Trends in Endocrinology & Metabolism

CellPress
REVIEWS

Review

Efferocytosis and Atherosclerosis: Regulation of Phagocyte Function by MicroRNAs

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There is evidence of the critical role of efferocytosis, the clearance of apoptotic cells (ACs) by phagocytes, in vascular cell homeostasis and protection against atherosclerosis. Specific microRNAs (miRs) can regulate atherogenesis by controlling the accumulation of professional phagocytes (e.g., macrophages) and nonprofessional phagocytes (i.e., neighboring tissue cells with the ability to acquire a macrophage-like phenotype) within the arterial wall, the differentiation of phagocytes into foam cells, the efferocytosis of apoptotic foam cells by phagocytes, and the phagocyte-mediated inflammatory response. A better understanding of the mechanisms involved in miR-regulated phagocyte function might lead to novel therapeutic antiatherosclerotic strategies. In this review, we try to shed light on the relationship between miRs and cellular players in the process of efferocytosis in the context of atherosclerotic plaque and their potential as molecular targets for novel antiatherosclerotic therapies.

Highlights

microRNAs (miRs) control several key processes that contribute to the maintenance of normal vascular biology.

Deregulations of several miRs have been suggested to be implicated in the pathophysiology of atherosclerosis.

The role of impaired efferocytosis in the development of atherosclerosis has recently been shown.

Different miRs may modulate atherosclerosis by regulating efferocytosis in apoptotic cells (ACs) and phagocytes.

MiRs that regulate efferocytosis could serve as potential molecular targets for designing novel antiatherosclerotic therapies.

Introduction

Efferocytosis (see Glossary), the process of removal of ACs by professional (e.g., macrophages) and nonprofessional phagocytes (i.e., neighboring tissue cells with the ability to acquire a macrophage-like phenotype); (Box 1 and Figure 1), is essential for human health, in that it is actively involved in maintaining homeostasis of tissues and organs by preventing the deleterious effects of cell necrosis [1–4]. Accordingly, efferocytosis leads to the clearance of ACs and reduces the release of proinflammatory mediators from dying cells. Conversely, impaired efferocytosis may result in a defective tissue homeostasis and contribute to the pathogenesis of various conditions, including atherosclerotic plaque formation/progression [2].

Some **microRNAs (miRs)** [5], a class of conserved small (19–25 nt) noncoding single-stranded RNAs, acting as critical post-transcriptional regulators of gene expression [6], control phagocyte accumulation, phagocyte differentiation into foam cells, efferocytosis of apoptotic foam cells by phagocytes, and phagocyte-mediated inflammatory response in the context of atherosclerotic plaque (Box 2 and Figure 2). An altered expression of some of these miRs may promote atherosclerotic progression [7]. Thus, miR-regulated phagocyte function may represent a potential therapeutic target in **atherosclerosis**. In this review, we attempt to clarify the relationship between miRs and cellular players in the process of efferocytosis as it pertains to the pathophysiology of atherosclerosis, and their potential as therapeutic targets for antiatherosclerotic therapies.

MiR Biogenesis, Function, and Therapeutic Modulation

MiRs are noncoding RNAs involved in the post-transcriptional regulation of gene expression. Primary miRs are transcribed from DNA sequences and processed into precursor miRs, and finally, mature miRs [8,9]. The expression of some miRs may be regulated by long noncoding RNAs [10]. MiRs generally downregulate gene expression by interacting with the 3′ untranslated

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 https://doi.org/10.1016/j.tem.2019.07.006

Box 1. Efferocytosis

Efferocytosis is the process of removal of ACs within tissues. In general, efferocytosis is divided into three main steps: recruitment, recognition, and engulfment/processing (see Figure 1 in main text). At the onset of apoptosis, apoptotic particles recruit phagocytes (recruitment). Then, phagocytes connect and interact with apoptotic particles (recognition). Due to this interaction, the engulfment proceeds through the rearrangement of phagocyte cytoskeleton, and decomposition of apoptotic particles can occur through the phagolysosome within phagocytes (engulfment/processing). Each of these steps occurs through a highly regulated balance between different soluble molecules and cellular pathways. The attraction of phagocytes to the site of cell death is regulated by soluble molecules released by the dying cells ('find-me' signals). The recognition of dying cells by phagocytes is mediated by the interaction between phagocyte receptors and surface molecules on dying cells ('eat-me' signals). The engulfment/processing of apoptotic bodies by phagocytes is regulated by intracellular molecular pathways. Notably, an efficient AC clearance involves the production of anti-inflammatory cytokines and the inhibition of proinflammatory cytokines [3].

region (3′ UTR) of target mRNAs and induce their degradation or block their translation. However, some miRs may interact with either noncoding regions other than 3′ UTR or coding sequences in the target mRNAs. In addition, in some circumstances, miRs can upregulate

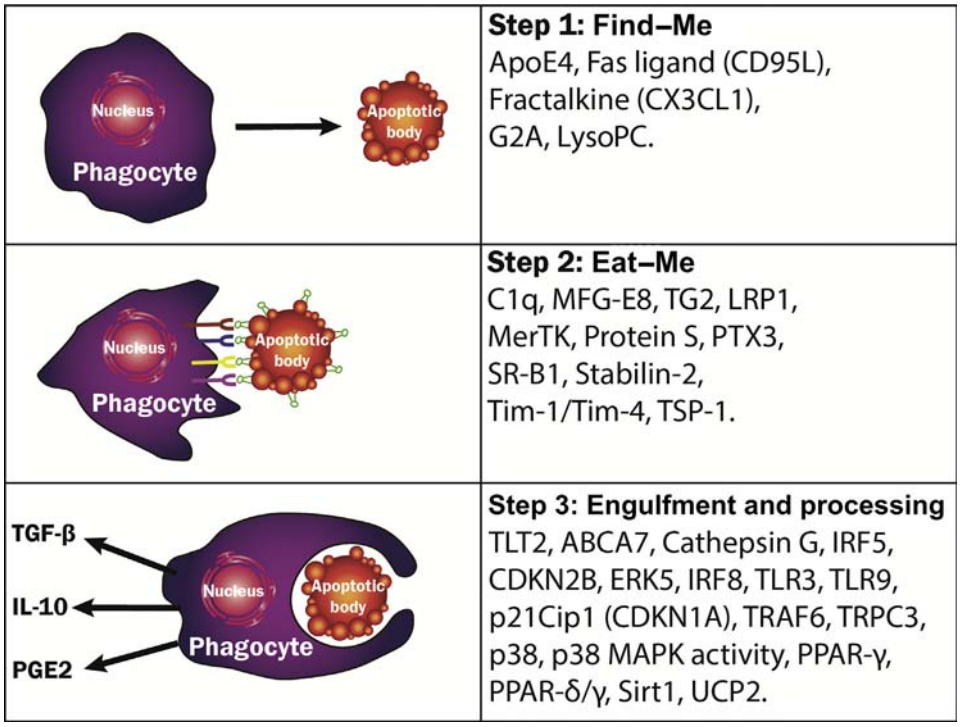

Figure 1. A Brief Illustration of Different Steps and Regulating Factors Involved in the Efferocytosis Process. Several molecular factors regulate efferocytosis, including soluble molecules attracting phagocytes to the site of cell death ('find-me' signals); (Step 1), surface proteins promoting the recognition of dying cells by phagocytes ('eat-me' signals); (Step 2), and molecular pathways involved in the engulfment and processing of apoptotic bodies (Step 3). Abbreviations: ABCA7, ATP-binding cassette transporter A7; ApoE, apolipoprotein E; CDKN2B, cyclin-dependent kinase inhibitor 2B; C1q, complement 1q; ERK, extracellular signal regulated kinase; G2A, G protein-coupled receptor; HMGB1, high-mobility group box 1 protein; IL-10, interleukin 10; IRF5, interferon regulatory factor 5; IRF8, interferon regulatory factor 8; LRP1, low-density lipoprotein receptor (LDLR)-related protein 1; LysoPC, lysophosphatidylcholine; MAPK, mitogen-activated protein kinase; MerTK, Mer tyrosine kinase; MFG-E8, Milk Fat Globule-EGF factor 8; p21Cip1 (CDKN1A), cyclin-dependent kinase inhibitor 1A; PGE2, prostaglandin E2; PPAR-δ/γ, peroxisome proliferator-activated receptor δ/γ; PTX3, pentraxin 3; Sirt1, sirtuin 1; SR-B1, scavenger receptor class B1; TG2, transglutaminase 2; TGF-β, transforming growth factor-β; Tim, T cell immunoglobulin- and mucin-domain-containing molecule; TLR3, toll-like receptor 3; TLR9, toll-like receptor 9; TLT2, TREM-like protein 2; TRAF6, tumor necrosis factor receptor-associated factor 6; TRPC3, transient receptor potential canonical 3; TSP-1, thrombospondin-1; UCP2, uncoupling protein 2.

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Box 2. Atherosclerotic Plaque Formation and Progression: The Role of Phagocytes

Upon exposure to oxidized low-density lipoproteins (oxLDLs) endothelial nitric oxide synthase (eNOS) is downregulated, leading to a reduced production of nitric oxide (NO). A reduced NO bioavailability promotes endothelial activation (expression of adhesion molecules and chemoattractant proteins by ECs) and monocyte chemotaxis. Upon transmigration into the arterial intima monocytes differentiate into macrophages. Subsequently, the uptake of oxLDLs by macrophages proceeds in an unrestricted manner. In addition, the uptake of oxLDLs by vascular smooth muscle cells (VSMCs), upon their differentiation into macrophage-like cells (loss of smooth muscle-specific markers and acquired expression of macrophage-specific markers), may occur. Within professional and nonprofessional phagocytes free cholesterol is formed due to lysosomal lipoprotein degradation and free cholesterol storage as cholesterol esters (CEs) occurs. Excessive cholesterol accumulation leads to the transformation of lipid-laden phagocytes into foam cells. The only mechanism to reduce excessive cholesterol accumulation by phagocytes and the formation of macrophage-derived foam cells is the reverse cholesterol transport (RCT), that is the high-density lipoprotein (HDL)-mediated cholesterol efflux from macrophages. Within atherosclerotic plaques foam cells eventually undergo apoptosis and necroptosis, and, if not effectively cleared by phagocyte-mediated efferocytosis, undergo secondary necrosis, contributing to the formation of the necrotic core. As the necrotic core grows and the fibrous cap gets thin, the plaque becomes more vulnerable to rupture [84,85].

Glossary

Apoptosis: a form of programmed cell death that contributes to homeostasis through removal of damaged cells or cells with a limited lifespan.
Atherosclerosis: a chronic inflammatory process that underlies coronary artery disease.
Efferocytosis: the process of clearance of dying and ACs by macrophages and other immune phagocytes.
MicroRNAs (MiRs): a class of conserved, small (19–25 nt), noncoding single-stranded RNAs acting as critical post-transcriptional regulators of gene expression.

gene expression by activating the translation of target mRNAs. A single miR may regulate multiple target mRNAs involved in different biological processes and a single mRNA may be regulated by multiple miRs.

MiR expression and function may be dysregulated in various pathological conditions, including atherosclerosis. Therefore, inhibition of miRs that are overexpressed, or replacement of miRs that are underexpressed, may represent potential therapeutic approaches in different diseases [11]. MiR inhibition is possible through antisense oligonucleotides containing the complementary sequences of the target endogenous mRNA (e.g., antimiR oligonucleotides, modified antimiR oligonucleotides, and antimiR peptides). MiR replacement can be achieved using genetic vectors (e.g., miR mimics).

However, some issues regarding the selective therapeutic modulation of miRs remain poorly clarified. First, since some miRs exert pleiotropic functions, their therapeutic modulation may potentially lead to nonselective and even unexpected biological effects. Secondly, since only a few miRs are tissue-specific, the selectivity of miR modulators for target cells (e.g., macrophages) is a crucial challenge to be faced in the drug development process. To date, almost exclusively, preclinical studies have evaluated the therapeutic antiatherosclerotic potential of miR mimetics and antagonists, and have shown promising results. Interventional studies investigating the efficacy and safety of treatment paradigms targeting miRs involved in efferocytosis within atherosclerotic lesions are awaited with great interest.

Efferocytosis and Atherosclerotic Plaque

Macrophages and vascular smooth muscle cells (VSMCs) acquiring a macrophage-like phenotype are key actors of efferocytosis in atherosclerosis plaques (Box 2 and Figure 2) [2]. Efferocytosis inhibits foam cell accumulation, thereby indirectly counteracting the production of reactive oxygen species (ROS) and proinflammatory mediators by foam cells and, thus, limiting the progression of atherosclerosis [12–16]. In addition, the elimination of apoptotic foam cells through efferocytosis directly prompts anti-inflammatory and -oxidant responses [i.e., production of anti-inflammatory cytokines, including interleukin (IL)-10 and transforming growth factor (TGF)-β, inactivation of nicotinamide adenine dinucleotide phosphate oxidase (NOX), and increased expression of heme oxygenase-1 (HO-1)] [17–19]. As a consequence of reduced production of proinflammatory and -oxidant mediators, along with an increased stimulation of anti-inflammatory and -oxidant responses, macrophage polarization from type 1 to type 2 macrophages is stimulated, the recruitment of additional phagocytes within atherosclerotic lesions is prevented, the phenotypic switching of VSMCs towards macrophage-like cells is

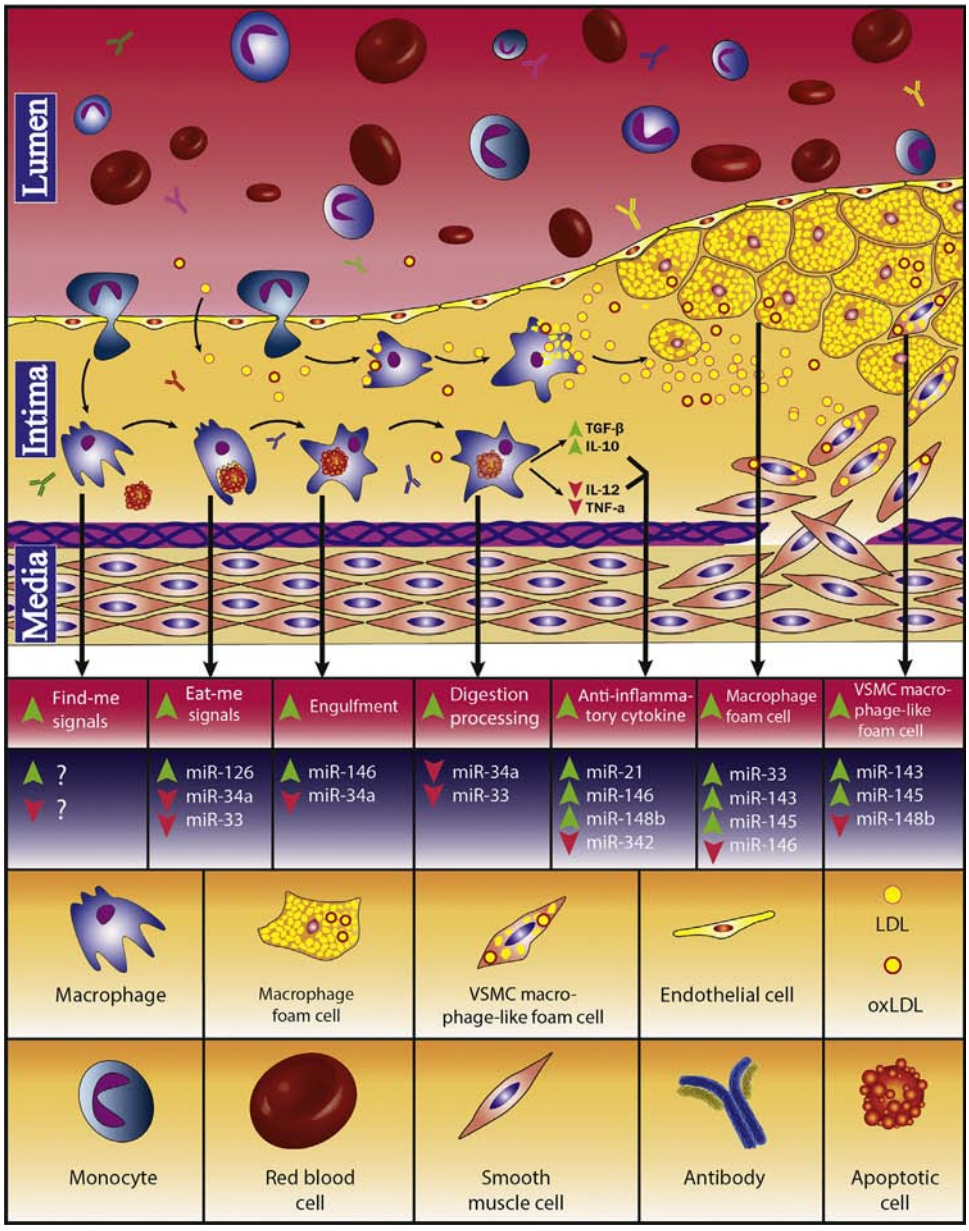

Figure 2. Regulation of Phagocyte Function within Atherogenesis by Different Types of MicroRNAs. Abbreviations: IL-10, interleukin 10; IL-12, interleukin 12; LDL, low-density lipoproteins; miR, microRNA; oxLDLs, oxidized low-density lipoproteins; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α; VSMCs, vascular smooth muscle cells.

reduced, cholesterol accumulation within atherosclerotic plaques is decreased, and the stability of atherosclerotic plaque is increased [20,21].

MiRs Regulating Phagocyte Function in Atherosclerosis

Among several miRs involved in the pathogenesis of atherosclerosis, only some miRs are directly involved in the regulation of phagocyte function (i.e., phagocyte accumulation within the arterial

wall, phagocyte differentiation into foam cells, efferocytosis of apoptotic foam cells by phagocytes, and a phagocyte-mediated inflammatory response). Phagocyte accumulation within the arterial wall is regulated by miR-126, miR-21, and miR-155 (Table 1 and Figure 2). Molecular pathways involved in phagocyte differentiation into foam cells, efferocytosis of apoptotic foam cells by phagocytes, and phagocyte-mediated inflammatory responses, which are strictly interconnected, are regulated by miR-126, -21, -155, -143, -145, -34a, -342-5p, -146, -33, and -148b (Table 1 and Figure 2). It is worth noting that some miRs may regulate phagocyte function at multiple levels (e.g., miR-126, -21, and -155) and may exert either proatherogenic, or antiatherogenic effects, depending on the pathogenetic step in which they are upregulated (e.g., miR-155) [17,22–26]. In addition, some miRs seem to have regulatory functions for other miRs (e.g., miR-342-5p regulates miR-155 expression).

MiRs Regulating Phagocyte Accumulation in Atherosclerotic Lesions

Endothelial activation, monocyte chemotaxis, and macrophage proliferation mediate phagocyte accumulation within early atherosclerotic lesions. Three miRs have been reported to have a crucial role in regulating this process, representing potential molecular targets of novel antiatherosclerotic strategies: miR-126, -21, and -155.

MiR-126 [27], which is overexpressed in damaged and activated endothelial cells (ECs), plays a crucial role in regulating monocyte chemotaxis and accumulation within early atherosclerotic lesions [28]. Accordingly, miR-126 suppresses the regulator of G protein signaling 16 (RGS16) function and increases the phosphorylation of extracellular signal-regulated kinase 1/2 (ERK1/2), leading to an increased production of chemokine C-X-C motif ligand 12 (CXCL12). CXCL12 and its receptor, C-X-C motif chemokine receptor 4 (CXCR4), induce the expression of anti-inflammatory cytokines and prevent monocyte chemotaxis in early atherosclerotic lesions [28]. Additionally, miR-126 indirectly inhibits monocyte chemotaxis by reducing endothelial activation through a decreased expression of vascular cell adhesion molecule 1 (VCAM-1) [29].

The expression of both strands of miR-21, including miR-21-3p and -21-5p, is increased in macrophages that accumulate within atherosclerotic lesions. MiR-21-3p has been shown to downregulate the expression of different circadian clock genes that control macrophage survival (R. Michael Blay, PhD thesis, Ludwig Maximilian University of Munich, 2016), including *circadian locomotor output cycles kaput (CLOCK)*; (R. Michael Blay, PhD thesis, Ludwig Maximilian University of Munich, 2016) and *brain and muscle Arnt-like protein-1 (BMAL1)*. The downregulation of these genes leads to a reduced expression of X-linked inhibitor of **apoptosis** (XIAP)-associated factor 1 (Xaf1), which inhibits the activation of caspase-3 by XIAP, an antiapoptotic protein [30]. This contributes to decreased macrophage apoptosis and to increased macrophage accumulation within atherosclerotic lesions.

MiR-155 inhibits macrophage accumulation in early atherosclerotic lesions by reducing colony-stimulating factor 1 (Csf1) receptor (Csf1r)-induced macrophage proliferation [17]. In addition, in the early phases of atherosclerosis, the upregulation of miR-155 in ECs leads to a reduced expression of endothelial nitric oxide synthase (eNOS), thereby indirectly promoting endothelial activation and monocyte chemotaxis [31].

MiRs Regulating Foam Cell Accumulation

Foam cell accumulation in the core of atherosclerotic plaques promotes the progression of atherosclerosis and is, in part, counteracted by phagocyte-mediated efferocytosis of apoptotic foam cells. Thus, different miRs regulating macrophage differentiation into foam cells and at

Table 1. The Main MiRs Involved in Efferocytosis and Atherosclerosis and the Effects Mediated by Their Direct Targets[a]

Increased miR levels (↑)/decreased miR levels (↓)	Effects on atherosclerosis progression	Direct/indirect effects on efferocytosis	Increased (↑)/reduced (↓) direct target expression	3′ UTR binding sequence	Professional/nonprofessional phagocyte	Effect on atherosclerosis	*In vivo/in vitro* model
↑miR-126 [28,37]	Inhibition	Promotion	↓ RGS16	5′-GCCAGTGTTTTTTGTGGTATGA-3′	Macrophage	Reduced macrophage-mediated inflammatory response	*APOE*$^{-/-}$ mice and human heart tissue
			↓ ADAM9	5′-AAUUAUAAGCUUUAAGGUA-3′			
↓miR-34a [5]	Unknown	Promotion	↑ AXL	Not available	Macrophage	Increased macrophage ability to engulf ACs	Resident murine and human tissue macrophages
			↑ SIRT1	Not available			
↑miR-21 [74]	Unknown	Promotion	↓ PTEN	Not available	Macrophage	Reduced macrophage-mediated inflammatory response	Peripheral blood monocyte-derived macrophages
			↓ PDCD4	Not available			
↓miR-21 (R. Michael Blay, PhD thesis, Ludwig Maximilian University of Munich, 2016)	Inhibition	Promotion	↑ BMAL1 ↑ CLOCK	5′-GCUGUU-3′ 5′-GCUGUU-3′	Macrophage	Reduced macrophage accumulation Reduced necrotic core formation	*miR-21*$^{-/-}$ mice
			↑ MBL2	5′-GAUGAGC-3′	Macrophage	Increased macrophage ability to engulf ACs	*miR-21*$^{-/-}$ mice
↑miR-155 [22]	Promotion	Inhibition	↓ HBP1	5′-AGCAUUAA-3′	Macrophage	Increased foam cell formation	Macrophages from atherosclerotic *APOE*$^{-/-}$ mice
↑miR-155 [50]			↓BCL6	5′CUGCAUUAG-3′		Increased macrophage-mediated inflammatory response	
↑miR-155 [31]			↑ eNOS	Not available	EC	Increased endothelial activation and monocyte chemotaxis	Human umbilical vein ECs

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Table 1. (continued)

Increased miR levels (↑)/decreased miR levels (↓)	Effects on atherosclerosis progression	Direct/indirect effects on efferocytosis	Increased (↑)/reduced (↓) direct target expression	3′ UTR binding sequence	Professional/nonprofessional phagocyte	Effect on atherosclerosis	*In vivo/in vitro* model
↑miR-155 [17]	Inhibition	Unknown	↓CSF1R	Not available	Macrophage	Reduced macrophage proliferation	*APOE*$^{-/-}$ mouse lesional macrophages
↑miR-342-5p [53]	Promotion	Inhibition	↓ AKT1	5′-GCACCCC-3′	Macrophage	Increased macrophage-mediated inflammatory response	*APOE*$^{-/-}$ mice
↑miR-33 [61]	Promotion	Inhibition	↓ATG5 ↓LAMP1 ↓PRKAA1	Not available	Macrophage	Increased foam cell formation	*LDLR*$^{-/-}$ mice
↓miR-33 [59]	Inhibition	Promotion	↑ ABCA1	5′-CAAUGCAA-3′	Macrophage	Increased cholesterol efflux	miR-33$^{-/-}$/*LDLR*$^{-/-}$ mice
			↑ ABCG1	5′-CGCAATGCAACGCAATGC-3′		Reduced foam cell formation	
↓miR-145/143 [68]	Inhibition	Promotion	↓ MYOCD	5′-ACUGGAC-3′	VSMC	Increased VSMC differentiation into macrophage-like cells	Mouse or human VSMCs
↓ miR-148b [71]	Promotion	Inhibition	↑ HSP90	5′-UGCACUG-3′	VSMC	Reduced VSMC proliferation and migration	Human VSMCs
↑miR-146 [66]	Inhibition	Promotion	↓ TLR4	5′-AAUUCAGUUGUC-3′	Macrophage	Reduced foam cell formation Reduced macrophage-mediated inflammatory response	oxLDL-stimulated macrophages

[a]Abbreviations: ATG5, autophagy related 5; BCL6, B cell leukemia/lymphoma 6; LAMP1, lysosomal-associated membrane protein 1; PRKAA1, protein kinase AMP-activated catalytic subunit alpha 1.

different steps during efferocytosis (i.e., recognition, engulfment, and digestion) may represent potential molecular targets for novel antiatherosclerotic therapies.

MiR-126 promotes macrophage-mediated efferocytosis by downregulating the expression of 'a disintegrin and metalloproteases' (ADAMs), membrane-anchored proteins of the zinc protease superfamily [32]. These proteins regulate the activity of different efferocytosis mediators, including transmembrane/extracellular proteins involved in the recognition and engulfment of ACs, such as MertK [33–35]. The proteolytic cleavage of MertK by ADAM domain-containing protein 9 (ADAM9) results in the production of an inactive soluble form of MertK (sMER), which may lead to defective recognition and engulfment of ACs, thereby impairing macrophage-mediated efferocytosis and promoting foam cell accumulation [36,37]. Instead, miR-126-mediated suppression of ADAM9 may restore macrophage-mediated efferocytosis and reduce foam cell accumulation in atherosclerotic plaques. Thus, potential antiatherosclerotic therapies aimed at reducing foam cell accumulation by improving macrophage-mediated efferocytosis may include miR-126 mimics [37].

MiR-21-5p inhibits macrophage-mediated efferocytosis by downregulating the expression of mannose-binding lectin 2 (MBL2); (R. Michael Blay, PhD thesis, Ludwig Maximilian University of Munich, 2016). MBL2 is a recognition receptor of the collectin family, which promotes the uptake of exogenous/endogenous danger debris by macrophages [38,39]. In the early phases of atherosclerosis, MBL2 expression on the surface of macrophages is crucial for the rapid clearance of apoptotic blebs and oxidized low-density lipoproteins (oxLDLs). Available evidence suggests that high expression levels of MBL2 on macrophages are atheroprotective [40]. Accordingly, MBL2 expression is reduced in murine models of atherosclerosis [41] and clinical studies investigating the relationship between MBL2 and atherosclerosis have shown a higher prevalence of loss-of-function *MBL2* genetic variants in patients with severe coronary artery disease compared with healthy controls [42,43].

Macrophage differentiation into foam cells may be enhanced by miR-155. MiR-155 downregulates the expression of high-mobility group (HMG)-box transcription protein 1 (HBP1), a transcription factor that controls macrophage phagocytic function [22,44,45]. The expression of miR-155 has been reported to be induced by oxLDLs. MiR-155-mediated downregulation of HBP1 has been associated with enhanced lipid uptake and ROS production by oxLDL-stimulated macrophages [22]. In addition, miR-155 has been shown to inhibit macrophage-mediated efferocytosis and to enhance foam cell accumulation within atherosclerotic lesions by suppressing the expression of Bcl6 [17]. Bcl6 is a potent transcription repressor, which may indirectly block the activation of RhoA, a small GTPase that regulates multiple cellular processes involving the actin cytoskeleton, including efferocytosis [46]. Bcl6 suppression by miR-155 leads to excess RhoA activation, which negatively affects macrophage cytoskeleton remodeling and impairs efferocytosis, thereby promoting the progression of atherosclerosis [17,47–49]. Accordingly, miR-155 deficiency in macrophages has been associated with increased Bcl6 expression and decreased progression of atherosclerosis in apolipoprotein E (ApoE) knockout ($ApoE^{-/-}$) mice [50]. In addition, a significant increase of the necrotic core area in advanced atherosclerotic lesions of low-density lipoprotein receptor knockout ($LDLR^{-/-}$) mice has been reported due to Bcl6 deficiency in macrophages [51].

There is evidence showing that miR-342-5p may indirectly inhibit macrophage-mediated efferocytosis by increasing the expression of miR-155 and suppressing the Bcl6/RhoA axis [17,46,52]. In fact, miR-342-5p upregulates miR-155 expression in macrophages by suppressing Akt1, which is an inhibitor of miR-155 [53].

MiR-34a inhibits macrophage-mediated efferocytosis by downregulating the expression of Axl, a receptor tyrosine kinase that is crucial for the recognition of ACs. In addition, miR-34a downregulates the expression of deacetylase sirtuin 1 (Sirt1) [5,54], which activates crucial regulators of the efferocytosis pathway, including AMP-activated protein kinase (AMPK) and liver X receptor-α (LXRα) [5,55,56]. Importantly, miR-34a expression by macrophages is itself downregulated by efferocytosis via various molecular signals, which are activated by AC lipid and nucleic acid digestion downstream of AMPK and LXRα. Consequently, a positive feedback loop that enhances the capacity of macrophages to remove ACs occurs due to efferocytosis and the subsequent downregulation in the expression of miR-34a [5]. This mechanism is crucial to fine-tune macrophage efferocytotic ability based on the presence, or absence, of ACs in different tissue environments.

MiR-33 increases macrophage differentiation into foam cells by suppressing the expression of different mediators of intracellular lipid metabolism and cholesterol efflux [57] within the reverse cholesterol transport (RCT) pathway [58], including ATP-binding cassette subfamily A member 1 (ABCA1) and ATP-binding cassette subfamily G member 1 (ABCG1) [59]. Consequently, cholesterol efflux has been shown to be increased, whereas the accumulation of cholesterol esters and other lipid subspecies has been reported to be reduced, in miR-33-deficient macrophages [59,60]. In addition, miR-33 may modulate macrophage differentiation into foam cells by regulating lipophagy [61]. Regulation of lipophagy by miR-33 occurs upstream of ABCA1-dependent cholesterol efflux. In fact, lipid catabolism through the acid lipolysis pathway is decreased by miR-33, leading to reduced formation of free cholesterol [61]. In aortic plaque macrophages of low-density lipoprotein receptor (LDLR)-deficient mice, lipophagy has been reported to be improved after treatment with miR-33 inhibitors due to the accumulation of key autophagy effectors, including microtubule-associated protein 1A/1B-light chain 3 (LC3) [62]. In addition, in different animal studies, miR-33 antagonism has been reported to decrease the progression of atherosclerosis [63–65].

MiR-146 plays a key role in the regulation of macrophage differentiation into foam cells by inhibiting toll-like receptor 4 (TLR4) and the activation of TLR4-dependent downstream signaling pathways [66]. TLR4 is activated by macrophage exposure to oxLDL. Upon TLR4 activation, the expression of crucial mediators of cytoskeleton rearrangement and lipid uptake is induced through the mitogen-activated protein kinase (MAPK) pathway. This facilitates lipid accumulation within macrophages and macrophage differentiation into foam cells [66].

Finally, some miRs (i.e., miR-143, -145, and -148b) indirectly control foam cell accumulation within atherosclerotic plaque, as well as the progression of atherosclerosis, by regulating VSMC proliferation, migration, and phenotype switching. MiR-143 and miR-145 enhance the expression of contractile proteins [e.g., myocardin (MYOCD)] in VSMCs [67], which downregulates the phenotypic switching of VSMCs towards macrophage-like foam cells [68,69]. Reduced expression of miR-143 and miR-145 has been associated with the downregulation of the MYOCD/serum response factor (SRF) complex in VSMCs, leading to an increase in VSMC differentiation into foam cells and to the expansion of the necrotic core of atherosclerotic plaques [70]. MiR-148b has been reported to reduce the expression of crucial mediators of the proliferation and migration of VSMCs [e.g., cell nuclear antigen (PCNA), ki-67, matrix metalloproteinase (MMP)-2, and MMP-9] via the downregulation of heat shock protein 90 (HSP90) [71–73], a ubiquitous molecular chaperone involved in cell signal transduction and transcriptional regulation. Accordingly, both proliferation and migration of VSMCs have been reported to be significantly inhibited by a miR-148b mimic [71,72]. Of significance, miR-148b-mediated inhibition of VSMC proliferation and migration seems to have a significant inhibitory effect on the progression of atherosclerosis. Importantly, miR-148b has been reported to be downregulated in carotid

atherosclerotic plaques from endarterectomized patients [71,72]. Therefore, miR-148b restoration may represent a potential antiatherosclerotic strategy.

MiRs Regulating the Phagocyte-Mediated Inflammatory Response

Some miRs regulating foam cell accumulation in atherosclerotic plaques (i.e., miR-21, -342-5p, -146, and -148b) are also involved in the modulation of a phagocyte-mediated inflammatory response. Therefore, their selective modulation might represent a novel therapeutic strategy to target inflammation in the context of atherosclerotic lesions.

MiR-21 modulates the macrophage-mediated inflammatory response by downregulating the expression of phosphatase and tensin homolog (PTEN) and programmed cell death 4 (PDCD4) [5]. The inhibition of these two molecular targets leads to the activation of net intracellular pathways resulting in the suppression of lipopolysaccharide (LPS)-induced nuclear factor kappa B (NF-κB) signaling pathways. This results in a reduced expression of tumor necrosis factor (TNF)-α [74], as well as an increase in the production of anti-inflammatory cytokines (e.g., IL-10).

MiR-342-5p modulates the macrophage-mediated inflammatory response [75] by suppressing the serine–threonine protein kinase Akt1. The inhibition of Akt1 stimulates the expression of different proinflammatory mediators, including nitric oxide synthase (Nos)2 and IL-6, thereby promoting the progression of atherosclerosis. Thus, not surprisingly, Akt1 loss in *ApoE*$^{-/-}$ mice has been associated with the expression of high levels of proinflammatory mediators and the continued progression of atherosclerosis [76].

MiR-146 plays a key role in the regulation of the macrophage-mediated inflammatory response, primarily by inhibiting TLR4-induced expression of proinflammatory mediators [e.g., IL-6, IL-8, monocyte chemoattractant protein-1 (MCP-1), and MMP-9] via the MAPK pathway and the IL-1 receptor associated kinase (IRAK)/TNF receptor associated factor 6 (TRAF6) axis-induced [66,77,78] production of anti-inflammatory cytokines (e.g., IL-10) [79]. However, other TLR4-dependent molecular pathways involved in the activation of the macrophage-mediated inflammatory response, including the p38 MAPK, NF-κB [78], c-Jun N-terminal kinase (JNK), and ERK1/2 [80], may be inhibited by miR-146 [81].

Finally, miR-148b inhibits the phagocyte-mediated inflammatory response in atherosclerotic plaques by suppressing HSP90-induced NF-κB activation and chemokine production [82]. Therefore, miR-148b restoration might be a potential strategy to selectively target inflammation in the context of atherosclerosis.

Concluding Remarks

Different miRs modulate atherosclerotic plaque formation and progression by regulating professional and nonprofessional phagocyte function (Figure 2). Thus, these miRs represent potential molecular targets for antiatherosclerotic therapy. Some miRs regulate the early phases of atherogenesis by modulating endothelial activation, monocyte chemotaxis, and macrophage proliferation (Table 1). Other miRs regulate atherosclerosis progression by modulating foam cell accumulation and the phagocyte-mediated inflammatory response (Table 1). The selective therapeutic modulation of some of these miRs has been attempted in various experimental studies with promising results. To date, the selectivity of delivery systems for miR-based therapies targeting macrophages remains a significant challenge in the drug development process. Various approaches are currently under investigation, including miR-carrying functionalized nanoparticles, which are specifically recognized by macrophage surface receptors. Future research is needed to investigate their feasibility and efficacy [82,83] (see Outstanding Questions).

Outstanding Questions

What are the detailed molecular mechanisms associated with miRs and their targets in different cell types involved in efferocytosis, as well as atherosclerotic plaque initiation, progression, and resolution?

How can novel therapies directed at miRs deal with nonselective and even unexpected biological effects of some miRs?

Since only a few miRs are tissue specific, how can selective delivery of therapeutic miR modulators to target cells (e.g., macrophages) be achieved in the drug development process?

How effective and safe are treatment paradigms targeting miRs involved in efferocytosis within atherosclerotic lesions?

References

1. Mundkur, L. *et al.* (2013) Mucosal tolerance to a combination of ApoB and HSP60 peptides controls plaque progression and stabilizes vulnerable plaque in Apob^{tm2Sgy}Ldlr^{tm1Her}/J mice. *PLoS One* 8, e58364
2. Tajbakhsh, A. *et al.* (2018) Efferocytosis in atherosclerotic lesions: malfunctioning regulatory pathways and control mechanisms. *Pharmacol. Ther.* 188, 12–25
3. Gheibi Hayat, S.M. *et al.* (2019) Efferocytosis: molecular mechanisms and pathophysiological perspectives. *Immunol. Cell Biol.* 97, 124–133
4. Abdolmaleki, F. *et al.* (2018) The role of efferocytosis in autoimmune diseases. *Front. Immunol.* 9, 1645
5. McCubbrey, A.L. *et al.* (2016) MicroRNA-34a negatively regulates efferocytosis by tissue macrophages in part via SIRT1. *J. Immunol.* 196, 1366–1375
6. Bartel, D.P. (2009) MicroRNAs: target recognition and regulatory functions. *Cell* 136, 215–233
7. McManus, D.D. and Freedman, J.E. (2015) MicroRNAs in platelet function and cardiovascular disease. *Nat. Rev. Cardiol.* 12, 711–717
8. Chau, B.N. *et al.* (2013) Therapeutic modulation of microRNAs. *Drug Discov. Today: Ther. Strateg.* 10, e127–e132
9. O'Brien, J. *et al.* (2018) Overview of microRNA biogenesis, mechanisms of actions, and circulation. *Front. Endocrinol. (Lausanne)* 9, 402
10. Fernandes, J.C.R. *et al.* (2019) Long non-coding RNAs in the regulation of gene expression: physiology and disease. *Non-Coding RNA* 5, 17
11. Christopher, A.F. *et al.* (2016) MicroRNA therapeutics: discovering novel targets and developing specific therapy. *Perspect. Clin. Res.* 7, 68–74
12. Seimon, T.A. *et al.* (2010) Atherogenic lipids and lipoproteins trigger CD36-TLR2-dependent apoptosis in macrophages undergoing endoplasmic reticulum stress. *Cell Metab.* 12, 467–482
13. Zhou, J. *et al.* (2005) Activation of the unfolded protein response occurs at all stages of atherosclerotic lesion development in apolipoprotein E-deficient mice. *Circulation* 111, 1814–1821
14. Liang, C.P. *et al.* (2012) Impaired MEK signaling and SERCA expression promote ER stress and apoptosis in insulin-resistant macrophages and are reversed by exenatide treatment. *Diabetes* 61, 2609–2620
15. Li, A.C. and Glass, C.K. (2002) The macrophage foam cell as a target for therapeutic intervention. *Nat. Med.* 8, 1235–1242
16. Moore, K.J. *et al.* (2013) Macrophages in atherosclerosis: a dynamic balance. *Nat. Rev. Immunol.* 13, 709–721
17. Wei, Y. *et al.* (2015) Regulation of *Csf1r* and *Bcl6* in macrophages mediates the stage-specific effects of microRNA-155 on atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* 35, 796–803
18. Lee, H.N. and Surh, Y.J. (2013) Resolvin D1-mediated NOX2 inactivation rescues macrophages undertaking efferocytosis from oxidative stress-induced apoptosis. *Biochem. Pharmacol.* 86, 759–769
19. Noda, M. *et al.* (2016) Fractalkine attenuates excito-neurotoxicity via microglial clearance of damaged neurons and antioxidant enzyme heme oxygenase-1 expression. *J. Biol. Chem.* 291, 14388
20. Frangogiannis, N.G. (2008) The immune system and cardiac repair. *Pharmacol. Res.* 58, 88–111
21. Brophy, M.L. *et al.* (2017) Eating the dead to keep atherosclerosis at bay. *Front. Cardiovasc. Med.* 4, 2
22. Tian, F.J. *et al.* (2014) Elevated microRNA-155 promotes foam cell formation by targeting HBP1 in atherogenesis. *Cardiovasc. Res.* 103, 100–110
23. Du, F. *et al.* (2014) MicroRNA-155 deficiency results in decreased macrophage inflammation and attenuated atherogenesis in apolipoprotein E-deficient mice. *Arterioscler. Thromb. Vasc. Biol.* 34, 759–767
24. Donners, M.M. *et al.* (2012) Hematopoietic miR155 deficiency enhances atherosclerosis and decreases plaque stability in hyperlipidemic mice. *PLoS One* 7, e35877
25. O'Neill, L.A. *et al.* (2011) MicroRNAs: the fine-tuners of toll-like receptor signalling. *Nat. Rev. Immunol.* 11, 163–175
26. Androulidaki, A. *et al.* (2009) The kinase Akt1 controls macrophage response to lipopolysaccharide by regulating microRNAs. *Immunity* 31, 220–231
27. Jansen, F. *et al.* (2012) Endothelial microparticle uptake in target cells is annexin I/phosphatidylserine receptor dependent and prevents apoptosis. *Arterioscler. Thromb. Vasc. Biol.* 32, 1925–1935
28. Zernecke, A. *et al.* (2009) Delivery of microRNA-126 by apoptotic bodies induces CXCL12-dependent vascular protection. *Sci. Signal.* 2, ra81
29. Harris, T.A. *et al.* (2008) MicroRNA-126 regulates endothelial expression of vascular cell adhesion molecule 1. *Proc. Natl. Acad. Sci. U. S. A.* 105, 1516–1521
30. Arora, V. *et al.* (2007) Degradation of survivin by the X-linked inhibitor of apoptosis (XIAP)-XAF1 complex. *J. Biol. Chem.* 282, 26202–26209
31. Lee, K.S. *et al.* (2014) Functional role of NF-κB in expression of human endothelial nitric oxide synthase. *Biochem. Biophys. Res. Commun.* 448, 101–107
32. Hamada, S. *et al.* (2012) MiR-126 acts as a tumor suppressor in pancreatic cancer cells via the regulation of ADAM9. *Mol. Cancer Res.* 10, 3–10
33. Seals, D.F. and Courtneidge, S.A. (2003) The ADAMs family of metalloproteases: multidomain proteins with multiple functions. *Genes Dev.* 17, 7–30
34. Moss, M.L. and Lambert, M.H. (2002) Shedding of membrane proteins by ADAM family proteases. *Essays Biochem.* 38, 141–153
35. Nguyen, K.Q. *et al.* (2014) Overexpression of MERTK receptor tyrosine kinase in epithelial cancer cells drives efferocytosis in a gain-of-function capacity. *J. Biol. Chem.* 289, 25737–25749
36. Tajbakhsh, A. *et al.* (2019) Effect of soluble cleavage products of important receptors/ligands on efferocytosis: their role in inflammatory, autoimmune and cardiovascular disease. *Ageing Res. Rev.* 50, 43–57
37. Suresh Babu, S. *et al.* (2016) MicroRNA-126 overexpression rescues diabetes-induced impairment in efferocytosis of apoptotic cardiomyocytes. *Sci. Rep.* 6, 36207
38. Ogden, C.A. *et al.* (2001) C1q and mannose binding lectin engagement of cell surface calreticulin and CD91 initiates macropinocytosis and uptake of apoptotic cells. *J. Exp. Med.* 194, 781–795
39. Fraser, D.A. and Tenner, A.J. (2010) Innate immune proteins C1q and mannan-binding lectin enhance clearance of atherogenic lipoproteins by human monocytes and macrophages. *J. Immunol.* 185, 3932–3939
40. Saevarsdottir, S. *et al.* (2005) Mannan binding lectin as an adjunct to risk assessment for myocardial infarction in individuals with enhanced risk. *J. Exp. Med.* 201, 117–125
41. Matthijsen, R.A. *et al.* (2009) Macrophage-specific expression of mannose-binding lectin controls atherosclerosis in low-density lipoprotein receptor-deficient mice. *Circulation* 119, 2188–2195
42. Madsen, H.O. *et al.* (1998) Association of mannose-binding-lectin deficiency with severe atherosclerosis. *Lancet* 352, 959–960
43. Best, L.G. *et al.* (2004) Prospective analysis of mannose-binding lectin genotypes and coronary artery disease in American Indians: the Strong Heart Study. *Circulation* 109, 471–475
44. Chen, Y.C. *et al.* (2010) Macrophage migration inhibitory factor is a direct target of HBP1-mediated transcriptional repression that is overexpressed in prostate cancer. *Oncogene* 29, 3067
45. Berasi, S.P. *et al.* (2004) HBP1 repression of the p47phox gene: cell cycle regulation via the NADPH oxidase. *Mol. Cell. Biol.* 24, 3011–3024
46. Pixley, F.J. *et al.* (2005) BCL6 suppresses RhoA activity to alter macrophage morphology and motility. *J. Cell Sci.* 118, 1873–1883
47. Bijkerk, R. *et al.* (2012) MicroRNA-155 functions as a negative regulator of RhoA signaling in TGF-β-induced endothelial to mesenchymal transition. *Microrna* 1, 2–10
48. Kong, W. *et al.* (2008) MicroRNA-155 is regulated by the transforming growth factor β/Smad pathway and contributes to epithelial cell plasticity by targeting RhoA. *Mol. Cell. Biol.* 28, 6773–6784

49. Feo, F. *et al.* (1976) Effect of a cholesterol-rich diet on cholesterol content and phagocytic activity of rat macrophages. *Agents Actions* 6, 135–142
50. Nazari-Jahantigh, M. *et al.* (2012) MicroRNA-155 promotes atherosclerosis by repressing *Bcl6* in macrophages. *J. Clin. Invest.* 122, 4190–4202
51. Barish, G.D. *et al.* (2012) The Bcl6-SMRT/NCoR cistrome represses inflammation to attenuate atherosclerosis. *Cell Metab.* 15, 554–562
52. Nakaya, M. *et al.* (2006) Opposite effects of rho family GTPases on engulfment of apoptotic cells by macrophages. *J. Biol. Chem.* 281, 8836–8842
53. Wei, Y. *et al.* (2013) The microRNA-342-5p fosters inflammatory macrophage activation through an Akt1- and microRNA-155-dependent pathway during atherosclerosis. *Circulation* 127, 1609–1619
54. Schober, A. and Weber, C. (2016) Mechanisms of microRNAs in atherosclerosis. *Annu. Rev. Pathol.: Mech. Dis.* 11, 583–616
55. Jiang, S. *et al.* (2013) Mitochondria and AMP-activated protein kinase-dependent mechanism of efferocytosis. *J. Biol. Chem.* 288, 26013–26026
56. A-Gonzalez, N. *et al.* (2009) Apoptotic cells promote their own clearance and immune tolerance through activation of the nuclear receptor LXR. *Immunity* 31, 245–258
57. Gerin, I. *et al.* (2010) Expression of miR-33 from an *SREBP2* intron inhibits cholesterol export and fatty acid oxidation. *J. Biol. Chem.* 285, 33652–33661
58. Rader, D.J. and deGoma, E.M. (2014) Future of cholesteryl ester transfer protein inhibitors. *Annu. Rev. Med.* 65, 385–403
59. Price, N.L. *et al.* (2017) Genetic dissection of the impact of miR-33a and miR-33b during the progression of atherosclerosis. *Cell Rep.* 21, 1317–1330
60. Mariño, G. and Kroemer, G. (2013) Mechanisms of apoptotic phosphatidylserine exposure. *Cell Res.* 23, 1247–1248
61. Ouimet, M. *et al.* (2017) MicroRNA-33 regulates macrophage autophagy in atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* 37, 1058–1067
62. Ouimet, M. *et al.* (2016) *Mycobacterium tuberculosis* induces the miR-33 locus to reprogram autophagy and host lipid metabolism. *Nat. Immunol.* 17, 677–686
63. Rayner, K.J. *et al.* (2011) Antagonism of miR-33 in mice promotes reverse cholesterol transport and regression of atherosclerosis. *J. Clin. Invest.* 121, 2921–2931
64. Rotllan, N. *et al.* (2013) Therapeutic silencing of microRNA-33 inhibits the progression of atherosclerosis in *Ldlr*$^{-/-}$ mice–brief report. *Arterioscler. Thromb. Vasc. Biol.* 33, 1973–1977
65. Marquart, T.J. *et al.* (2010) MiR-33 links SREBP-2 induction to repression of sterol transporters. *Proc. Natl. Acad. Sci.* 107, 12228–12232
66. Yang, K. *et al.* (2011) MiR-146a inhibits oxidized low-density lipoprotein-induced lipid accumulation and inflammatory response via targeting toll-like receptor 4. *FEBS Lett.* 585, 854–860
67. Cordes, K.R. *et al.* (2009) MiR-145 and miR-143 regulate smooth muscle cell fate and plasticity. *Nature* 460, 705–710
68. Vengrenyuk, Y. *et al.* (2015) Cholesterol loading reprograms the microRNA-143/145–myocardin axis to convert aortic smooth muscle cells to a dysfunctional macrophage-like phenotype. *Arterioscler. Thromb. Vasc. Biol.* 35, 535–546
69. Rangrez, A.Y. *et al.* (2011) MiR-143 and miR-145: molecular keys to switch the phenotype of vascular smooth muscle cells. *Circ. Cardiovasc. Genet.* 4, 197–205
70. Moore, K.J. and Tabas, I. (2011) Macrophages in the pathogenesis of atherosclerosis. *Cell* 145, 341–355
71. Zhang, X. *et al.* (2017) Down-regulation of hsa-miR-148b inhibits vascular smooth muscle cells proliferation and migration by directly targeting HSP90 in atherosclerosis. *Am. J. Transl. Res.* 9, 629–637
72. Cimino, D. *et al.* (2013) MiR148b is a major coordinator of breast cancer progression in a relapse-associated microRNA signature by targeting ITGA5, ROCK1, PIK3CA, NRAS, and CSF1. *FASEB J.* 27, 1223–1235
73. Barbareschi, M. *et al.* (1992) Tumour suppressor gene products, proliferation, and differentiation markers in lung neuroendocrine neoplasms. *J. Pathol.* 166, 343–350
74. Das, A. *et al.* (2014) Engulfment of apoptotic cells by macrophages: a role of microRNA-21 in the resolution of wound inflammation. *J. Immunol.* 192, 1120–1129
75. Nazari-Jahantigh, M. *et al.* (2015) MicroRNA-specific regulatory mechanisms in atherosclerosis. *J. Mol. Cell. Cardiol.* 89, 35–41
76. Fernandez-Hernando, C. *et al.* (2007) Loss of Akt1 leads to severe atherosclerosis and occlusive coronary artery disease. *Cell Metab.* 6, 446–457
77. Lee, J.Y. *et al.* (2003) Reciprocal modulation of toll-like receptor-4 signaling pathways involving MyD88 and phosphatidylinositol 3-kinase/AKT by saturated and polyunsaturated fatty acids. *J. Biol. Chem.* 278, 37041–37051
78. Taganov, K.D. *et al.* (2006) NF-κB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. *Proc. Natl. Acad. Sci. U. S. A.* 103, 12481–12486
79. Seibold, K. and Ehrenschwender, M. (2015) p62 regulates CD40-mediated NFκB activation in macrophages through interaction with TRAF6. *Biochem. Biophys. Res. Commun.* 464, 330–335
80. Cekic, C. *et al.* (2009) Selective activation of the p38 MAPK pathway by synthetic monophosphoryl lipid A. *J. Biol. Chem.* 284, 31982–31991
81. Lin, Y.C. *et al.* (2010) Anti-inflammatory actions of Syk inhibitors in macrophages involve non-specific inhibition of toll-like receptors-mediated JNK signaling pathway. *Mol. Immunol.* 47, 1569–1578
82. Sharma, G. *et al.* (2010) Targeting of macrophage foam cells in atherosclerotic plaque using oligonucleotide-functionalized nanoparticles. *Nano Life* 1, 207–214
83. Beldman, T.J. *et al.* (2017) Hyaluronan nanoparticles selectively target plaque-associated macrophages and improve plaque stability in atherosclerosis. *ACS Nano* 11, 5785–5799
84. Abdolmaleki, F. *et al.* (2019) Atherosclerosis and immunity: a perspective. *Trends Cardiovasc. Med.* 29, 363–371
85. Tabas, I. and Bornfeldt, K.E. (2016) Macrophage phenotype and function in different stages of atherosclerosis. *Circ. Res.* 118, 653–667