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## Review Article

## Oxidative stress in atherosclerosis: The role of microRNAs in arterial remodeling



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## ABSTRACT

Atherosclerosis is the underlying condition in most cardiovascular diseases. Among the highly specific cellular and molecular responses, endothelial dysfunction plays a key role in disease initiation and progression. These events coincide with the occurrence of oxidative stress. Increased reactive oxygen species production and oxidation of low-density lipoprotein are detected throughout atherosclerosis progression. MicroRNAs (miRNAs) have emerged as important regulators of gene expression that posttranscriptionally modify cellular responses and function. Accumulating studies indicate an integrated miRNA network in the molecular mechanisms that control cellular homeostasis, vascular inflammation, and metabolism. Experimental models of atherosclerosis highlight a direct link between altered miRNA expression profiles and the pathophysiology of the disease and identify putative miRNA candidates for the development of novel therapeutic strategies. In this review, we provide an overview of the role of miRNA regulatory networks in oxidative stress in atherosclerosis and arterial remodeling and discuss their potential therapeutic implications.

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## Introduction

MicroRNAs (miRNAs) have emerged as powerful regulators of cardiovascular diseases. The majority of miRNA genes are initially transcribed by RNA polymerase II as primary transcripts that undergo maturation by sequential cleavage by the RNase III endonucleases Drosha and Dicer. The mature miRNAs are small noncoding molecules (20–25 nucleotides) that incorporate into the RNA-induced silencing complex (RISC) and specifically guide it to bind to target mRNA sequences usually in the 3' untranslated region (UTR) [1]. Multiple mechanisms of miRNA-mediated gene repression have been described. Binding of the RISC to the target mRNA can promote the degradation of the mRNA and/or inhibit its translation, whereas binding of the miRNA complexes to the promoter of target genes may induce epigenetic changes to the chromatin and lead to transcriptional silencing [2,3]. MiRNA networks are particularly important under stress conditions to maintain homeostasis of biological systems. MiRNAs have numerous targets often within the same functional pathway [4]. Their effects on individual genes can be modest. However, miRNAs alter cellular responses via a coordinated effect on multiple targets. In the cardiovascular system, miRNAs have been shown to control key aspects of development and pathophysiology [5]. Signature patterns of miRNAs have been associated with distinct pathologies, and miRNA-based therapeutic manipulations have been proposed as promising strategies for cardiovascular diseases [6–8]. Here we review the role of miRNAs in the pathogenesis of atherosclerosis and the subsequent arterial remodeling and discuss their potential therapeutic utility.

## Atherosclerosis and oxidative stress

In the vessel wall, endothelial cells (ECs), smooth muscle cells (SMCs), and macrophages are sources of free radicals. A reduction in nitric oxide (NO) bioavailability, attributed to a dysfunction of the endothelial nitric oxide synthase (eNOS) enzyme and activity, contributes to atherosclerosis [9]. Moreover, oxygen undergoes univalent reduction by NADH/NAD(P)H and xanthine oxidase (XO) to form superoxide. In the vasculature, superoxide is thought to be

mainly derived from membrane-bound NAD(P)H oxidase and mitochondria, whereas additional superoxide is generated by XO catalyzing hypoxanthine and xanthine to uric acid [10]. In atherosclerosis, a profound imbalance of pro- and antioxidants causes oxidative stress.

Despite the compelling evidence for a proatherogenic role of oxidative stress, stimuli that are supposed to reduce oxidative stress are not consistently associated with a reduction in atherosclerosis. For example, antioxidant treatments failed to offer significant protection in animal models and if an improvement was observed, it was mainly attributed to increased NO bioavailability or the induction of cytoprotective and anti-inflammatory enzymes such as heme oxygenase 1 [11]. Oxidative processes such as lipid oxidation, however, were largely unaffected. Thus, it is still not entirely clear how oxidative stress accelerates atherosclerosis.

## MiRNAs in atherosclerosis and vascular remodeling (Tables 1 and 2)

Accumulating evidence supports the notion that endothelial homeostasis, innate and adaptive immunity, lipid metabolism, and local blood flow patterns are among the key regulatory processes in atherogenesis [12–14].

### Endothelial cell activation

Endothelial dysfunction leads to increased endothelial permeability, upregulation of endothelial adhesion molecules, and inflammatory cell infiltration into the arterial wall. This cascade of events ultimately results in atherosclerosis [15] and occurs at preferred sites in the arterial tree that are associated with disturbed laminar flow, notably, arterial branch points and bifurcations. Thus, hemodynamic forces influence the initiation of atherogenesis [16]. The arterial endothelium, the interface between the circulating blood and the vessel wall, is particularly susceptible. In areas with complex patterns of blood flow, ECs display a higher turnover, decreased eNOS expression, and increased cytokine and

**Table 1**  
MiRNAs exhibiting atheroprotective functions.

MiRNA	Cell type	Target gene	Function affected	Ref.
MiR-10a	ECs	HOXA1	Predisposition to vascular inflammation	[21]
MiR-19a	ECs	Cyclin D1	Proliferation of ECs	[24]
MiR-125-5p	Monocytes	ORP9	Lipid uptake, cytokine expression	[79]
MiR-126	ECs	KLF2, VCAM-1	VEGF signaling.	[22,26,27]
MiR-133	SMCs	Sp1	Proliferation of SMCs	[55,56]
MiR-143/145	SMCs	KLF4, KLF5	Proliferation of SMCs	[47–53]
MiR-146a/b	Monocytes, macrophages	IRAK1, TRAF6	Proinflammatory cytokine production	[38–41]
MiR-146a	Macrophages	TLR4	Lipid accumulation, inflammatory response	[80]
MiR-147	Macrophages	TNF $\alpha$ , IL-6, PYHIN, UBCH8, ZBP1	Cytokine production	[42]
MiR-181b	ECs	Importin- $\alpha$ 3	Inflammatory response	[25]
MiR-9, miR-187, miR-125a, miR-99b, let-7e	Monocytes, neutrophils	NF- $\kappa$ B1	Inflammatory response	[43]
Let-7g	Monocytes	LOX-1	OxLDL internalization.	[78]

**Table 2**  
MiRNAs exhibiting proatherogenic functions.

MiRNA	Cell type	Target gene	Function affected	Ref.
MiR-21	ECs	PPAR $\alpha$	Derepression of AP-1, inflammatory response	[19]
MiR-21	SMCs	PTEN, Bcl-2, PDCD4	Proliferation of SMCs, senescence	[57,59,63]
MiR-33a/b	Macrophages	ABCA1, ABCG1, NPC1, CROT, CPT1a, HADHB	Lipid metabolism	[64–70]
MiR-92a	ECs	KLF2, KLF4	Anti-inflammatory and vasodilatory properties of ECs	[18,23]
MiR-122	Hepatocytes		Lipid metabolism	[72–75]
MiR-125b	Macrophages	IRF4	Inflammatory response of macrophages, responsiveness to IFN- $\gamma$	[34,36]
MiR-144	Macrophages	ABCA1	Cholesterol efflux	[81,82]
MiR-146a	SMCs	KLF4	Proliferation of SMCs	[61,62]
MiR-155	Macrophages	IKK $\epsilon$ , FADD, Ripk-1	Inflammatory response	[34,35]
MiR-200c	ECs	ZEB1	Senescence	[29,30]
MiR-217, miR-34a	ECs	Sirt1	Senescence	[31,32]
MiR-221, miR-222	SMCs	c-kit, p27, p57	Proliferation of SMCs, contractile gene transcription.	[58,60]
MiR-342-5p	Macrophages	Akt1/miR-155	Inflammatory stimulation of macrophages	[37]
MiR-633	ECs		Induces a proinflammatory phenotype in ECs and monocyte adhesion	[20]
MiR-758	Macrophages	ABCA1	Cholesterol efflux	[71]

adhesion molecule production, consistent with a proatherogenic phenotype [17].

#### MiRNAs associated with a proatherosclerotic EC phenotype

**MiR-92a:** MiR-92a seems to contribute to the endothelial heterogeneity associated with atherosusceptible areas. In these regions, endothelial miR-92a coregulates Krüppel-like-factors 2 and 4 (KLF2 and KLF4), critical regulators of endothelial homeostasis. Two conserved sites of miR-92a were identified in the KLF2 and KLF4 3' UTR and reporter gene assays confirmed that miR-92a directly targets both transcription factors [18] and modulates the anti-inflammatory and vasodilatory properties of the endothelium.

**MiR-21:** MiR-21 is also upregulated by disturbed flow and induces a proinflammatory profile of ECs through downregulation of peroxisome proliferators-activated receptor- $\alpha$  (PPAR $\alpha$ ). As a result derepression of activator protein 1 (AP-1) and enhanced adhesion molecule and cytokine expression are observed [19].

**MiR-633:** Furthermore, a distinct miRNA signature is identified in ECs exposed to oscillatory stress in vitro. MiR-633 displays the most pronounced differences in expression and regulates the disturbed flow-induced proinflammatory phenotype of ECs and monocyte adhesion to the endothelium [20].

#### MiRNAs associated with protective EC phenotype.

**MiR-10a:** In response to mechanical forces in atherosusceptible areas miR-10a is downregulated and the inflammatory response directly modulated through the degradation of I $\kappa$ B $\alpha$  and nuclear factor  $\kappa$ B (NF- $\kappa$ B) signaling. HOXA1 is directly targeted by miR-10a and mediates the expression of cytokines (MCP-1, IL-6, IL-8) and adhesion molecules (VCAM-1, E-selectin). Thus, loss of endothelial miR-10a expression is linked to the predisposition of vascular inflammation in areas of disturbed flow [21].

**MiR-126:** In contrast, areas of high laminar flow are protected from atherosclerosis. In these areas, ECs tend to align to the direction of flow, exhibit a quiescent phenotype, and express significantly lower inflammatory mediators [13]. The atheroprotective flow induces the expression of miR-126 that subsequently activates VEGF signaling, through KLF2 upregulation [22]. Interestingly, downregulation of miR-92a in response to high laminar flow is an essential step for the induction of KLF2 [23].

**MiR-19a:** Suppression of EC proliferation by shear stress is, at least in part, miRNA mediated. MiR-19a directly downregulates cyclin D1 mRNA and protein expression in ECs exposed to laminar flow in a parallel plate flow system. Inhibition of the endogenous miR-19a attenuates the antiproliferative effect of flow, suggesting that miR-19a is important for endothelial homeostasis [24].

**MiR-181b:** Activation of ECs by proinflammatory stimuli that promote leukocyte recruitment is another trigger of vascular remodeling. Cytokines such as tumor necrosis factor  $\alpha$  (TNF $\alpha$ ) alter the miRNA profile of ECs to orchestrate the upregulation of inflammatory mediators. Nuclear translocation of the NF- $\kappa$ B dimer is an essential step in the inflammatory process and largely depends on a subset of importins—the  $\alpha$  subgroup. Recent studies uncovered a novel function of miR-181b in the endothelium that targets a distinct member of importins, the importin- $\alpha$ 3, and thus regulates the NF- $\kappa$ B-mediated inflammation in the vessel wall. TNF $\alpha$  treatment in ECs results in downregulation of miR-181b that facilitates the induction of VCAM-1, ICAM-1, and E-selectin [25]. To add to the complexity of the miRNA-mediated regulation, miR-126, miR-155, miR-221, miR-222, miR-17-3p, and miR-31 also control the expression of VCAM-1 [26,27], ICAM-1, and E-selectin [28], suggesting a highly coordinated manner of regulation of the inflammatory response by the miRNA networks.

**MiRNAs associated with vascular aging.** Aging plays an important part in the development and progression of atherosclerotic lesions. EC senescence in particular has a profound effect on cell apoptosis and disruption of the endothelial monolayer, rendering the atherosclerotic plaque prone to rupture.

**MiR-200c:** In vitro, increased transcription of the miR-200c/141 cluster was observed in response to oxidative stress. Upregulation of the mature miR-200c levels in ECs induced endothelial growth arrest, apoptosis, and senescence, mainly owing to zinc-finger enhancer binding 1 (ZEB1) transcription factor inhibition [29]. ZEB transcription factors and miR-200 were previously shown to be reciprocally linked in a feedback loop that tightly controls their expression and regulates cell survival and senescence [30].

**MiR-217:** MiR-217 also controls senescence. MiR-217 expression progressively increases with aging in endothelial cells. It is detected in human atherosclerotic lesions and negatively correlated with Sirt1 [31]. Sirt1 is an NAD<sup>+</sup>-dependent class III histone deacetylase that is upregulated under caloric restriction and has both antioxidant and anti-inflammatory effects. Excessive reactive oxygen species (ROS) and aging are known to decrease Sirt1 levels. Although the link between

oxidative stress and miR-217 expression is yet to be established, reporter assays indicated that miR-217 binds to the 3' UTR and directly regulates Sirt1 expression.

**MiR-34a:** In addition to miR-217, miR-34a was reported to target Sirt1 in ECs. High levels of miR-34a were observed in aging endothelium that correlated with low levels of Sirt1. A miR-34a binding site in the 3' UTR of Sirt1 and direct targeting were detected, although a potential ROS-dependent regulation of miR-34a expression in EC was not investigated [32].

#### *Leukocyte infiltration: oxidative stress-responsive miRNAs*

Adhesion of leukocytes to the inflamed endothelium is critical for the development of atherosclerosis. NF- $\kappa$ B activation plays a pivotal role in the inflammatory response.

After cleavage by Dicer, short double-stranded RNA duplexes are generated from the miRNA precursors. Based on their stability one of the strands is biologically active and represents the mature miRNA, and the inactive strand, the so-called miRNA star strand (miRNA\*), is usually degraded. Oxidative stress seems to selectively stabilize the miRNA\* of specific miRNAs [4]. In macrophages, a panel of oxidative stress-responsive miRNAs, namely miR-27a\*, miR-27b\*, miR-29b\*, miR-24-2\*, and miR-21\*, was identified and shown to modulate the NF- $\kappa$ B pathway, suggesting that macrophage functions may be affected [33].

#### *MiR-155 and miR-125b*

Proinflammatory stimuli result in differential miRNA expression in monocytes. MiR-155 and miR-125b show the most pronounced changes after lipopolysaccharide (LPS), interferon- $\gamma$  (IFN- $\gamma$ ), or TNF $\alpha$  stimulation in an NF- $\kappa$ B-dependent manner and have opposing effects on TNF $\alpha$  production [34]. Overexpression of miR-125b induces an activated phenotype in macrophages and elevates responsiveness to IFN- $\gamma$ , whereas anti-miR-125b treatment decreases CD80 surface expression, at least partially by controlling IRF4 levels [35,36]. On the other hand, miR-155 results in enhanced translation of TNF $\alpha$ , most probably by affecting the stability of its transcript, and IKK $\epsilon$ , FADD, and Ripk1—key mediators in the inflammatory response—were validated as direct targets of miR-155. Increased TNF $\alpha$  expression that could render them more susceptible to endotoxin shock was observed in mice overexpressing miR-155 in B cells, indicating that miR-155 exerts both positive and negative effects on LPS signaling. A cross talk between miR-342-5p and miR-155 was recently reported to affect the inflammatory activation of macrophages in atherosclerotic lesions. Increased expression of miR-342-5p macrophages in early atherosclerotic lesions suppressed the expression of Akt1 and led to upregulation of miR-155 and increased nitro-oxidative stress [37].

#### *MiR-146a/b*

The expression of miR-146a/b in monocytes is strongly affected by proinflammatory stimuli such as LPS through an NF- $\kappa$ B-dependent mechanism. The adaptor molecules IRAK1 and TRAF6 are bona fide miR-146a targets, suggesting a novel mechanism of negative-feedback regulation of Toll-like receptor (TLR) and NF- $\kappa$ B activation [38,39]. Of note, the expression of miR-146a in peripheral blood mononuclear cells is significantly increased in patients with acute coronary syndrome [40]. Targeted deletion of miR-146a in mice leads to hyperresponsiveness to LPS and excessive production of proinflammatory cytokines (TNF $\alpha$ , IL-6). Stimulation of miR-146a-deficient macrophages with LPS results in an overproduction of TNF $\alpha$  and IL-6 proteins. In contrast, monocytes with ectopic expression of miR-146a display a diminished inflammatory

response [41]. In addition to acting as a molecular brake on inflammation, miR-146a also controls immune cell proliferation as the miR-146a-null mice develop a spontaneous autoimmune disorder and die prematurely.

#### *MiR-147*

In a similar manner, miR-147 displays potent anti-inflammatory properties and attenuates the expression of proinflammatory cytokines in macrophages stimulated with TLR2, TLR3, and TLR4 ligands, whereas its expression is dependent on NF- $\kappa$ B and IRF3 signaling. Transfection of miR-147 mimics into peritoneal macrophages significantly decreases LPS-induced TNF $\alpha$  and IL-6 production as well as the expression of several other LPS-induced genes, such as PYHIN, UBCH8, and ZBP1, and importantly the expression level of miR-147 as well. Overall, miR-147 has emerged as part of a negative-feedback loop that can inhibit the proinflammatory response of macrophages and prevent excessive inflammation [42].

#### *Other miRNAs*

Additional miRNAs (miR-9, miR-187, miR-125a, miR-99b, and let-7e) are induced by LPS in human monocytes and neutrophils. Upregulation of miR-9 is consistently observed in monocytes after activation with LPS, TNF $\alpha$ , and IL-1 $\beta$  but not IFN- $\gamma$ . A conserved miR-9 binding site on the NF- $\kappa$ B1 transcript and a miR-9-dependent inhibition were identified. Thus, miR-9 was proposed to be part of an inhibitory feedback loop in the inflammatory response, acting at the level of NF- $\kappa$ B1 [43].

Infiltration of monocytes in the lesion sites is fundamental for the development of atherosclerosis. Distinct monocyte subsets have been implicated in the pathogenesis of the disease. The potential correlation between miRNA signatures in monocytic subsets and atherosclerotic plaque has been investigated and miR-99b, miR-152, and miR-422a expression was reported in monocytes and atherosclerotic plaques, but not in healthy vessels. This finding may imply monocyte accumulation in the vessel wall and novel candidate miRNAs that could serve as targets for therapeutic interventions [44].

#### *Smooth muscle cell proliferation and migration*

Neointima formation is a common feature of vessel remodeling. In response to endothelial dysfunction and inflammatory cell infiltration, SMCs migrate from the media to the intima and proliferate to form neointimal lesions. The switch from a contractile to a synthetic proliferative phenotype in SMCs is controlled by miRNAs.

#### *MiR-143/145*

The miR-143/145 cluster is a cardiac and smooth muscle cell-specific cluster [45] and its expression is directly regulated by serum response factor (SRF) and Nkx2.5 and the Jag-1/Notch pathway [46]. Several lines of evidence suggest that this cluster is essential for the acquisition of the contractile phenotype, SMC differentiation, and the structural integrity of the aorta. SMCs from miR-143/145-null mice maintain a proliferative state that favors neointima formation [47]. The mutant mice developed neointimal lesions in the absence of hyperlipidemia, lipid depositions, and foam cells. Angiotensin-converting enzyme was identified as a direct target of this miRNA cluster and its inhibition could partially rescue the phenotype in miR-143/145-null mice. Further support to this notion was provided by studies in rats using a model of carotid artery balloon injury. A critical role of miR-145 was demonstrated in SMC phenotype and neointima formation. KLF5 emerged as the direct target of miR-145 that mediated the observed phenotypic modulation [48]. Additionally, transforming

growth factor- $\beta$  stimulation was shown to favor the contractile phenotype of SMCs in vitro through an upregulation of miR-143/145 that targeted KLF4 expression [49,50]. Maintaining the differentiated state of SMCs is critical in various pathologies such as atherosclerosis, restenosis, and aneurysm, and downregulation of miR-143/145 is a common feature. Likewise, structural modifications of the aorta due to incomplete differentiation were reported in miR-143/145-null mice [51]. Moreover, these mice display significant reduction in blood pressure probably due to diminished vascular rigidity, whereas in response to vascular injury they have impaired neointimal formation and reduced migratory activity of SMCs. Modulation of cytoskeletal assembly is considered to contribute to this phenotype, as miR-143/145 [52] were shown to affect the release of the myocardin-related transcription factors from actin, their nuclear translocation, and their interaction with SRF [53].

#### MiR-133

MiR-133, a miRNA highly abundant in skeletal and cardiac muscle [54] but also detectable in the aorta, was recently reported to prevent the phenotypic switching of SMCs in vitro and in vivo. Gain- and loss-of-function experiments revealed that miR-133 directly regulates the transcription factor Sp-1 and its downstream target KLF4 [55]. Thus, miR-133 decreases SMC proliferation in vitro and in vivo and its expression is markedly reduced after balloon injury in the carotid artery. Likewise, expression levels of miR-133 were correlated with plaque stability in humans. Comparison of the miRNA profile of symptomatic and asymptomatic atherosclerotic plaques revealed a significant upregulation of miR-133 in symptomatic plaques [56].

#### Other miRNAs

Unlike miR-133, miR-21, miR-221, and miR-222 promote SMC survival, proliferation, and dedifferentiation. Identified as differentially expressed and localized in SMCs in neointimal lesions these miRNAs have distinct functions: miR-21 by modulating apoptosis via regulation of PTEN and Bcl-2; miR-221 and miR-222 by controlling proliferation via targeting p27 (Kip1) and p57 (Kip2) [57–59]. The pleiotropic effects of platelet-derived growth factor (PDGF) seem to be mediated at least in part by miR-221. Induced by PDGF-BB treatment in SMCs, this miRNA specifically targets c-kit and p27/Kip1 and inhibits SMC-specific contractile gene transcription [60]. In a similar manner, miR-146a—a miRNA that is upregulated in human atherosclerotic plaques—was shown to promote SMC proliferation and neointimal hyperplasia through a feedback loop with KLF4 [61,62]. As far as oxidative stress and SMC apoptosis are concerned, an unexpected role for ROS-mediated upregulation of miR-21 was uncovered. Programmed cell death 4 was identified as a direct target of miR-21. Interestingly, upregulation of miR-21 expression inhibited ROS-mediated SMC apoptosis and death and had an antiapoptotic albeit modest effect [63].

#### MiRNA-mediated lipid metabolism and atherosclerosis

The link between hypercholesterolemia and atherosclerosis is well established. The role of miRNAs in regulating lipid metabolism has only been discovered recently.

#### MiR-33a/b

Two intronic miRNAs, miR-33a and miR-33b, have been identified as critical regulators of lipid metabolism. Encoded within the sterol response element binding protein genes (SREBP2 and SREBP1), they represent a feedback loop that fine-tunes fatty acid and cholesterol levels [64,65]. Their targets include genes involved in cholesterol export and high-density lipoprotein (HDL) synthesis

(ABCA1, ABCG1, and NPC1) and fatty acid oxidation (CROT, CPT1a, HADHB) [66,67]. Antagonism of miR-33 in low-density lipoprotein (LDL) receptor-null mice gave promising results for the treatment of atherosclerosis. These mice show increased levels of circulating HDL, enhanced reverse cholesterol transport, and regression of established atherosclerotic plaques [68,69]. In nonhuman primates, systemic administration of anti-miR-33 induced hepatic expression of ABCA1 and resulted in a sustained increase in plasma HDL [70].

#### MiR-758

ABCA1 is a critical transporter in cholesterol efflux and thus its regulation was further interrogated using bioinformatic analysis. It was shown that its 3' UTR may also be targeted by miR-758, a miRNA that is repressed in cholesterol-loaded macrophages. Experimental evidence confirmed that miR-758 posttranscriptionally regulates ABCA1 expression in macrophages, hepatocytes, and astrocytes and has a significant impact on cholesterol efflux [71].

#### MiR-122

Remarkable effects on plasma cholesterol levels were obtained by manipulating miR-122 expression as well. MiR-122 is one of the most abundant miRNAs in the liver and regulates lipid metabolism [72]. Targeting miR-122 in mice resulted in a sustained decrease in plasma cholesterol, in both HDL and LDL fractions [73,74]. These findings were extended to nonhuman primates, in which antagonism of miR-122 in African green monkeys led to significantly lower cholesterol in the circulation without apparent toxicity [75].

#### Let-7g

After increased endothelial permeability, the exacerbated ROS production by activated macrophages leads to lipoprotein oxidation (oxLDL) and deposition of modified lipoproteins in the arterial wall, a key event in the progression of the disease [76]. OxLDL can further activate ECs that adapt a proinflammatory phenotype, leading to leukocyte recruitment. As oxLDL and other lipid-loaded monocytes accumulate they are transformed into foam cells that form the fatty streaks in the intima [77]. The scavenger receptor lectin-like oxidized low density receptor-1 (LOX-1) mediates the binding and internalization of oxLDL into cells and plays an active role in atherosclerosis by stimulating a proinflammatory phenotype. A negative feedback regulation between let-7g and LOX-1 that affects proliferation and cell migration was recently reported. Serum levels of let-7g were significantly reduced in subjects with hypercholesterolemia compared to controls [78].

#### MiR-125-5p/miR-146a

In circulating cells, stimulation with oxLDL resulted in a distinct miRNA profile and a marked upregulation of miR-125a-5p and miR-146a. In human peripheral blood monocytes manipulation of miR-125a-5p levels led to changes in lipid uptake and the secretion of cytokines. Although the molecular mechanisms involved are still not clear it was proposed that the oxysterol-binding protein-like 9, with its diverse functions in lipid metabolism, is directly targeted by miR-125a-5p [79]. MiR-146a, on the other hand, inhibits oxLDL-mediated lipid accumulation and inflammation in macrophages through direct targeting of TLR4 [80].

#### MiR-144

Two independent groups simultaneously identified the role of miR-144 in cholesterol metabolism, via suppression of ABCA1. A novel pathway that involves activation of the liver X nuclear receptors or the bile acid receptor farnesoid X receptor increased expression of miR-144, and downregulation of ABCA1 was shown to regulate plasma HDL cholesterol [81,82]. In mice, overexpression of

miR-144 appears to regulate both macrophage cholesterol efflux and HDL biogenesis in the liver.

#### Secreted miRNAs and atheroprotection

A novel concept of intercellular communication via secreted vesicles has been recently reported. Several lines of evidence suggest that various stimuli induce the release of vesicles that carry distinct miRNA signatures, implying active packaging of these carriers that could facilitate specific functions. The specificity of cellular secretion of miRNAs was reported in monocytes after stimulation with LPS, H<sub>2</sub>O<sub>2</sub>, or oleic acid/palmitic acid and treatment with advanced glycation end products. Remarkably, more pronounced differences were observed in the secreted than in the cellular miRNA pool [83]. In a pioneering study using next-generation sequencing Diehl et al. [84] reported a distinct miRNA pattern of expression in microparticles derived from stimulated or unstimulated monocytes or endothelial cells compared to the miRNA cellular pool. These findings provided further support to the notion that microparticles may act as transport vehicles delivering specific miRNAs. Cellular export to protein and lipoprotein complexes has also been described. Argonaute (Ago)-miRNA complexes were detected in the circulation and their miRNA profile was reliably characterized [85], indicating that functional miRNA-induced silencing complexes are released from the cells. In lipoproteins, a specific miRNA pool was detected. The miRNA content identified in HDL complexes from atherosclerotic individuals was shown to induce differential gene expression in hepatocytes, indicating that HDL may participate in a miRNA-mediated mechanism of intercellular communication [86], although it is still not clear if this is a common or rather specialized mechanism [87].

#### MiR-126

MiR-126, residing in endothelial cell-derived apoptotic bodies, was shown to induce the degradation of RGS16, induce CXCR4 signaling, and enhance CXCL12, which can mobilize progenitor cells. This was proposed to promote CXCR4-dependent mobilization and recruitment of stem cell antigen-1-positive progenitor cells to atherosclerotic plaques in mice and has led to a decrease in diet-induced atherosclerosis as well as collar-induced plaque formation [88]. Packaging of miR-126 in microvesicles seems to be tightly regulated. A significant reduction in the miR-126 content of endothelial particles released under hyperglycemic conditions was detected *in vitro* in ECs. This regulatory mechanism was further confirmed in a population study. In diabetic individuals, reduced miR-126 expression was detected in circulating particles compared to healthy controls [6]. In addition to endothelial particles, in an intervention study in humans, a positive correlation between miR-126 and platelet microparticles was observed on day 2 after ischemia/reperfusion injury, suggesting that miR-126 is responsive to the vascular stimulation [8]. An intriguing role for the endothelial secreted miR-126 in SMC turnover was recently revealed in low shear stress. MiR-126 emerged as a paracrine mediator that can shift gene expression and function in SMCs toward a proatherogenic phenotype [89].

#### MiR-150

A miRNA-mediated cross talk between monocytes and endothelial cells in atherosclerosis has also been demonstrated. MiR-150 secreted from monocytic cells was shown to enhance endothelial cell migration. Monocytic cells secrete miR-150 and its levels in microvesicles increase in response to inflammatory stimuli. Circulating microvesicles from patients with atherosclerosis contain increased levels of miR-150. Treatment of endothelial

cells with these vesicles resulted in enhanced migration. A direct target of miR-150, the transcription factor c-Myb, was found to mediate this response [83]. c-Myb controls cell proliferation, lineage commitment, and migration.

#### MiR-143/145

EC and SMC cross talk is also important to maintain SMCs in a contractile and atherosclerotic state. A recent study suggests that ECs communicate with SMCs through vesicles that transfer miR-143/145 and confer atheroprotection. The concept that secreted miRNAs can induce a phenotype switch in SMCs is intriguing, as miR-143/145 concentrations are already high in cultured SMCs, even in the absence of ECs. It is currently unclear how ECs could deliver sufficient amounts of miR-143/miR145 [90] to prevent an SMC switch to a synthetic, proliferative, and proatherogenic phenotype. In principle, exosomes and other vesicles may act as carriers of genetic information in the form of miRNAs and could be exploited for the development of novel therapeutic approaches. However, the exact mechanism of targeted cellular uptake is still unknown.

#### Circulating miRNAs

Blood cells are probably the most important contributors to circulating miRNAs. Consequently, changes in blood cell counts and hemolysis will have a significant influence on circulating miRNA levels. Moreover, a larger proportion of plasma microvesicles are derived from platelets with only a smaller percentage coming from endothelial cells. As shown in interventional studies in healthy volunteers, ischemia/reperfusion injury results in a rise of miRNAs that are abundant in platelets and platelet microparticles, such as miR-223, -126, -24, -21, and -197. In contrast, antiplatelet therapy is associated with decreased shedding of platelet microparticles and a corresponding reduction of platelet miRNAs in plasma [91]. Nonetheless, comparisons of Ago1- and Ago2-associated miRNAs in plasma and full blood have revealed significant differences in the miRNA profiles [92], indicating that some circulating miRNAs originate from other organs, i.e., liver (miR-122), muscle (miR-133a), or heart (miR-208a). Heart-specific miRNAs are released after myocardial infarction and implicated as potential biomarkers similar to troponins [93,94]. Effects of heparin, however, have to be taken into account when designing studies to investigate the relation of circulating microRNAs to acute cardiovascular events or coronary intervention [95,96].

#### MiRNA therapeutics

MiRNAs have unique functions and are promising candidates for novel therapeutic interventions. They have a small size and highly conserved sequence and miRNA networks exert a mild effect at baseline but pronounced biological functions under stress. Their ability to target pathways instead of individual effectors and thus overcome potential redundancy mechanisms is a particularly useful feature in designing novel miRNA-based therapeutic strategies [97]. On the other hand given the multiplicity of miRNA targets it would be hard to avoid “off-target” effects. In addition, only a few of the studied miRNAs are tissue specific and hence targeting specific organs may be particularly challenging [98]. A variety of chemistries for miRNA inhibitors have been developed and the efficacy, specificity, and toxicity of these constructs are being tested extensively in animal models [99,100]. There are several issues that need to be addressed regarding their cellular uptake, storage, and mode of action, whereas data from large animal studies are required before therapeutic inhibition of

miRNAs can enter any clinical trials for cardiovascular diseases. Even so, miRNA therapeutics is a fast-evolving field that could offer opportunities for novel therapeutic approaches.

## Conclusions

Although increased ROS production has been observed in cardiovascular diseases, attenuation of oxidative stress has so far not translated into a successful therapeutic intervention. In the vascular setting, additional studies are required to further characterize the function and regulation of miRNAs responsive to oxidative stress. Nevertheless, substantial progress has been made in understanding the role of miRNAs in targeting the various aspects of atherogenesis and arterial remodeling. The concept has emerged that miRNAs take part in positive as well as negative regulatory loops and may function as networks rather than individual regulators. Identifying the critical miRNAs that govern the processes of response to vascular injury and resolution of inflammation is a formidable task. It requires an understanding of the temporal and spatial relationship of miRNA expression in the various cell types that interact in the vessel wall. If miRNA mimics/inhibitors could be delivered effectively in a targeted rather than a systemic manner, miRNA therapeutics may represent novel opportunities to halt the development of atherosclerosis.

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