of the most dominant miRNAs expressed in ECs and has long been a research hotspot. In the past few years, scientists found that miR-126 was upregulated concomitantly with the blockade of PI3K/Akt/NF- κ B signaling.⁷² In addition, miR-126 is suggested to reduce the production of ROS and the expression of TNF- α by targeting TRAF7. TRAF7 is responsible for binding to the TNF receptor and inducing ROS production; therefore, TRAF7 inhibition protects ECs from oxidative stress and

subsequent inflammation and apoptosis.⁷³ Similarly, miR-10a significantly inhibits inflammation in ECs. Knock out of miR-10a significantly increases the phosphorylation of I κ B α and aggravates inflammation. In addition, a miR-10a deficiency leads to upregulated expression of monocyte chemoattractant protein (MCP)-1, IL-6, IL-8, VCAM-1 and E-selectin. Moreover, miR-10a suppresses the expression of two promoters of I κ B α degradation, mitogen-activated kinase kinase kinase 7 (MAP3K7) and β -transducin repeat-containing gene (β TRC).⁷⁴ Notably, miR-92a attenuates endothelial inflammation by targeting KLF2/4 and SOCS5 (Fig. 3).⁷⁵

In addition to its role in macrophages, miR-146 also functions as a potential inhibitor of EC activation and inflammation. We have mentioned several times that miR-146a represses NF- κ B signaling pathways. The inhibition of HuR, a mediator of NF- κ B signaling, by miR-146 may also support the atheroprotective role of miR-146. Additionally, miR-146 suppresses both mitogen-activated protein kinase (MAPK) and the JNK/AP-1 pathway.⁷⁶ In contrast to its obscure role in macrophages, miR-155 produced by ECs clearly exerts a protective effect. In ECs, myosin light chain kinase mediates the opening of cell–cell junctions between ECs and increases the permeability of ECs in response to proinflammatory factors, which incapacitates the endothelial barrier and promotes monocyte migration. However, a deficiency in myosin light chain kinase caused by miR-155

targeting reduces atherosclerosis, proving the atheroprotective role of miR-155 in mice.⁷⁷

Based on considerable evidence, miR-181 is another predominant atheroprotective miRNA. In mice, miR-181b inhibits NF- κ B signaling and reduces atheromas; *in vitro*, various proinflammatory factors reduce miR-181b expression in endothelial cells. Generally, both miR-181-5p and miR-181-3p reduce the expression of genes involved in inflammation, such as adhesion molecules and inhibitors of the inflammatory signaling pathway. They also suppress the recruitment of macrophages into lesions. Previous studies have suggested that miR-181b inhibits importin- α , which is important in the translocation of the transcription factor NF- κ B (whereas overexpression of miR-181b in leukocytes has no effect on NF- κ B signaling).⁷⁸ However, a recent study by another group showed that miR-181-5p and miR-181-3p cooperatively inhibit NF- κ B signaling by binding to TGF- β -activated kinase 1-binding protein (TAB2) and NF- κ B essential modulator (NEMO), respectively.⁷⁹

VCAM, ICAM and E-selectin are all adhesion molecules mediating adhesion between leukocytes and endothelial cells. Their expression in ECs and monocytes is strictly controlled by multiple miRNAs. For example, miR-17-3p and miR-31 suppress ICAM-1 and E-selectin expression, respectively, miR-126-3p inhibits VCAM-1 expression, and miR-221/222 target ICAM-1, VCAM-1 and monocyte chemotactic protein 1 (MCP-1).^{80–82} Moreover, miR-296 targets ICAM-1

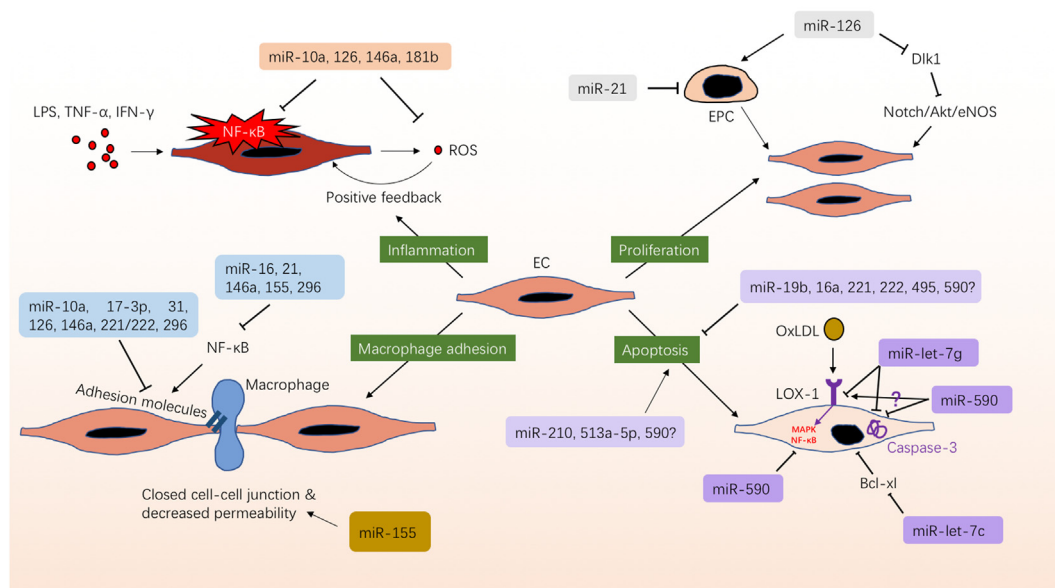


Figure 3 The role of miRNAs in regulating EC activities involved in initiating atherosclerosis, including inflammation, macrophage adhesion, proliferation and apoptosis. Proinflammatory cytokines activate ECs and induce the production of ROS by ECs, forming a positive feedback loop that amplifies inflammation. This process is targeted by miR-10a, 126, 146a and 181b. Macrophage–EC adhesion requires the expression of adhesion molecules on the surface of ECs and macrophages and requires separated ECs to allow macrophages to enter the intima. Many adhesion molecules are targets of NF- κ B signaling, and their expression is either directly or indirectly regulated by miRNAs. EC proliferation is regarded as a protective activity that restores an integral endothelium and prevents atherosclerosis. MiR-21 inhibits this process by inhibiting the proliferation of EPCs, while miR-126 promotes EC proliferation by activating Notch/Akt/eNOS signaling. EC apoptosis is a proatherosclerotic process that exacerbates inflammation. MiRNAs regulate EC apoptosis by targeting the receptor LOX-1, downstream MAPK/NF- κ B signaling, the antiapoptotic factor Bcl, and the protease Caspase-3.

and CX3C chemokine receptor 1 (CX3CR1) and subsequently suppresses monocyte adhesion.⁴⁰ KLF2/4 are transcription factors induced by ECs under high shear stress. Both of these proteins help maintain tight intercellular junctions, reduce permeability and attenuate inflammation in ECs. However, miR-103, the direct suppressor of KLF2/4 expression, enhances monocyte adhesion to ECs.⁸³ CXCL1 ligand 1 (CXCL1) is one of the chemokines required for macrophage adhesion that is secreted by ECs upon activation, and miR-103 increases CXCL1 expression and aggravates atherosclerosis.⁸³ MiR-146 targets HuR, which inhibits endothelial nitric oxide synthase (eNOS) responsible for producing NO that prevents leukocyte-EC adhesion.⁷⁶ In addition, miR-146 indirectly inhibits the expression of adhesion molecules by inhibiting NF- κ B signaling.⁷⁶ Notably, miR-146 itself potently and directly decreases adhesion molecule levels.⁷⁶ Since both E-selectin and VCAM-1 are target genes of the transcription factor NF- κ B, miRNAs that inhibit the nuclear translocation of NF- κ B or target other adaptor proteins in the NF- κ B signaling pathway all prevent monocytes from adhering to ECs.

The proliferation of ECs is one of the most crucial processes in the repair of vascular injury and ameliorating the progression of atherosclerosis. To date, an increasing number of miRNAs have been shown to regulate EC proliferation, and miR-126 is the most frequently discussed miRNA. Indeed, hyperlipidemic conditions impair the proliferation of ECs and the resulting insufficient endothelial repair exacerbate atherosclerosis. Fortunately, miR-126-5p counteracts this process and promotes EC proliferation by suppressing delta-like 1 (Dlk1), as suggested by Schober A et al.⁸⁴ Dlk1 inhibits Notch/Akt/eNOS signaling associated with EC proliferation. This finding explains the role of miR-126-5p in maintaining vascular homeostasis and protecting vessels from atherosclerosis. Schober A et al also showed that miR-126-5p knockout mice exhibit impaired endothelial repair, further proving the antiatherosclerotic effect of miR-126-5p.⁸⁴ Additionally, increased miR-126 expression activates endothelial progenitor cells (EPCs), which are derived from mature ECs and contribute to endothelial repair and proliferation.⁸⁵ EPCs are the main cellular source of EC regeneration and vessel recovery. The TGF- β /BMP signaling pathway plays a vital role in the differentiation of EPCs but suppresses their proliferation. Importantly, miR-21 downregulates WW domain-containing protein 1 (WWP1) and activates the TGF- β /BMP signaling pathway, ultimately inhibiting EPC proliferation and contributing to the initiation and progression of atherosclerosis.⁸⁶

The accumulation of oxLDL in endothelial cells drives their apoptosis through diverse mechanisms involving multiple signaling pathways. Apoptotic ECs are new proinflammatory stimuli for the endothelium that exacerbate endothelial injury. OxLDL-induced EC apoptosis also contributes to plaque rupture and coagulation, accelerating the progression of atherosclerosis and leading to ischemic stroke. The receptor for oxLDL on ECs is lectin-like LDL receptor-1 (LOX-1). Once bound to oxLDL, LOX-1 transduces signals to downstream mediators, including MAPK and NF- κ B, leading to endothelial activation, dysfunction and ultimately apoptosis. LOX-1 deficiency has been implicated in decreased atherosclerosis.⁸⁷ Several miRNAs have been found to regulate the expression of LOX-1. For example,

miR-let-7g targets LOX-1 and caspase-3 (a key protease involved in apoptosis that initiates the cleavage and activation of many other proteases) and inhibits EC apoptosis.^{88,89}

In addition to LOX-1, other molecules important for modulating EC apoptosis are also potential targets of miRNAs. For example, miR-let-7c is suggested to enhance apoptosis in ECs by inhibiting the expression of Bcl-xL.⁹⁰ By targeting 3'-phosphoinositide-dependent kinase-1 (PDK1), miR-210 also enhances EC apoptosis.⁹¹ TNF- α -induced EC apoptosis is initiated by miR-513a-5p through its inhibition of X-linked inhibitor of apoptosis (XIAP) expression.⁹² Xue Y et al suggested that peroxisome proliferator-activated receptor γ coactivator 1 α (PGC-1 α) is a common target of miR-19b, miR-221 and miR-222, and the deficiency of these miRNAs leads to ROS accumulation and ultimately EC apoptosis.⁹³ However, in the same year, Qin B et al observed that E-Twenty-Six-1 (Ets-1) and its downstream target p21 are also possible targets of miR-221/222.⁹⁴ This evidence indicates that miR-221/222 inhibit EC apoptosis through more than one pathway. By inhibiting these two targets, miR-221/222 attenuate atherosclerosis.⁹⁴ Likewise, miR-590 has multiple targets to reduce EC apoptosis. For example, miR-590 reduces the oxLDL-induced expression of pro-apoptotic factors such as p53 and Bax.⁹⁵ By reducing the activation of caspase-3, miR-590 upregulates Bcl-2, a well-known cell apoptosis inhibitor.⁹⁵ Additionally, miR-590 decreases MAPK activation and NF- κ B translocation.⁹⁵ Moreover, upregulated miR-590 reverses the overexpression of ROS and upregulates LOX-1, which are all proatherosclerotic assaults.⁹⁵ MiR-26a is another suppressor of EC apoptosis that directly targets transient receptor potential cation channel 6 (TRPC6).⁹⁶ MiR-495 not only suppresses EC apoptosis but also induces proliferation by targeting C-C ligand 2 (CCL2).⁹⁷

MiRNAs regulate the proliferation and migration of VSMCs in atherosclerosis

Under normal conditions, VSMCs are contractile and non-proliferative cells. After exposure to an insult, they begin to proliferate and migrate, which contributes to atherosclerosis. Previous studies have shown that VSMC proliferation exerts adverse effects on atherosclerosis, but in recent years, scientists have gradually recognized the role of VSMC proliferation in preventing the progression and rupture of atherosclerotic plaques.¹⁷ Although researchers are still debating whether the proliferation of VSMCs is beneficial or harmful, VSMC proliferation undeniably improves the stability of plaques to some extent. For example, the repeatedly mentioned miR-21 promotes the proliferation of VSMCs. Downregulated miR-21 levels are accompanied by unstable plaques.⁹⁸ In turn, the delivery of miR-21 mimics to the lesion area leads to a thicker fibrous cap and increased plaque stability, probably because miR-21 directly targets repressor element-1 (RE-1) silencing transcription (REST), a suppressor of VSMC proliferation.⁹⁸

The proliferation of VSMCs is often accompanied by migration. Some miRNAs that promote or inhibit the proliferation of VSMCs also exert the same effect on VSMC migration. For example, the expression of miR-34a is

elevated in response to PDGF-BB and TGF- α .⁹⁹ Both factors are proinflammatory signals important for inducing inflammation in macrophages, ECs and VSMCs in the pathogenesis of atherosclerosis. The upregulated miR-34a in turn inhibits both VSMC proliferation and migration by targeting Notch1, completing a negative feedback loop that attenuates atherosclerosis.¹⁰⁰ Other similar examples are listed in Table 1.

MiRNAs critically regulate plaque stability

The integrity of plaques depends primarily on the stiffness of the fibrous cap, which is critically maintained by the

extracellular matrix (ECM). The molecules contributing to the fibrous cap mainly include collagen and elastin. A balance of ECM synthesis and degradation is critically important to maintain the integrity of this cap. In the intima, VSMCs synthesize ECM and macrophages are responsible for secreting various enzymes (such as matrix metalloproteinases and MMP) that degrade ECM.¹²⁹ Both insufficient production and overdegradation of ECM would lead to a weakened fibrous cap. Therefore, the proliferation, activation, senescence and apoptosis of macrophages, ECs and VSMCs all influence plaque stability. These unstable plaques are usually characterized by a thin fibrous cap, a necrotic core rich in extracellular lipids, macrophage infiltration and excess inflammation, increased calcification, and local

Table 1 MiRNAs involved in regulating VSMC proliferation and their targets.

MicroRNA	Role in VSMC proliferation (and probably migration)	Target	References
21	positive	REST, PTEN	98,101
22-3p	negative	HMGB1	102
24	negative	HMGB1	103
34a	negative	Notch1	100
34c	negative	SCF	104
124	negative	S100A4	105
126-3p	negative	LRP6	106
126-5p	positive	Dlk1	84
129-5p	negative	Wnt5a	107
132	negative	LRRFIP	108
133a	positive	IGF-1R	109
135b-5p	positive	MEF2C	110
136	positive	PPP2R	111
138	positive	SIRT1	112
141	negative	PAPP-A	113
143/145	positive	Klf4, myocardin, Elk-1	114
146a/b	positive	SMAD4	115
147b	positive	YY1	116
148b	positive	HSP90	117
155	negative	ATR1	118
185	negative	STIM1	119
214	negative	NCKAP1	120
221/222	positive	p27(Kip1), p57(Kip2)	121
362-3p	negative	ADAMTS1	122
365b-3p	negative	ADAMTS1	123
378a-5p	positive	CDK1	124
379	negative	IGF-1	125
448	positive	MEF2C	126
499a-3p	positive	MEF2C	110
503	negative	INSR	127
599	negative	TGFB2	128

Some of these miRNAs are also associated with VSMCs migration, such as miR-22-3p, 24, 34a, 135b-5p, 138, 147b, 148b, 178a-5p, 362-3p, 365b-3p, 379, 448, 499a-3p, 503, and 599. Those miRNAs important in both VSMCs proliferation and migration tends to have same effect on both processes, either promoting or suppressing. Abbreviations: REST, RE1-silencing transcription factor; PTEN, phosphatase and tensin homolog; HMGB1, high mobility group box-1; SCF, stem cell factor; S100A4, S100 calcium-binding protein A4; LRP6, lipoprotein receptor related protein 6; Dlk1, Notch1 inhibitor delta-like 1 homolog; LRRFIP, leucine-rich repeat (in Flightless 1) interacting protein-1; IGF-1R, insulin-like growth factor-1 receptor; MEF2C, myocyte enhancer factor 2C; PPP2R2A, protein phosphatase; SIRT1, sirtuin silent information regulator 1; PAPP-A, pregnancy-associated plasma protein A; Klf4, Kruppel-like factor 4; YY1, Yin Yang 1; HSP90, heat shock protein 90; ATR1, angiotensin II 1 type receptor; STIM1, stromal interaction molecule 1; NCKAP1, NCK associated protein 1; ADAMTS1, a disintegrin and metalloproteinase with thrombospondin motifs 1; CDK1, cyclin-dependent kinase; MEF2C, myocyte-enhancer factor 2; INSR, insulin receptor; TGFB2, transforming growth factor B2.

vascular degeneration.¹³⁰ These unstable plaques most commonly rupture at the shoulder area of the plaque, which contains fewer VSMCs and more macrophages.¹³¹

Several miRNAs regulate plaque stability through diverse mechanisms. Matrix metalloproteinase (MMP) is the main degrader of ECM and increases the risk of plaque rupture. However, ECM degradation also makes room for proliferating VSMCs, which facilitates VSMC proliferation and migration to some extent.³¹ Therefore, a reasonable hypothesis is that some MMPs promote plaque stability, while others render plaques prone to rupture. For example, overexpressed MMP-2 and MMP-9 may be associated with unstable plaques, but MMP-14 may lead to stable plaques in individuals with advanced atherosclerosis.¹³² This difference may be because MMP-14 promotes VSMC proliferation, which thickens the fibrous cap and stabilizes the plaque.¹³³ Knock out of miR-24 significantly upregulates MMP-14 expression in foam cells and enlarges the plaque size.¹³⁴ MiR-133a suppresses the expression of MMP-9 in ECs, indicating its role in increasing plaque stability.¹³⁵ MiR-181b negatively regulates tissue inhibitor of metalloproteinase-3 (TIMP-3) and elastin production, in turn increasing collagen degradation and leading to plaque instability.¹³⁶ MiR-497 is indirectly associated with decreased MMP-9 levels because of its negative effect on MAP kinase kinase 1 (MEK1), a key member of the MAPK/ERK family that is an important regulator of MMP-9 expression.¹³⁷

Theoretically, the apoptosis of macrophages, foam cells, VSMCs, and ECs all contributes to the expansion of the necrotic core and softens the plaque. The vitality and normal activities of VSMCs are the key to maintaining a tough plaque. In this respect, the previously mentioned miRNAs prevent the apoptosis of VSMCs, and miRNAs inducing proliferation of VSMCs significantly protect the plaque from rupture. For example, the previously mentioned study by Jin H et al reported an important role for miR-21 in stabilizing the fibrous cap of an atheroma. In unstable areas, the concentration of miR-21 is significantly decreased.⁸⁵ In addition, knock out of miR-21 leads to insufficient VSMC proliferation, and local delivery of miR-21 reverses this situation.⁸⁵ These miRNAs increase VSMC survival and prevent plaque rupture. For example, by targeting adenomatous polyposis coli (APC, a tumor suppressor), miR-210 regulates Wnt signaling and ensures VSMC survival, ultimately stabilizing advanced plaques.¹³⁸

However, not all evidence of miRNAs regulating plaque stability has fully explained their clear mechanisms. For example, the increased p27^{Kip1} expression caused by a loss of miR-221/222 may result in plaque rupture, as suggested by Bazan HA et al.¹³⁹ MiR-92a and miR-155 have been reported to reduce plaque stability, while 143/145 and miR-494 stabilize plaques.^{68,75,130,140} These findings require an in-depth investigation to further clarify the role of miRNAs in regulating atherosclerosis, specifically plaque stability.

The prospect of miRNAs in clinical use as biomarkers and preventive targets of imminent atherosclerotic ischemic stroke

Numerous studies have indicated simultaneous changes in miRNA levels, such as miR-21, miR-27b, miR-130a, miR-210,

and miR-221, after plaque rupture in either the lesional area or serum or both.¹³⁰ This result suggests that miRNAs are potential biomarkers of atherosclerotic ischemic stroke. Unfortunately, to date, most studies have only reported synchronous changes in the levels of several miRNAs upon plaque rupture but have failed to define the sequential order and logical relations between these two events. Therefore, we are unable to equate the upregulation or downregulation of these miRNAs with imminent atherosclerotic ischemic stroke. Scientists must also further clarify which miRNAs are atheroprotective and which induce rupture. Although we have provided some examples in this article, more evidence is definitely required since some miRNAs have multiple and sometimes contradictory roles, such as miR-155.

Indeed, miRNA-based therapy is a current hotspot of drug development in various areas. After all, miRNAs play vital roles in both the initiation of atherosclerotic ischemic stroke and processes occurring after injury, such as pathological changes in neurons, rescue of the penumbra zone and subsequent neural tissue repair. Obviously, miRNAs have great potential as therapeutic targets. However, only a few drugs have been approved by the Food and Drug Administration (FDA) to date. One example is mipomersen sodium an antisense oligonucleotide drug effective at reducing LDL-C, apoB and total cholesterol levels that was approved in 2013, and it has been proven to be effective in patients with homozygous familial hypercholesterolemia.¹⁴¹ This treatment represents a good start, but we are still far from developing miRNA mimics or inhibitors specifically targeting those proteins that are only important for initiating atherosclerosis and plaque rupture. As described in this review, many miRNAs have more than one protein target, and each protein has its own role in different gene networks that are involved in diverse cell activities. This situation inevitably results in side effects and becomes an obstacle to the clinical application of miRNA mimics or inhibitors. In conclusion, miRNAs represent potential biomarkers of impending atherosclerotic ischemic stroke. Delivery of miRNA mimics or inhibitors may be a prospective treatment for preventing atherosclerotic ischemic stroke.

Author contributions

Contributions of each author to the manuscript:

Study design: J.X. and Q.J.

Data Collection & Analysis: Q.J. and Y.L.

Manuscript preparation: Q.J.

Manuscript modification: J.X., Q.Z. and Q.W.

Conflict of interests

We declare that we have no financial and personal relationships with other people or organizations that might inappropriately influence our work. No professional or other personal interest of any nature or kind in any product, service and/or company exists that could be construed as influencing the position presented in, or the review of, the manuscript.

Acknowledgements

This study did not receive any financial or material support.

Data availability statement

All data have been provided. We do not have any experimental data or pictures due to the article type of this review.

Abbreviations

ABCA1	ATP binding cassette transporter A1
ACAT-1	acyl coenzyme a-cholesterol acyltransferase
APC	adenomatous polyposis coli
Apo	apolipoprotein
Bcl6	B-cell lymphoma 6
βTRC	β-transducin repeat-containing gene
CARHSP1	calcium-regulated heat stable protein 1
CCL2	C–C ligand 2
CX3CR1	CX3C chemokine receptor 1
CXCL1	CXC ligand 1
DLK1	delta-like 1
ECM	extracellular matrix
ECs	endothelial cells
eNOS	endothelial nitric oxide synthase
Ets-1	E-Twenty-Six-1
EPCs	endothelial progenitor cells
HBP1	HMG-box transcription factor 1
ICAM-1	intercellular adhesion molecule
IFN	interferon
IL	interleukin
IRAK1	IL receptor-associated kinase 1
KLF13	Krueppel-like factor 13
LOX-1	lectin-like LDL receptor-1
LPGAT1	lysophosphatidylglycerol acyltransferase 1
LPS	lipopolysaccharide
MAPK	mitogen-activated protein kinase
MAP3K7	mitogen-activated kinase kinase kinase 7
MCP-1	monocyte chemoattractant protein-1
MEK1	MAP kinase kinase 1
MIF	macrophage inhibitory factor
MKK3	mitogen-activated protein kinase kinase 3
MMP	matrix metalloproteinases
MTP	microsomal triglyceride transfer protein
NEMO	NF-κB essential modulator
Nos2	nitric oxide synthase 2
OxLDL	oxidized low-density lipoprotein
PDCD4	programmed cell death 4
PK1	3'-phosphoinositide-dependent kinase-1
PGC-1α	peroxisome proliferator-activated receptor γ coactivator 1α
Pknox1	Pbx/knotted 1 homeobox
PPAR-γ	peroxisome proliferator-activated receptor γ
REST	repressor element-1 (RE-1) silencing transcription
RISC	RNA-induced silencing complex
SHIP1	Src homology 2 domain-containing inositol-5-phosphatase-1
SOCs	suppressor of cytokine signaling
TAB2	TGF-β-activated kinase 1-binding protein

TGF-β	transforming growth factor-β
TIMP-3	tissue inhibitor of metalloproteinase-3
TNF	tumor necrosis factor
TRAF6	TNF receptor-associated factor 6
TRPC6	transient receptor potential cation channel 6
VCAM-1	vascular cell adhesion molecule-1
VSMCs	vascular smooth muscle cells
WWP1	WW domain-containing protein 1
XIAP	X-linked inhibitor of apoptosis

References

1. Katan M, Luft A. Global burden of stroke. *Semin Neurol.* 2018; 38(2):208–211.
2. World Health Organization. *The World Health Report 2004: Changing History.* Geneva: World Health Organization; 2004.
3. Beal CC. Gender and stroke symptoms: a review of the current literature. *J Neurosci Nurs.* 2010;42(2):80–87.
4. Wang W, Jiang B, Sun H, et al. Prevalence, incidence, and mortality of stroke in China: results from a nationwide population-based survey of 480 687 adults. *Circulation.* 2017; 135(8):759–771.
5. Sacco RL, Kasner SE, Broderick JP, et al. An updated definition of stroke for the 21st century: a statement for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke.* 2013;44(7):2064–2089.
6. Murphy TH, Li P, Betts K, Liu R. Two-photon imaging of stroke onset in vivo reveals that NMDA-receptor independent ischemic depolarization is the major cause of rapid reversible damage to dendrites and spines. *J Neurosci.* 2008;28(7): 1756–1772.
7. Rink C, Khanna S. MicroRNA in ischemic stroke etiology and pathology. *Physiol Genomics.* 2011;43(10):521–528.
8. Berghuan SC, Bodde MC, Jukema JW. Pathophysiology and treatment of atherosclerosis: Current view and future perspective on lipoprotein modification treatment. *Neth Heart J.* 2017;25(4):231–242.
9. Bobryshev YV, Ivanova EA, Chistiakov DA, Nikiforov NG, Orekhov A N. Macrophages and their role in atherosclerosis: pathophysiology and transcriptome analysis. *Biomed Res Int.* 2016;2016:9582430.
10. Watson MG, Byrne HM, Macaskill C, Myerscough MR. A two-phase model of early fibrous cap formation in atherosclerosis. *J Theor Biol.* 2018;456:123–136.
11. Moore KJ, Sheedy FJ, Fisher EA. Macrophages in atherosclerosis: a dynamic balance. *Nat Rev Immunol.* 2013;13(10): 709–721.
12. Orecchioni M, Ghosheh Y, Pramod AB, Ley K. Macrophage polarization: different gene signatures in M1(LPS+) vs. Classically and M2(LPS-) vs. Alternatively activated macrophages. *Front Immunol.* 2019;10:1084.
13. Niu X, Schulert GS. Functional regulation of macrophages phenotypes by microRNAs in inflammatory arthritis. *Front Immunol.* 2019;10:2217.
14. Gao H, Cao M, Chen P, et al. TNF-α promotes human antibody-mediated complement-dependent cytotoxicity of porcine endothelial cells through downregulating P38-mediated Occludin expression. *Cell Commun Signal.* 2019;17(1):75.
15. Pryma CS, Ortega C, Dubland JA, Francis GA. Pathways of smooth muscle foam cell formation in atherosclerosis. *Curr Opin Lipidol.* 2019;30(2):117–124.
16. Ikeda U, Ikeda M, Seino Y, et al. Expression of intercellular adhesion molecule-1 on rat vascular smooth muscle cells by pro-inflammatory cytokines. *Atherosclerosis.* 1993;104(1–2): 61–68.

17. Bennett MR, Sinha S, Owens GK. Vascular smooth muscle cells in atherosclerosis. *Circ Res*. 2016;118(4):692–702.
18. Zhang M, Wu JF, Chen WJ, et al. MicroRNA-27a/b regulates cellular cholesterol efflux, influx and esterification/hydrolysis in THP-1 macrophages. *Atherosclerosis*. 2014;234(1):54–64.
19. Krutzfeldt J, Rajewsky N, Braich R, et al. Silencing of microRNAs in vivo with 'antagomirs'. *Nature*. 2005;438(7068):685–689.
20. Shen WJ, Azhar S, Kraemer FB. SR-B1: a Unique multifunctional receptor for cholesterol influx and efflux. *Annu Rev Physiol*. 2018;(80):95–116.
21. Wagschal A, Najafi-Shoushtari SH, Wang L, et al. Genome-wide identification of microRNAs regulating cholesterol and triglyceride homeostasis. *Nat Med*. 2015;21(11):1290–1297.
22. Rotllan N, Price N, Pati P, Goedeke L, Fernández-Hernando C. MicroRNAs in lipoprotein metabolism and cardiometabolic disorders. *Atherosclerosis*. 2016;246:352–360.
23. Jiang H, Zhang J, Du Y, et al. MicroRNA-185 modulates low density lipoprotein receptor expression as a key posttranscriptional regulator. *Atherosclerosis*. 2015;243(2):523–532.
24. Pan S, Yang X, Jia Y, et al. Intravenous injection of microvesicle-delivery miR-130b alleviates high-fat diet-induced obesity in C57BL/6 mice through translational repression of PPAR- γ . *J Biomed Sci*. 2015;22:86.
25. Jiang C, Ting AT, Seed B. PPAR- γ agonists inhibit production of monocyte inflammatory cytokines. *Nature*. 1998;391(6662):82–86.
26. Huang N, Wang J, Xie W, et al. MiR-378a-3p enhances adipogenesis by targeting mitogen-activated protein kinase 1. *Biochem Biophys Res Commun*. 2015;457(1):37–42.
27. Goedeke L, Rotllan N, Ramirez CM, et al. MiR-27b inhibits LDLR and ABCA1 expression but does not influence plasma and hepatic lipid levels in mice. *Atherosclerosis*. 2015;243(2):499–509.
28. Soh J, Iqbal J, Queiroz J, Fernandez-Hernando C, Hussain MM. MicroRNA-30c reduces hyperlipidemia and atherosclerosis in mice by decreasing lipid synthesis and lipoprotein secretion. *Nat Med*. 2013;19(7):892–900.
29. Zhou L, Irani S, Sirwi A, Hussain MM. MiRNAs regulating apolipoprotein B-containing lipoprotein production. *Biochim Biophys Acta*. 2016;1861(12 Pt B):2062–2068.
30. Zhou L, Hussain MM. Human microRNA-548 decreases hepatic apoB secretion and lipid synthesis. *Arterioscler Thromb Vasc Biol*. 2017;37(5):786–793.
31. Yin R, Zhang C, Hou Y, Wang X. MicroRNA let-7g and atherosclerosis plaque stabilization. *World J Cardiovasc Dis*. 2017;7:24–36.
32. Liang X, Xu Z, Yuan M, et al. MicroRNA-16 suppresses the activation of inflammatory macrophages in atherosclerosis by targeting PDCD4. *Int J Mol Med*. 2016;37(4):967–975.
33. Canfran-Duque A, Rotllan N, Zhang X, et al. Macrophage deficiency of miR-21 promotes apoptosis, plaque necrosis, and vascular inflammation during atherogenesis. *EMBO Mol Med*. 2017;9(9):1244–1262.
34. Baatsch I, Nazari-Jahantigh M, Kumbrink J, Weber C, Schober A. Macrophage-microRNA-147 protects against atherosclerosis in mice. *Arterioscler Thromb Vasc Biol*. 2019;39(Suppl_1):A553.
35. Mussbacher M, Salzmann M, Brostjan C, et al. Cell type-specific roles of NF- κ B linking inflammation and thrombosis. *Front Immunol*. 2019;10:85.
36. Sun SC. The non-canonical NF- κ B pathway in immunity and inflammation. *Nat Rev Immunol*. 2017;17(9):545–558.
37. Feinberg MW, Moore K J. MicroRNA regulation of atherosclerosis. *Circ Res*. 2016;118(4):703–720.
38. Cheng HS, Sivachandran N, Lau A, et al. MicroRNA-146 represses endothelial activation by inhibiting pro-inflammatory pathways. *EMBO Mol Med*. 2013;5(7):1017–1034.
39. Feng J, Li A, Deng J, et al. MiR-21 attenuates lipopolysaccharide-induced lipid accumulation and inflammatory response: potential role in cerebrovascular disease. *Lipids Health Dis*. 2014;13:27.
40. Li H, Ouyang XP, Jiang T, Zheng XL, He PP, Zhao GJ, et al. MicroRNA-296: a promising target in the pathogenesis of atherosclerosis? *Mol Med*. 2018;24(1):12.
41. Nazari-Jahantigh M, Wei Y, Noels H, et al. MicroRNA-155 promotes atherosclerosis by repressing Bcl6 in macrophages. *J Clin Invest*. 2012;122(11):4190–4202.
42. Sun HX, Zeng DY, Li RT, et al. Essential role of microRNA-155 in regulating endothelium-dependent vasorelaxation by targeting endothelial nitric oxide synthase. *Hypertension*. 2012;60(6):1407–1414.
43. Yao R, Ma YL, Liang W, et al. MicroRNA-155 modulates Treg and Th17 cells differentiation and Th17 cell function by targeting SOCS1. *PLoS One*. 2012;7(10):e46082.
44. Huang RS, Hu GQ, Lin B, Lin ZY, Sun CC. MicroRNA-155 silencing enhances inflammatory response and lipid uptake in oxidized low-density lipoprotein-stimulated human THP-1 macrophages. *J Invest Med*. 2010;58(8):961–967.
45. Li X, Kong D, Chen H, et al. MiR-155 acts as an anti-inflammatory factor in atherosclerosis-associated foam cell formation by repressing calcium-regulated heat stable protein 1. *Sci Rep*. 2016;6:21789.
46. Calippe B, Douin-Echinard V, Laffargue M, et al. Chronic estradiol administration in vivo promotes the proinflammatory response of macrophages to TLR4 activation: involvement of the phosphatidylinositol 3-kinase pathway. *J Immunol*. 2008;180(12):7980–7988.
47. Zhang Y, Zhang HE, Liu Z. MicroRNA-147 suppresses proliferation, invasion and migration through the AKT/mTOR signaling pathway in breast cancer. *Oncol Lett*. 2016;11(1):405–410.
48. Jahantigh MN, Baatsch I, Csaba G, Zimmer R, Hoelper S, Krueger M, et al. MiR-147 deficiency in macrophages exacerbates atherosclerosis in mice. *Eur Heart J*. 2018;39(suppl 1):3783.
49. Wang J, Bai X, Song Q, et al. MiR-223 inhibits lipid deposition and inflammation by suppressing toll-like receptor 4 signaling in macrophages. *Int J Mol Sci*. 2015;16(10):24965–24982.
50. Wei Y, Nazari-Jahantigh M, Chan L, et al. The microRNA-342-5p fosters inflammatory macrophage activation through an Akt1- and microRNA-155-dependent pathway during atherosclerosis. *Circulation*. 2013;127(15):1609–1619.
51. Banerjee S, Xie N, Cui H, et al. MicroRNA let-7c regulates macrophage polarization. *J Immunol*. 2013;190(12):6542–6549.
52. Saha B, Bruneau JC, Kodys K, Szabo G. Alcohol-induced miR-27a regulates differentiation and M2 macrophage polarization of normal human monocytes. *J Immunol*. 2015;194(7):3079–3087.
53. Ponomarev ED, Veremeyko T, Barteneva N, Krichevsky AM, Weiner HL. MicroRNA-124 promotes microglia quiescence and suppresses EAE by deactivating macrophages via the C/EBP- α -PU.1 pathway. *Nat Med*. 2011;17(1):64–70.
54. Banerjee S, Cui H, Xie N, et al. Mir-125a-5p regulates differential activation of macrophages and inflammation. *J Biol Chem*. 2013;288(49):35428–35436.
55. Bras JP, Silva AM, Calin GA, Barbosa MA, Santos SG, Almeida MI, et al. MiR-195 inhibits macrophages pro-inflammatory profile and impacts the crosstalk with smooth muscle cells. *PLoS One*. 2017;12(11):e0188530.
56. Wei Y, Zhu M, Schober A. Macrophage microRNAs as therapeutic targets for atherosclerosis, metabolic syndrome, and cancer. *Int J Mol Sci*. 2018;19(6):1756.
57. Thulin P, Wei T, Werngren O, et al. MicroRNA-9 regulates the expression of peroxisome proliferator-activated receptor δ in human monocytes during the inflammatory response. *Int J Mol Med*. 2013;31(5):1003–1010.

58. Lv LL, Feng Y, Wu M, et al. Exosomal miRNA-19b-3p of tubular epithelial cells promotes M1 macrophage activation in kidney injury. *Cell Death Differ.* 2020;27(1):210–226.
59. Barnett RE, Conklin DJ, Ryan L, et al. Anti-inflammatory effects of miR-21 in the macrophage response to peritonitis. *J Leukoc Biol.* 2016;99(2):361–371.
60. Ouimet M, Ediriweera HN, Gundra UM, et al. MicroRNA-33-dependent regulation of macrophage metabolism directs immune cell polarization in atherosclerosis. *J Clin Invest.* 2015;125(12):4334–4348.
61. Chaudhuri AA, So AY, Sinha N, et al. MicroRNA-125b potentiates macrophage activation. *J Immunol.* 2011;187(10):5062–5068.
62. Audsley MD, Moseley GW. Paramyxovirus evasion of innate immunity: diverse strategies for common targets. *World J Virol.* 2013;2(2):57–70.
63. Zhao L, Liu YW, Yang T, et al. The mutual regulation between miR-214 and A2AR signaling plays an important role in inflammatory response. *Cell Signal.* 2015;27(10):2026–2034.
64. Sala F, Aranda JF, Rotllan N, et al. MiR-143/145 deficiency attenuates the progression of atherosclerosis in Ldlr^{-/-} mice. *Thromb Haemost.* 2014;112(4):796–802.
65. Goedeke L, Rotllan N, Canfrán-Duque A, et al. MicroRNA-148a regulates LDL receptor and ABCA1 expression to control circulating lipoprotein levels. *Nat Med.* 2015;21(11):1280–1289.
66. Meiler S, Baumer Y, Toulmin E, Seng K, Boisvert WA. MicroRNA 302a is a novel modulator of cholesterol homeostasis and atherosclerosis. *Arterioscler Thromb Vasc Biol.* 2015;35(2):323–331.
67. Mandolini C, Santovito D, Marcantonio P, et al. Identification of microRNAs 758 and 33b as potential modulators of ABCA1 expression in human atherosclerotic plaques. *Nutr Metab Cardiovasc Dis.* 2015;25(2):202–209.
68. Donners MM, Wolfs IM, Stöger LJ, et al. Hematopoietic miR-155 deficiency enhances atherosclerosis and decreases plaque stability in hyperlipidemic mice. *PLoS One.* 2012;7(4):e35877.
69. Chen YC, Zhang XW, Niu XH, et al. Macrophage migration inhibitory factor is a direct target of HBP1-mediated transcriptional repression that is overexpressed in prostate cancer. *Oncogene.* 2010;29(21):3067–3078.
70. Berasi SP, Xiu M, Yee AS, Paulson KE. HBP1 repression of the gene: cell cycle regulation via the NADPH oxidase. *Mol Cell Biol.* 2004;24(7):3011–3024.
71. Tian FJ, An LN, Wang GK, et al. Elevated microRNA-155 promotes foam cell formation by targeting HBP1 in atherogenesis. *Cardiovasc Res.* 2014;103(1):100–110.
72. Yuan X, Chen J, Dai M. Paeonol promotes microRNA-126 expression to inhibit monocyte adhesion to ox-LDL-injured vascular endothelial cells and block the activation of the PI3K/Akt/NF- κ B pathway. *Int J Mol Med.* 2016;38(6):1871–1878.
73. Wang Y, Wang F, Wu Y, et al. MicroRNA-126 attenuates palmitate-induced apoptosis by targeting TRAF7 in HUVECs. *Mol Cell Biochem.* 2015;99(1–2):123–130.
74. Fang Y, Shi C, Manduchi E, Civelek M, Davies P F. MicroRNA-10a regulation of proinflammatory phenotype in atherosusceptible endothelium in vivo and in vitro. *Proc Natl Acad Sci U S A.* 2010;107(30):13450–13455.
75. Loyer X, Potteaux S, Vion AC, et al. Inhibition of microRNA-92a prevents endothelial dysfunction and atherosclerosis in mice. *Circ Res.* 2014;114(3):434–443.
76. Cheng HS, Sivachandran N, Lau A, et al. MicroRNA-146 represses endothelial activation by inhibiting pro-inflammatory pathways. *EMBO Mol Med.* 2013;5(7):1017–1034.
77. Sun C, Wu MH, Yuan SY. Nonmuscle myosin light-chain kinase deficiency attenuates atherosclerosis in apolipoprotein E-deficient mice via reduced endothelial barrier dysfunction and monocyte migration. *Circulation.* 2011;124(1):48–57.
78. Sun X, He S, Wara AKM, et al. Systemic delivery of MicroRNA-181b inhibits nuclear factor- κ B activation, vascular inflammation, and atherosclerosis in apolipoprotein E-deficient mice. *Circ Res.* 2014;114(1):32–40.
79. Su Y, Yuan J, Zhang F, et al. MicroRNA-181a-5p and microRNA-181a-3p cooperatively restrict vascular inflammation and atherosclerosis. *Cell Death Dis.* 2019;10(5):365.
80. Suárez Y, Wang C, Manes TD, Pober JS. Cutting edge: TNF-induced microRNAs regulate TNF-induced expression of E-selectin and intercellular adhesion molecule-1 on human endothelial cells: feedback control of inflammation. *J Immunol.* 2010;184(1):21–25.
81. Li P, Wei J, Li X, et al. 17 β -Estradiol enhances vascular endothelial Ets-1/miR-126-3p expression: the possible mechanism for attenuation of atherosclerosis. *J Clin Endocrinol Metab.* 2017;102(2):594–603.
82. Chistiakov DA, Sobenin IA, Orekhov AN, Bobryshev Y V. Human miR-221/222 in physiological and atherosclerotic vascular remodeling. *Biomed Res Int.* 2015;2015:354517.
83. Hartmann P, Zhou Z, Ntarelli L, et al. Endothelial Dicer promotes atherosclerosis and vascular inflammation by miRNA-103-mediated suppression of KLF4. *Nat Commun.* 2016;7:10521.
84. Schober A, Nazari-Jahantigh M, Wei Y, et al. MicroRNA-126-5p promotes endothelial proliferation and limits atherosclerosis by suppressing Dlk1. *Nat Med.* 2014;20(4):368–376.
85. Chistiakov DA, Orekhov AN, Bobryshev YV. The role of miR-126 in embryonic angiogenesis, adult vascular homeostasis, and vascular repair and its alterations in atherosclerotic disease. *J Mol Cell Cardiol.* 2016;97:47–55.
86. Zuo K, Li M, Zhang X, et al. MiR-21 suppresses endothelial progenitor cell proliferation by activating the TGF β signaling pathway via downregulation of WWP1. *Int J Clin Exp Pathol.* 2015;8(1):414–422.
87. Mehta JL, Chen J, Hermonat PL, Romeo F, Novelli G. Lectin-like, oxidized low-density lipoprotein receptor-1 (LOX-1): a critical player in the development of atherosclerosis and related disorders. *Cardiovasc Res.* 2006;69(1):36–45.
88. Ding Z, Wang X, Schnackenberg L, et al. Regulation of autophagy and apoptosis in response to ox-LDL in vascular smooth muscle cells, and the modulatory effects of the microRNA hsa-let-7g. *Int J Cardiol.* 2013;168(2):1378–1385.
89. Zhang Y, Chen N, Zhang J, Tong Y. Has-let-7g miRNA targets caspase-3 and inhibits the apoptosis induced by ox-LDL in endothelial cells. *Int J Mol Sci.* 2013;14(11):22708–22720.
90. Qin B, Xiao B, Liang D, Li Y, Jiang T, Yang H. MicroRNA let-7c inhibits Bcl-xl expression and regulates ox-LDL-induced endothelial apoptosis. *BMB Rep.* 2012;45(8):464–469.
91. Li Y, Yang C, Zhang L, Yang P. MicroRNA-210 induces endothelial cell apoptosis by directly targeting PDK1 in the setting of atherosclerosis. *Cell Mol Biol Lett.* 2017;22:3.
92. Shin S, Moon KC, Park KU, Ha E. MicroRNA-513a-5p mediates TNF- α and LPS induced apoptosis via downregulation of X-linked inhibitor of apoptotic protein in endothelial cells. *Biochimie.* 2012;94(6):1431–1436.
93. Xue Y, Wei Z, Ding H, et al. MicroRNA-19b/221/222 induces endothelial cell dysfunction via suppression of PGC-1 α in the progression of atherosclerosis. *Atherosclerosis.* 2015;241(2):671–681.
94. Qin B, Cao Y, Yang H, Xiao B, Lu Z. MicroRNA-221/222 regulate ox-LDL-induced endothelial apoptosis via Ets-1/p21 inhibition. *Mol Cell Biochem.* 2015;405(1–2):115–124.
95. Bao MH, Li JM, Zhou QL, et al. Effects of miR-590 on oxLDL-induced endothelial cell apoptosis: roles of p53 and NF- κ B. *Mol Med Rep.* 2016;13(1):867–873.
96. Zhang Y, Qin W, Zhang L, et al. MicroRNA-26a prevents endothelial cell apoptosis by directly targeting TRPC6 in the setting of atherosclerosis. *Sci Rep.* 2015;5:9401.

97. Liu D, Zhang XL, Yan CH, et al. MicroRNA-495 regulates the proliferation and apoptosis of human umbilical vein endothelial cells by targeting chemokine CCL2. *Thromb Res*. 2015; 135(1):146–154.
98. Jin H, Li DY, Chernogubova E, et al. Local delivery of miR-21 stabilized fibrous caps in vulnerable atherosclerotic lesions. *Mol Ther*. 2018;26(4):1040–1055.
99. Wang H, Jin Z, Pei T, et al. Long noncoding RNAs C2dat1 enhances vascular smooth muscle cell proliferation and migration by targeting MiR-34a-5p. *J Cell Biochem*. 2019;120(3): 3001–3008.
100. Chen Q, Yang F, Guo M, et al. miRNA-34a reduces neointima formation through inhibiting smooth muscle cell proliferation and migration. *J Mol Cell Cardiol*. 2015;89(Part A):75–86.
101. Jiang Q, Han Y, Gao H, Tian R, Li P, Wang C, et al. Ursolic acid induced anti-proliferation effects in rat primary vascular smooth muscle cells is associated with inhibition of microRNA-21 and subsequent PTEN/PI3K. *Eur J Pharmacol*. 2016;781:69–75.
102. Huang SC, Wang M, Wu WB, et al. Mir-22-3p inhibits arterial smooth muscle cell proliferation and migration and neointimal hyperplasia by targeting HMGB1 in arteriosclerosis obliterans. *Cell Physiol Biochem*. 2017;42(6):2492–2506.
103. Yang J, Chen L, Ding J, et al. MicroRNA-24 inhibits high glucose-induced vascular smooth muscle cell proliferation and migration by targeting HMGB1. *Gene*. 2016;586(2): 268–273.
104. Choe N, Kwon JS, Kim YS, et al. The microRNA miR-34c inhibits vascular smooth muscle cell proliferation and neointimal hyperplasia by targeting stem cell factor. *Cell Signal*. 2015;27(6):1056–1065.
105. Choe N, Kwon DH, Shin S, et al. The microRNA miR-124 inhibits vascular smooth muscle cell proliferation by targeting S100 calcium-binding protein A4 (S100A4). *FEBS Lett*. 2017; 591(7):1041–1052.
106. Jansen F, Stumpf T, Proebsting S, et al. Interacellular transfer of miR-126-3p by endothelial microparticles reduces vascular smooth muscle cell proliferation and limits neointima formation by inhibiting LRP6. *J Mol Cell Cardiol*. 2017;104: 43–52.
107. Zhang Y, Liu Z, Zhou M, Liu C. MicroRNA-129-5p inhibits vascular smooth muscle cell proliferation by targeting wnt5a. *Exp Ther Med*. 2016;12(4):2651–2656.
108. Choe N, Kwon JS, Kim JR, et al. The microRNA miR-132 targets Lrrfip1 to block vascular smooth muscle cell proliferation and neointimal hyperplasia. *Atherosclerosis*. 2013;229(2): 348–355.
109. Gao S, Wassler M, Zhang L, et al. MicroRNA-133a regulates insulin-like growth factor-1 receptor expression and vascular smooth muscle cell proliferation in murine atherosclerosis. *Atherosclerosis*. 2014;232(1):171–179.
110. Xu Z, Han Y, Liu J, et al. MiR-135b-5p and miR-499a-3p promote cell proliferation and migration in atherosclerosis by directly targeting MEF2C. *Sci Rep*. 2015;5:12276.
111. Zhang CF, Kang K, Li XM, Xie BD, et al. MicroRNA-136 promotes vascular muscle cell proliferation through the ERK1/2 pathway by targeting PPP2R2A in atherosclerosis. *Curr Vasc Pharmacol*. 2015;13(3):405–412.
112. Xu J, Li L, Yun HF, Han YS. MiR-138 promotes smooth muscle cells proliferation and migration in db/db mice through down-regulation of SIRT1. *Biochem Biophys Res Commun*. 2015; 463(4):1159–1164.
113. Zhang Y, Chen B, Ming L, et al. MicroRNA-141 inhibits vascular smooth muscle cell proliferation through targeting PAPP-A. *Int J Clin Exp Pathol*. 2015;8(11):14401–14408.
114. Cordes KR, White MP, Sheehy NT, et al. MiR-145 and miR-143 regulate smooth muscle cell fate and plasticity. *Nature*. 2009; 460(7256):705–710.
115. Xue L, Luo S, Ding H, et al. Upregulation of miR-146a-5p is associated with increased proliferation and migration of vascular smooth muscle cells in aortic dissection. *J Clin Lab Anal*. 2019;33(4):e22843.
116. Yue Y, Lv W, Zhang L, Kang W. MiR-147b influences vascular smooth muscle cell proliferation and migration via targeting YY1 and modulating Wnt/ β -catenin activities. *Acta Biochim Biophys Sin (Shanghai)*. 2018;50(9):905–913.
117. Zhang X, Shi H, Wang Y, Hu J, Sun Z, Xu S. Down-regulation of hsa-miR-148b inhibits vascular smooth muscle cells proliferation and migration by directly targeting HSP90 in atherosclerosis. *Am J Transl Res*. 2017;9(2):629–637.
118. Yang LX, Liu G, Zhu GF, et al. MicroRNA-155 inhibits angiotensin II-induced vascular smooth muscle cell proliferation. *J Renin Angiotensin Aldosterone Syst*. 2014;15(2):109–116.
119. Fang M, Li Y, Wu Y, Ning Z, Wang X, Li X, et al. MiR-185 silencing promotes the progression of atherosclerosis via targeting stromal interaction molecule 1. *Cell Cycle*. 2019; 18(6-7):682–695.
120. Afzal TA, Luong LA, Chen D, et al. NCK associated protein 1 modulated by miRNA-214 determines vascular smooth muscle cell migration, proliferation, and neointima hyperplasia. *J Am Heart Assoc*. 2016;5(12):e004629.
121. Liu X, Cheng Y, Zhang S, Lin Y, Yang J, Zhang C. A necessary role of miR-221 and miR-222 in vascular smooth muscle cell proliferation and neointimal hyperplasia. *Circ Res*. 2009; 104(4):476–487.
122. Li M, Liu Q, Lei J, Wang X, Chen X, Ding Y, et al. MiR-362-3p inhibits the proliferation and migration of vascular smooth muscle cells in atherosclerosis by targeting ADAMTS1. *Biochem Biophys Res Commun*. 2017;493(1):270–276.
123. Qu Y, Zhang N. MiR-365b-3p inhibits the cell proliferation and migration of human coronary artery smooth muscle cells by directly targeting ADAMTS1 in coronary atherosclerosis. *Exp Ther Med*. 2018;16(5):4239–4245.
124. Liu S, Yang Y, Jiang S, et al. MiR-378a-5p regulates proliferation and migration in vascular smooth muscle cell by targeting CDK1. *Front Genet*. 2019;10:22.
125. Li K, Wang Y, Zhang A, Liu B, Jia L. MiR-379 inhibits cell proliferation, invasion, and migration of vascular smooth muscle cells by targeting insulin-like factor-1. *Yonsei Med J*. 2017;58(1):234–240.
126. Zhang R, Sui L, Hong X, Yang M, Li W. MiR-448 promotes vascular smooth muscle cell proliferation and migration in through directly targeting MEF2C. *Environ Sci Pollut Res Int*. 2017;24(28):22294–22300.
127. Bi R, Ding F, He Y, et al. miR-503 inhibits platelet-derived growth factor-induced human aortic vascular smooth muscle cell proliferation and migration through targeting the insulin receptor. *Biomed Pharmacother*. 2016;84:1711–1716.
128. Xie B, Zhang C, Kang K, Jiang S, Kai Kang, Shulin J. miR-599 inhibits vascular smooth muscle cells proliferation and migration by targeting TGF β 2. *PLoS One*. 2015;10(11): e0141512.
129. Rudijanto A. The role of vascular smooth muscle cells on the pathogenesis of atherosclerosis. *Acta Med Indones*. 2007; 39(2):86–93.
130. Koroleva IA, Nazarenko MS, Kucher AN. Role of microRNA in development of instability of atherosclerotic plaques. *Biochemistry (Moscow)*. 2017;82(11):1380–1390.
131. Plutzky J. Atherosclerotic plaque rupture: emerging insights and opportunities. *Am J Cardiol*. 1999;84(1A):15J–20J.
132. Liu XQ, Mao Y, Wang B, et al. Specific matrix metalloproteinases play different roles in intraplaque angiogenesis and plaque instability in rabbits. *PLoS One*. 2014;9(9): e107851.
133. Johnson JL. Metalloproteinases in atherosclerosis. *Eur J Pharmacol*. 2017;816:93–106.

134. Di Gregoli K, Jenkins N, Salter R, White S, Newby AC, Johnson J L. MicroRNA-24 regulates macrophage behavior and retards atherosclerosis. *Arterioscler Thromb Vasc Biol.* 2014;34(9):1990–2000.
135. Kurozumi S, Yamaguchi Y, Kurosumi M, Ohira M, Matsumoto H, Horiguchi J. Recent trends in microRNA research into breast cancer with particular focus on the associations between microRNAs and intrinsic subtypes. *J Hum Genet.* 2017;62(1):15–24.
136. Di Gregoli K, Mohamad Anuar NN, Bianco R, et al. MicroRNA-181b controls atherosclerosis and aneurysms through regulation of TIMP-3 and elastin. *Circ Res.* 2017;120(1):49–65.
137. Wang YY, Li H, Wang XH, Yuan M, Li G P. Probucol inhibits MMP-9 expression through regulating miR-497 in HUVECs and apoE knockout mice. *Thromb Res.* 2016;140:51–58.
138. Eken SM, Jin H, Chernogubova E, et al. MicroRNA-210 enhances fibrous cap stability in advanced atherosclerosis lesions. *Circ Res.* 2017;120(4):633–644.
139. Bazan HA, Hatfield SA, O'Malley CB, Brooks AJ, Lightell D Jr, Woods TC, et al. Acute loss of miR-221 and miR-222 in the atherosclerotic plaque shoulder accompanies plaque rupture. *Stroke.* 2015;46(11):3285–3287.
140. Wezel A, Welten SM, Razawy W, et al. Inhibition of microRNA-494 reduces carotid artery atherosclerotic lesion development and increases plaque stability. *Ann Surg.* 2015;262(5):841–848.
141. Thomas GS, Cromwell WC, Ali S, Chin W, Flaim JD, Davidson M. Mipomersen, an apolipoprotein B synthesis inhibitor, reduces atherogenic lipoproteins in patients with severe hypercholesterolemia at high cardiovascular risk: a randomized, double-blind, placebo-controlled trial. *J Am Coll Cardiol.* 2013;62(23):2178–2184.