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REVIEW

MicroRNA regulation of macrophages in human pathologies

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Abstract Macrophages play a crucial role in the innate immune system and contribute to a broad spectrum of pathologies, like in the defence against infectious agents, in inflammation resolution, and wound repair. In the past several years, microRNAs (miRNAs) have been demonstrated to play important roles in immune diseases by regulating macrophage functions. In this review, we will summarize the role of miRNAs in the differentiation of monocytes into macrophages, in the classical and alternative activation of macrophages, and in the regulation of phagocytosis and apoptosis. Notably, miRNAs preferentially target genes related to the cellular cholesterol metabolism, which is of key importance for the inflammatory activation and phagocytic activity of macrophages. miRNAs functionally link various mechanisms involved in macrophage activation and contribute to initiation and resolution of inflammation. miRNAs represent promising diagnostic and therapeutic targets in different conditions, such as infectious diseases, atherosclerosis, and cancer.

Keywords Immune disorders · MicroRNA · Macrophage · Lipid metabolism · Inflammation

Abbreviations

M-CSF	Macrophage colony stimulating factor
GM-CSF	Granulocyte-macrophage colony-stimulating factor
CSF1R	Macrophage colony stimulating factor receptor
PU.1	Purine-rich PU-box-binding protein 1
SREBP	Sterol regulatory element binding protein
PRRs	Pattern-recognition receptors
TLRs	Toll-like receptors
IFN-γ	Interferon gamma
IL	Interleukin
TNF	Tumor necrosis factor
ROS	Reactive oxygen species
NF-κB	Nuclear factor-κB
LPS	Lipopolysaccharide
PPARγ	Peroxisome proliferator-activated receptor gamma
JAK	Janus kinases
STAT1	Signal transducers and activators of transcription 1
PI3K	Phosphatidylinositol 3-kinase
Akt	Thymoma viral proto-oncogene
HIF-1	Hypoxia-inducible factor-1
OXPHOS	Oxidative phosphorylation
miRNAs	MicroRNAs
UTR	Untranslated region
C/EBP-α	CCAAT/enhancer binding protein alpha
ACVR1B	Activin A receptor type IB
Ets E26	Avian leukemia oncogene
Bmpr2	Bone morphogenetic protein receptor type-2
OxLDL	Oxidized low-density lipoprotein
SOCS1	Suppressor of cytokine signaling 1
Bcl6	B cell leukemia/lymphoma 6
FADD	Fas-associated death domain-containing protein
Ccl2	Chemokine (C–C motif) ligand 2
Erk	Mitogen-activated protein kinase 1
APOE	Apolipoprotein E

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TRAF6	TNF receptor-associated factor 6
IRAK1	IL-1 receptor-associated kinase 1
IRF5	Interferon regulatory factor 5
TMEM49	Transmembrane protein 19
PDCD4	Programmed cell death 4
PTEN	Phosphatase and tensin homolog
Egr2	Early growth response 2
LXR	Liver X receptor
ABCA1	ATP binding cassette subfamily A member 1
ABCG1	ATP binding cassette subfamily G member 1
ACAT1	Acetyl-CoA acetyltransferase 1
CE	Cholesteryl ester

Introduction

The eponymous function of macrophages is phagocytosis, for instance, of microorganisms, foreign material, or apoptotic cells, which plays a major role in the defence against infectious agents, in development, tissue homeostasis, inflammation resolution, and wound repair [1–3]. Tissue-resident macrophages exist in various tissues throughout the body where they are involved in immune surveillance (for instance, in the liver, lung, brain, and epidermis) and in tissue homeostasis through bone remodelling and regulation of haematopoiesis [4] (Fig. 1). In mice, most tissue-resident macrophages, including liver Kupffer cells and alveolar, splenic, and peritoneal macrophages, originate from embryonic precursors and self-renew by proliferation, whereas intestinal macrophages, which are physiologically exposed to microbial products, are constantly replaced by circulating monocytes [5–8]. In addition, inflammation triggers the accumulation of recruited macrophages derived from circulating monocytes, which are released from reservoirs in blood and bone marrow [2, 9, 10] (Fig. 1).

The differentiation of monocytes into macrophages is morphologically characterized by an increase in cell size and vacuole content, plasma membrane extensions, and an increased number of mitochondria, and functionally by an enhanced phagocytosis capacity and inflammatory activation [11, 12]. Growth factors, like macrophage colony stimulating factor (M-CSF) and granulocyte–macrophage colony-stimulating factor (GM-CSF), induce the production of monocytes in the bone marrow, the differentiation of monocytes into macrophages and the proliferation of resident and recruited macrophages during inflammation [13–15]. M-CSF alone is sufficient for the differentiation of monocytes into macrophages and the surface expression of the M-CSF receptor (CSF1R) driven by the myeloid transcription factor purine-rich PU-box-binding protein 1 (PU.1) increased during the differentiation process [16–18]. M-CSF-mediated monocyte-to-macrophage differentiation is associated with the differential regulation of 2 % of the transcripts, including cell cycle genes and genes related to lipid and arachidonate metabolism [19]. Moreover, increased sterol regulatory element binding protein (SREBP)-1c-dependent fatty acid synthesis promotes phagocytosis and the development of cellular organelles,

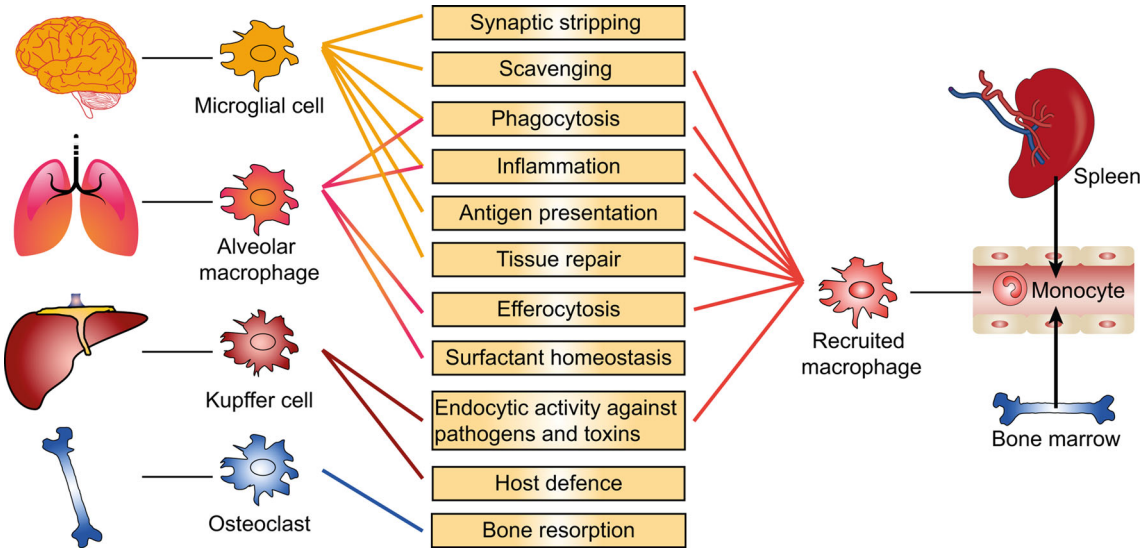

Fig. 1 Macrophages perform important homeostatic and inflammatory functions. Tissue-resident macrophages exist in various tissues throughout the body where they maintain tissue homoeostasis, surveil the local microenvironment, and promote the accumulation of recruited macrophages in response to injuries or infections. Circulating monocytes from reservoirs in blood, spleen and bone marrow differentiate into macrophages at the inflammatory sites where they remove invading organisms or tissue debris, and promote inflammation resolution upon polarization into different subtypes of activated macrophages

such as lysosomes, the endoplasmic reticulum, and the Golgi network during macrophage differentiation [20].

To identify pathogens, macrophages express pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs), that recognize conserved motifs (so called pathogen-associated molecular patterns) on bacteria, fungi, and viruses [21]. PRRs like C-type lectin receptors and scavenger receptors trigger the phagocytosis of microbes, whereas TLRs, such as TLR2 and TLR4, may identify specific pathogens in phagosomes, which activate signalling pathways that promote phagosome maturation and inflammatory gene expression, and result in enhanced killing of infectious agents through oxidative and non-oxidative mechanisms [22–24]. Phagocytosis and lysosomal digestion of pathogens can lead to the presentation of processed antigens by major histocompatibility complex class II molecules on the surface of macrophages, which regulates the adaptive immune response by activating CD4+ T cells [25]. Conversely, T cells derived cytokines, such as interferon gamma (IFN-γ) and interleukin (IL)-17, greatly increase the antimicrobial activity of macrophages, which thus become a main effector cell type of the adaptive immune system [25]. In addition to phagocytosis, activated macrophages promote inflammation by secreting numerous factors, such as cytokines, proteinases, bioactive lipids, reactive oxygen and nitrogen intermediates, and complement components [26, 27] (Fig. 1). However, the effects of recruited macrophages are tightly controlled to prevent tissue destruction. Moreover, a phenotypic switch of inflammatory macrophages into a phenotype that promotes inflammation resolution is essential for the termination of the immune response [28].

The heterogeneity of recruited macrophages

The functional phenotypes of recruited macrophages range between two extremes, the pro-inflammatory and anti-inflammatory subtype, which contribute to different stages of the inflammatory response [29–33]. The initiation phase of an inflammatory response is characterized by pro-inflammatory macrophages, which secrete inflammatory cytokines, such as IL-12, IL-1β and tumor necrosis factor (TNF)-α, and exhibit increased microbicidal activity through production of reactive oxygen species (ROS) and reactive nitrogen species [33–35]. Anti-inflammatory macrophages play important roles in inflammation resolution and tissue repair by secreting anti-inflammatory cytokines, such as IL-10, which suppresses the activation of the nuclear factor-κB (NF-κB) signalling pathway [30, 34, 36]. In vitro, IL-4-mediated macrophage polarization into an anti-inflammatory phenotype (also known as alternatively activated or M2 macrophages) has only a limited effect on gene expression, whereas pro-inflammatory macrophage polarization (also known as classically activated or M1 macrophages) by lipopolysaccharide (LPS) and IFN-γ stimulation alters ~5 % of the macrophage transcriptome [19]. These changes of the gene expression during macrophage polarization are largely driven by the activation of transcription factors, such as NF-κB and peroxisome proliferator-activated receptor gamma (PPARγ) [37, 38]. In M1 macrophages, LPS-induced TLR4 signalling activates the NF-κB pathway and IFN-γ triggers Janus kinases (JAK)-mediated dimerization of signal transducers and activators of transcription 1 (STAT1), which results in increased expression of inflammatory genes and metabolic reprogramming [38, 39]. In addition to M-CSF-driven signalling via the phosphatidylinositol 3-kinase (PI3K)-thymoma viral proto-oncogene (Akt) pathway, IL-4 promotes anti-inflammatory gene expression and M2 polarization by activating STAT6 and PPARγ [37]. Notably, Akt1 activation promotes a M2 phenotype, whereas Akt2 induces a M1 macrophage subtype, indicating that the effect of Akt on macrophage polarization is isoform-specific [40, 41]. Moreover, activation of the transcription factors nuclear receptor subfamily 4 group A member 1 and Krüppel-like factor 4 promotes a M2 phenotype [42–46]. In contrast to anti-inflammatory macrophages, pro-inflammatory cells promote atherosclerosis and a switch from an anti-inflammatory into a pro-inflammatory macrophage phenotype in adipose tissue increases adipocyte dysfunction and drives obesity-induced insulin resistance, demonstrating that these macrophage subtypes have opposing roles in chronic inflammatory diseases [47–51]. In contrast to the effect of GM-CSF, treatment with M-CSF promotes the differentiation into macrophages with characteristics of the M2 phenotype, which may be the default pathway in macrophage differentiation [19].

In addition to the production and secretion of pro-inflammatory cytokines, energy metabolism differs fundamentally between M1 and M2 macrophages [35]. Activation of the transcription factor hypoxia-inducible factor-1 (HIF-1) by inflammatory activation shifts ATP synthesis from mitochondrial oxidative phosphorylation (OXPHOS) to aerobic glycolysis in macrophages and results in increased production of ROS through the mitochondrial electron transport chain by utilizing fatty acids, which results in increased NF-κB activation and enhanced bactericidal activity [52–57]. Moreover, nitric oxide production in inflammatory macrophages may contribute to mitochondrial ROS production by inhibiting cytochrome c oxidase [58–60]. M2 macrophages produce ATP by utilizing long-chain fatty acids in OXPHOS, which promotes an anti-inflammatory phenotype and improves macrophage survival [53, 55, 61–63], indicating that differences in

nutrient utilization control macrophage activation and the phenotypic switch of macrophages during the progression of inflammation [35].

Resolution of inflammation requires the removal of tissue debris and apoptotic leukocytes (mainly neutrophils) by macrophages [64]. In contrast to infectious pathogens, apoptotic cells are engulfed through a distinct set of macrophage receptors, such as MERTK and CD36, which detect phosphatidylserine exposed on the surface of apoptotic cells [64, 65]. Induction of an M2 phenotype increases MERTK expression and enhances the clearance of apoptotic cells by macrophages (also known as efferocytosis) [66]. Moreover, the removal of apoptotic cells promotes anti-inflammatory gene expression in the efferocyte and thus contributes to inflammation resolution [67, 68]. Defective efferocytosis may lead to secondary necrosis of apoptotic leukocytes and the release of intracellular antigens, such as high-mobility group protein 1 and DNA, from the dying cells, which trigger an inflammatory response in macrophages and promote self-perpetuating chronic inflammation [69]. Thus, impaired efferocytosis may contribute to chronic inflammatory diseases, such as atherosclerosis and lung inflammation, systemic lupus erythematosus, and rheumatoid arthritis [70, 71]. Therefore, tight control of the different aspects of macrophage function is critical for the proper initiation and resolution of inflammation.

Although the M1 and M2 macrophage polarization concept helps to describe the immune response in a variety of diseases, a much broader transcriptional repertoire in macrophages exists and results in numerous functional subtypes, including macrophages (termed Mox macrophages) with impaired phagocytic and reduced inflammatory activity that develop in response to oxidized phospholipids and CXCL4-induced macrophages (termed M4 macrophages) characterized by reduced phagocytosis and a pro-inflammatory cytokine profile, [32, 72–76]. In vivo, a huge diversity of conditions activate macrophages and may result in an infinite spectrum of phenotypes driven by a highly dynamic network of transcriptional programs [33, 76–78]. Gene expression processes are intrinsically noisy and the regulation of transcriptional programs by transcription factors can result in large phenotypic variation [79, 80]. The inherently random nature of gene expression may greatly contribute to the enormous phenotypic heterogeneity of macrophages [81–83]. Short, non-coding microRNAs (miRNAs) negatively regulate gene expression by interacting with the 3′-UTR of mRNAs and thereby participate in negative feedback loops or incoherent feed-forward motifs that adapt gene expression networks. Thus, miRNAs confer phenotypic robustness by regulating transcriptional noise and may thereby play a crucial role in macrophage activation [84–86].

MicroRNA biogenesis and function

miRNAs are processed in a two-step process from RNA polymerase II-generated primary transcripts (pri-miRNAs) characterized by a hairpin structure with a double stranded stem [87]. In the nucleus, the endoribonucleolytic enzyme Drosha cleaves the pri-miRNAs at the 5′ and 3′ end into miRNA precursors (pre-miRNAs) that possess a 2-nt overhang at the 3′ end. After translocation of the pre-miRNAs into the cytoplasma by GTP-dependent binding to Exportin 5, the RNase III endonuclease Dicer recognizes the 2-nt overhang and cleaves the pre-miRNAs near the terminal loop of the hairpin into 21–25 nt long miRNA duplexes. Dicer assembles with RNA binding proteins, like TAR RNA binding protein or protein activator of interferon induced protein kinase EIF2AK2 (also known as PACT), and Argonaute (AGO) proteins to form a RNA-induced silencing complexes (RISCs)-loading complex, which mediates the transfer of miRNA duplexes from Dicer to AGO proteins in an ATP-dependent manner [88, 89]. In contrast to siRNA duplexes, which are separated by AGO2-mediated cleavage of one strand of the duplex (called slicing), the central mismatches in the miRNA duplexes prevent slicing [90]. Alternatively, the separation of the miRNA duplexes occurs through a process called unwinding in which the N-terminal end of the AGO proteins wedges into the miRNA double strand, presumably due to ATP-independent conformational change of the AGO protein, and thereby releases one of the strands (the passenger strand) from the RISC [91]. The mature RISC, a complex of an AGO protein and a single-stranded miRNA, is capable of target RNA binding. The first monophosphate and nucleotide of the 5′ end of the miRNA is anchored to the AGO proteins, which results in the presentation of the nucleotides 2–5 to recognize complementary RNA sequences. The canonical target sites interact through Watson–Crick complementary bases with the nucleotides 2–8 at the 5′ end of miRNAs (termed the "seed" sequence) and are usually located at the beginning or the end of long 3′ untranslated region (UTR) in the target mRNA. To silence the expression of the target mRNA, GW182 family proteins are recruited to the RISC, and inhibit mRNA translation or promote mRNA deadenylation and degradation by the exonuclease XRN1 [92]. Due to the short miRNA recognition sequence, an individual miRNA can target usually more than one mRNA and, conversely, one mRNA transcript contains binding sites for multiple miRNAs [93]. Therefore, mRNAs or other RNA molecules, such as long non-coding RNAs, which contain the same miRNA target site compete with each other for the binding to the same miRNA and thus one miRNA target can regulate the expression level of the competing endogenous

RNAs in trans [94–96]. Of note, in quiescent cells, not all expressed miRNAs bind to targets and target-free miRNAs are stored in small AGO2 complexes until cells are activated [97, 98].

Considering the regulatory function of miRNAs in the cellular response to environmental fluctuation, miRNAs are essential regulatory elements for macrophage phenotype reprogramming in response to various inflammatory stimuli [99–102]. In this Review, we will discuss how miRNAs regulate the macrophage phenotype and function during an inflammatory response.

Role of microRNAs in recruited macrophages

MicroRNAs regulate monocyte-to-macrophage differentiation

A large number of miRNAs are differentially regulated during the differentiation of monocytes into macrophages, for example, miR-24, -30b, -142-3p, and 199a-5p are down-regulated in this process [103, 104] (Fig. 2). Although miR-24, -30b and -142-3p have different seed sequences and are transcribed from different genes, all three miRNAs impair macrophage differentiation and activation. Overexpression of miR-24, miR-30b, or miR-142-3p in macrophages inhibits phagocytosis, reduces the expression of the mannose receptor (CD206) and inflammatory cytokines, such as IL-12 and TNF-α, and limits activation of PKC and NF-κB [103, 105, 106]. Similarly, PU.1-mediated down-regulation of miR-199a-5p promotes macrophage differentiation and proliferation by derepressing the type I serine/threonine kinase receptor of the transforming growth factor beta family activin A receptor type IB (ACVR1B) (also known as ALK4) and the CCAAT/enhancer binding protein alpha (C/EBP-α) expression [104, 107]. ACVR1B mediates signalling of the activin/inhibin/nodal pathway by heterodimerization with the type II receptors ACTRIIA/B and phosphorylation of SMAD2/3 proteins [107], and promotes activin A-induced pro-inflammatory macrophage polarization [108]. Although these miRNAs regulate monocyte differentiation in vitro, there is a lack of studies to demonstrate their roles in vivo.

Expression and secretion of microRNAs in macrophage polarization

miRNAs are essential regulatory elements of macrophage polarization. Depletion of miRNAs by deletion of the *Dicer* gene leads to a marked reduction in the expression of pro-inflammatory genes, such as IL-6, TNF-α and IL-12, in macrophages stimulated with various TLR agonists [109]. Moreover, miRNA transcription is differentially regulated in M1 and M2 macrophages. MiR-155 and miR-147 are selectively up-regulated in M1 macrophages stimulated by LPS and IFN-γ [110–112], whereas the expression of miRNAs, like miR-125b-5p and miR-99a-5p, is increased in IL-4-treated macrophages [76]. Interestingly, IL-4 treatment also induces exosomal secretion of those miRNAs for which the number of mRNA targets decreased owing to transcription changes during alternative macrophage activation. For example, down-regulation of the miR-218-5p target Lyz2 in IL-4-stimulated macrophages

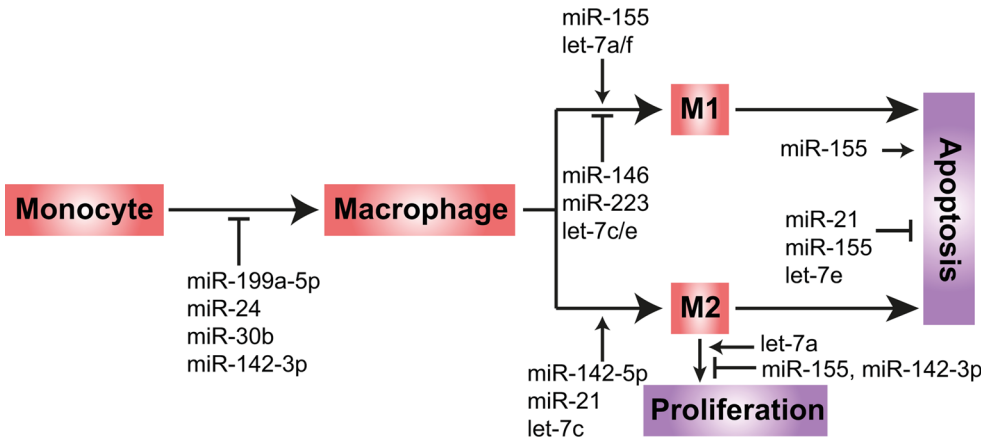

Fig. 2 Role of miRNAs in monocyte-to-macrophage differentiation and macrophage polarization. Differentiation of monocytes to macrophages is inhibited by miR-199a-5p, miR-24, miR-30b and miR-142-3p. M1 macrophage polarization requires miR-155 and let-7a/f, but is inhibited by miR-146, miR-223 and let-7c/e. Although miR-21 has been reported to play both pro- and anti-inflammatory roles, the latter role is more prominent. In addition, miR-142-5p and let-7c promote M2 macrophage polarization. In contrast to let-7a, miR-155 and miR-142-3p inhibit macrophage proliferation. miR-155 has both pro- and anti-apoptotic roles, whereas miR-21 and let-7e negatively regulate macrophage apoptosis

causes enrichment of miR-218-5p in exosomes, whereas miR-16-5p, which does not target Lyz2, is not enriched in exosomes [113]. Thus, the exosomal secretion of miRNAs may represent a mechanism by which macrophages rapidly adapt their miRNA pool to changes of the transcriptome. Moreover, exosomal miRNAs secreted from macrophages may be transferred to other types of cells, like endothelial cells, and suppress mRNA targets in the recipient cell [113]. Conversely, anti-inflammatory miRNAs, such as miR-10a, are transferred from endothelial cells to monocytes through endothelial cell-derived extracellular vesicles, thereby repressing monocyte inflammatory activation [114]. Although secretion and uptake of miRNAs via microvesicles may contribute to macrophage polarization, their roles in the development of macrophage subtypes in vivo are currently incompletely understood.

The pro-inflammatory M1 microRNA: miR-155

A high miR-155 expression level is characteristic for M1 macrophages [110–112] (Fig. 3). LPS and mildly oxidized low-density lipoprotein (moxLDL) bind to TLR4 and increase miR-155 expression by activating the transcription factors E26 avian leukemia oncogene 2 (Ets2) and NF-κB, which bind to the promoter of miR-155 host gene BIC [115–118]. Moreover, IFN-γ up-regulates miR-155 by activating the JAK/STAT1 pathway in epithelial cells and tumour cells [119–121]. The different members of the Akt family have opposing effects on miR-155 expression. The M2-kinase Akt1 and the M1-kinase Akt2 down-regulates and up-regulates the expression of miR-155 in macrophages, respectively [40, 122]. Notably, miR-342-5p is a positive regulator of miR-155 expression level by suppressing the expression of Akt1 in M1 macrophages [102]. By contrast, the endogenous competitive RNA bone morphogenetic protein receptor type-2 (Bmpr2) prevents targeting of Akt1 by miR-342-5p in unstimulated macrophages. The transcriptional suppression of Bmpr2 during M1 polarization increases the availability of miR-342-5p and thereby leads to increased targeting of Akt1. Therefore, the competition between Bmpr2 and Akt1 for the binding to miR-342-5p regulates the expression of miR-155 (Fig. 3). Additionally, the YY1/histone deacetylase2/4 complex and the anti-inflammatory transcription factor Kruppel-like factor 2 transcriptionally repress miR-155 in oxLDL-treated macrophages [117, 123].

In vitro, *Mir155* knockout and inhibition of miR-155 reduce the expression level of pro-inflammatory mediators, such as TNF-α, chemokine (C–C motif) ligand 2 (Ccl2) and IL-6 in macrophages treated with LPS or moxLDL and IFN-γ [110, 118, 122, 124]. Overexpression of miR-155 increases lipid uptake and ROS production in macrophages treated with oxLDL [117]. In addition, pro- and anti-apoptotic effects of miR-155 have been described. miR-155 inhibits oxLDL- and DNA damage-induced apoptosis of macrophage [125, 126]. By contrast, miR-155 is required for ESAT-6-induced apoptosis of macrophages [127]. However, miR-155 has no effect on the apoptosis of lesional macrophages in atherosclerosis and basal macrophage apoptosis [110]. These controversial effects of miR-155 on macrophage apoptosis might be related to distinct roles of miR-155 targets in the different apoptosis signalling pathways studied.

Several targets of miR-155 have been identified in macrophages, including suppressor of cytokine signaling 1 (SOCS1) and B cell leukemia/lymphoma 6 (Bcl6), which

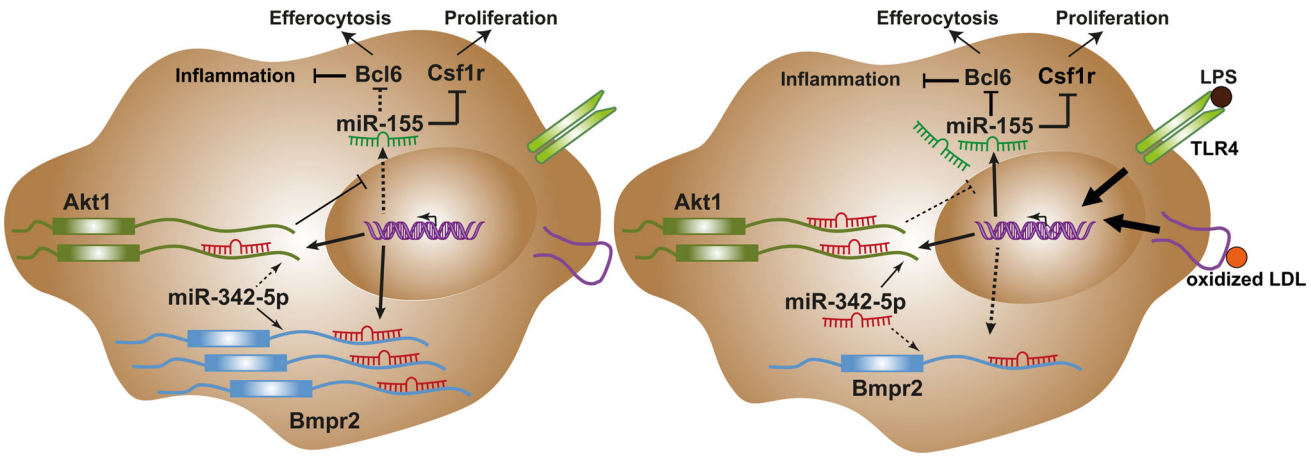

Fig. 3 miR-155 and miR-342-5p form a functional pair to regulate macrophage activation. Bone morphogenetic protein receptor type-2 (Bmpr2) and thymoma viral proto-oncogene 1 (Akt1) compete for binding to miR-342-5p in resting macrophages. Transcriptional down-regulation of Bmpr2 in activated macrophages promotes the targeting of Akt1 by miR-342-5p and thereby up-regulates miR-155. Whereas basal miR-155 expression reduces macrophage proliferation through targeting of Csf1r, increased miR-155 levels in activated macrophages promote inflammatory gene expression and inhibit efferocytosis by targeting Bcl6

mediate the pro-inflammatory effects of miR-155 [110, 124]. Notably, disrupting the interaction between miR-155 and SOCS1 by mutating the miR-155 target site in the 3′-UTR of SOCS1 in mice impairs the immunological functions of T cells in vivo, suggesting a critical role of this single miRNA-mRNA interaction [128]. Additionally, miR-155 represses efferocytosis via targeting Bcl6, an inhibitor of the efferocytosis suppressor ras homolog gene family, member A [99] (Fig. 3). Several targets, including Fas-associated death domain-containing protein (FADD) and Trp53inp1 may mediate the anti-apoptotic role of miR-155, whereas SOCS1 mediates the pro-apoptotic role of miR-155 [125–127]. Targeting HMG-box transcription factor 1 contributes to the effect of miR-155 on foam cell formation after oxLDL treatment [117]. Furthermore, miR-155 inhibits macrophage proliferation by targeting Csf1r [99, 129].

miR-155 expression is increased in both murine and human lesions during the development of atherosclerosis, probably due to the existence of IFN-γ and modified LDL in the lesions which could activate the lesional macrophages [99, 110, 118]. miR-155 promotes CCL2 level by down-regulating Bcl6 in lesional macrophages, thereby enhancing the progression of atherosclerosis [110, 118]. By contrast, hematopoietic miR-155 inhibits the development of early atherosclerosis [130]. These controversial findings may be due to a stage-dependent effect of miR-155 during the progression of atherosclerosis, because increased miR-155 levels in macrophages from advanced lesions is associated with enhanced inflammation [99], suggesting a phenotypic switch from M2- to M1-like macrophages. In addition, miR-155 represses Csf1r expression, reduces lesional macrophage content and proliferation and decreases lesion size at the early stage of atherosclerosis [99]. However, in advanced lesions, the role of miR-155-regulated Csf1r expression in lesional macrophage accumulation is limited owing to the down-regulation of M-CSF. On the other hand, targeting of Bcl6 by miR-155 fosters atherosclerosis due to impaired macrophage efferocytosis and necrotic core formation at the late stage [99]. The stage-dependent role of miR-155 suggests that blocking the interaction between miR-155 and Bcl6 might be a potential therapeutic strategy for atherosclerosis.

The anti-inflammatory M2 microRNA: miR-223-3p

miR-223-3p is evolutionary conserved among various species and primarily expressed in hematopoietic cells [131]. Although its expression is down-regulated during monocyte-to-macrophage differentiation [132], miR-223-3p is still one of the most abundant miRNAs in macrophages [112, 133]. Moreover, miR-223-3p is highly enriched in microvesicles released from GM-CSF-treated human monocytes and vesicle-mediated transfer of miR-223-3p to recipient myeloid cells increases macrophage differentiation and survival [134]. In contrast to macrophage differentiation, miR-223-3p expression increases continuously during granulopoiesis and plays an essential role in the regulation of granulocyte differentiation and function by modestly suppressing hundreds of targets [135, 136]. Knockout of the *Mir223* gene in mice increases the number of immature and hyperactive neutrophils in the circulation, while the proportion of monocytes in the peripheral blood and spleen is not altered [137]. Myeloid-specific transcription factors, such as PU.1 and C/EBP-α, induce the expression of miR-223-3p [133]. Accordingly, IL-4 stimulation induces miR-223-3p expression in murine macrophages in a PPARγ-dependent manner [137, 138], whereas LPS treatment down-regulates the expression level of miR-223-3p [137, 139].

The functional effects of miR-223-3p in macrophages are characterized by reduced expression of inflammatory cytokine, e.g. IL-1β, and of PPARγ in IL-4-treated macrophages [137]. In addition to the effect on Il1β mRNA expression, miR-223-3p limits IL-1β protein expression by targeting the inflammasome component Nlrp3 in macrophages [140, 141]. Overexpression of miR-223-3p can also reduce the expression of IL-6 and IL-1β in LPS-treated murine macrophages [137, 139]. Several targets of miR-223-3p have been identified in macrophages, including the Pbx/knotted 1 homeobox (Pknox1, also known as Prep-1), RAS p21 protein activator (GTPase activating protein) 1 (RASA1), nuclear factor of activated T cells 5 (NFAT5), STAT3, and IKKα, which might mediate the anti-inflammatory effects of miR-223-3p [132, 137, 139]. In murine macrophages, miR-223-3p-mediated targeting of NFAT5 and RASA1 results in reduced LPS-induced M1 polarization and enhanced IL-4-mediated M2 polarization [138], and targeting of STAT3 has been implicated in the regulation of IL-6 and IL-1β by miR-223-3p [139]. Pknox1 mediates the up-regulation of the anti-inflammatory cytokine IL-10 in macrophages that engulf apoptotic cells [68]; however, Pknox1 also increases the expression of IL-1β in LPS-treated (but not IL-4-treated) macrophages [137]. Thus, targeting of Pknox1 may explain the effects of miR-223-3p on IL-1β production in inflammatory macrophages. In contrast to the Pknox1 and RASA1 binding sites, the validated murine Nfat5 and Stat3 binding sites for miR-223-3p are not conserved in human [139].

The anti-inflammatory effects of miR-223-3p on macrophages may play a role in the pathogenesis of tuberculosis, because monocytes and monocyte-derived macrophages from patients with tuberculosis express higher levels of miR-223-3p and are less inflammatory than those from healthy subjects [142]. In mice, knockout of the *Mir223* gene in bone marrow cells increases adipose tissue

inflammation and insulin resistance, presumably owing to the shift of the adipose tissue macrophage phenotype towards an inflammatory M1 subtype [137]. Moreover, activation of the Csf1r/mitogen-activated protein kinase 1 (Erk)/Ets signaling pathway increases the expression of miR-223-3p in oncogenic M2-type tumor-infiltrating myeloid cells (TIMs) [143]. Conditional knockout of *Dicer* in myeloid cells, which leads to down-regulation of miR-223-3p in TIMs, reduces metastatic tumor burden, suggesting that the anti-inflammatory effects of miR-223-3p on macrophages may promote cancer [143].

The anti-inflammatory M1 microRNA: miR-146

The miR-146 family includes two members, miR-146a and miR-146b, which are encoded by genes located on different chromosomes. Although the sequences differ by two nucleotides, the seed sequences of miR-146a and miR-146b are identical, indicating that these two miRNAs target the same genes. LPS-induced TLR activation and stimulation with pro-inflammatory cytokines, like IL-1β and TNF-α, up-regulate the expression of miR-146a via pro-inflammatory NF-κB signaling, whereas Akt2 represses miR-146a expression in macrophages [111, 144, 145]. Moreover, apolipoprotein E (APOE) promotes miR-146a expression by up-regulating the transcription factor PU.1 [146]; however, the mechanism by which APOE induces PU.1 expression is unclear. In a negative feedback loop, the up-regulation of miR-146a results in endotoxin tolerance and limits IL-1β-induced inflammatory activation by repressing TNF receptor-associated factor 6 (TRAF6) and IL-1 receptor-associated kinase 1 (IRAK1) [144, 147]. Moreover, the pro-inflammatory effect of AKT2 is mediated by the downregulation of miR-146a and consequently the derepression of TRAF6 and interferon regulatory factor 5 (IRF5) [145]. In addition, miR-146a-mediated suppression of NOTCH1 decreases IL-6 production in macrophages by inhibiting LPS-induced NOTCH signalling [148–150]. These studies indicate that miR-146a plays an anti-inflammatory role by regulating a negative feedback loop comprising the TLR4-NF-κB pathway in vitro (Fig. 2).

In mice, knockout of the *Mir146a* gene results in hyperresponsivity to LPS owing to increased production of TNF-α, IL-6 and IL-1β in bone marrow-derived macrophages [151]. Moreover, the effect of miR-146 treatment has been studied in several mouse models of inflammatory diseases. In hyperlipidemic mice, treatment with miR-146a mimics reduces Ly-6C^{high} monocytosis, the lesional macrophage number, the macrophage inflammatory response, and atherosclerosis [146]. Systemic delivery of miR-146a encapsulated in polyethylenimine nanoparticles inhibits renal fibrosis and macrophage infiltration in the kidney by inhibiting pro-fibrotic and inflammatory signaling pathways following unilateral ureteral obstruction [152]. The administration of miR-146a and miR-146b mimics prevents liver graft injury probably by reducing NF-κB-mediated inflammation through targeting TRAF6 and IRAK1 in Kupffer cells [153]. Taken together, delivery of miR-146 mimics may be a therapeutic approach to limit inflammation by negatively regulating TLR4-NF-κB signaling in macrophages.

The anti-apoptotic and anti-inflammatory M1- and M2 microRNA: miR-21

The human pri-miR-21 is located immediately downstream of the vacuole membrane protein-1 gene [also known as transmembrane protein 19 (TMEM49)] and transcriptionally regulated by its own promoter in the 10th intron of the TMEM49 gene [154]. Processing of the pre-miR-21 results in two mature miRNA, miR-21-5p (miR-21) and miR-21-3p (miR-21*). While the human miR-21 sequence is evolutionary highly conserved between species, miR-21* is mainly expressed in humans and rodents. In contrast to most miRNAs, miR-21-5p is overexpressed in several cancer cell types [155, 156] and promotes cancer development mainly by inhibiting apoptosis but also by increasing proliferation [157–160]. LPS treatment induces miR-21 expression in murine macrophages mediated by the TLR adaptor protein MyD88 and downstream activation of NF-κB signalling [161]. In M2-macrophages, stimulation with IL-10 and CSF1R-induced PI3K and $ERK_{1/2}$ signalling up-regulate miR-21 expression [112, 162]. Moreover, engulfment of apoptotic cells increases miR-21 expression in macrophages [163].

Overexpression of miR-21 promotes IL-10 and represses IL-1β expression in macrophages [161–163], indicating an anti-inflammatory role of miR-21. In line with its anti-apoptotic effects, miR-21 inhibits glucose-induced macrophage apoptosis by blocking caspase3 activation [164]. The anti-apoptotic and anti-inflammatory effects of miR-21 (Fig. 2) are mediated by suppression of programmed cell death 4 (PDCD4) or phosphatase and tensin homolog (PTEN) [161, 162, 164–168]. Whereas PDCD4 promotes apoptosis via inhibiting p53 transcription or the translation of anti-apoptotic proteins, PTEN induces apoptosis by blocking the pro-survival PI3K/Akt pathway [169, 170]. In addition, PDCD4 is required for LPS-induced NF-κB activation and IL-6 production, whereas PTEN can promote both pro- and anti-inflammatory macrophage activation maybe due to the diverse contribution of the Akt isoforms to macrophage polarization [40, 161, 162, 171, 172]. Although both miR-21 and miR-21* are up-regulated in human atherosclerotic plaques [173, 174] and the circulating miR-21 levels are reduced in patients with

unstable plaques [173], the role of the differential regulation of miR-21 in macrophages is currently incompletely understood in vivo.

The chameleon-like let-7 microRNA family: c and e versus a and f

In humans, the let-7 miRNA family contains 13 members (let-7a-1/-2/-3, let-7b, let-7c, let-7d, let-7e, let-7f-1/-2, let-7g, let-7i, and miR-98) that share the same seed sequence. The let-7 miRNAs are expressed from nine different chromosomes and individual let-7 family members, such as let-7a and let-7f, are encoded in multiple genomic loci [175]. Human let-7a is expressed from three paralogs into the precursor miRNAs let-7a-1–let-7d (which includes let-7a-1, let-7f-1, and let-7d), let-7a-2, and let-7a-3/let-7b [176, 177]. Let-7 miRNAs are abundantly expressed in various cell types, including macrophages. LPS stimulation regulates the expression of the various let-7 miRNAs differently. In murine macrophages, LPS treatment down-regulates the expression of let-7a and let-7c (through polycomb repressor complex 2-mediated histone trimethylation), and up-regulates let-7e expression by activating Akt1 [122, 178–180]. By contrast, in human macrophages, LPS increases the expression of let-7a and decreases the expression of let-7f [181, 182]. Moreover, infection of macrophages with *Salmonella typhimurium* and *Mycobacterium tuberculosis* down-regulates the expression of several let-7 family members, such as let-7a and let-7f, presumably mediated by LPS and mycobacterium-derived ESAT-6, respectively [179, 183]. These in vitro results demonstrate that the regulation of the individual let-7 family members varies in macrophages and also differs between humans and mice.

The effect of let-7 family members on the macrophage activation is not uniform. Interestingly, the 3′-UTRs of the pro-inflammatory IL-6 and the anti-inflammatory IL-10, which both contain only few conserved miRNA binding sites, are both targeted by let-7a and let-7d [179]. Accordingly, *Salmonella* infection post-transcriptionally increases the expression of these cytokines in macrophages presumably by down-regulating let-7 family members. In LPS-treated murine macrophages, the up-regulation of let-7e suppresses not only the expression of IL-6, but also the expression of several other pro-inflammatory cytokines, such as TNF-α, CCL2, and CCL3, clearly demonstrating an anti-inflammatory role of let-7e [122]. This more general anti-inflammatory effect of let-7e may be mediated by the suppression of TLR4 through interaction of let-7e with a non-canonical binding site in the murine TLR4 3′-UTR containing three G:U wobbles in the seed match [122]. Although let-7i can also target human TLR4 through a canonical binding site that is not conserved in mice, it is unclear whether the interaction of let-7i with TLR4 plays a role in the inflammatory activation of macrophages [184]. Notably, let-7e can also target caspase 3 and may thus increase the survival of inflammatory macrophages [185]. The anti-inflammatory effect of let-7 family members like let-7c may be mediated by the targeting of the transcription factor C/EBP-δ, which contains a highly conserved binding site in its 3′-UTR sequence [178]. NF-κB activation in LPS-treated macrophages up-regulates C/EBP-δ transcription, which in turn amplifies NF-κB-induced IL-6 and TLR4 expression [186, 187]. Moreover, compared to other let-7 family members, let-7c greatly suppresses the expression of the p21-activated kinase 1, a serine/threonine kinase that is essential for LPS-induced NF-κB activation [180]. Accordingly, down-regulation of let-7c in LPS-treated macrophages mediates the increased expression of several inflammatory cytokines, such as TNF-α, IL-6, IL-1β, IL-12, and of iNOs [178, 180]. Conversely, high expression levels of let-7c in M2-type macrophages promote the IL-4-induced up-regulation of M2 markers and contribute to the high efferocytosis capability of M2 macrophages by increasing the expression of CD36, indicating that let-7c plays an important role in alternative macrophage polarization [178].

In contrast to the anti-inflammatory role of some let-7 family members, let-7f induces the expression of inflammatory cytokines, such as IL-1β, TNF-α, chemokine (C-X-C motif) ligand 1, and CCL2, and of iNOs in macrophages infected with *M. tuberculosis* [183]. Thus, suppression of let-7f expression in infected macrophages enhances the survival of *M. tuberculosis* and may promote mycobacterial disease [183]. Let-7f increases the inflammatory activation of macrophages by increased NF-κB activation owing to the targeting of tumor necrosis factor alpha-induced protein 3 (also known as A20), which negatively regulates TLR signaling by deubiquitinylation of TRAF6 [183, 188]. In addition, another negative regulator of NF-κB activation, NFKB inhibitor interacting Ras-like protein 2 (NKIRAS2, also known as κB-Ras2), contains a highly conserved binding site for let-7 miRNAs in its 3′-UTR. Let-7a can target NKIRAS2 in human macrophages and may thus generate a positive feed-forward loop in which NF-κB-mediated up-regulation of let-7a promotes NF-κB activation by suppressing NKIRAS2 [182, 189]. Additionally, let-7a enhances the proliferation of macrophages stimulated with LPS/IFN-γ by inducing S phase entry, which is mediated by increased expression of cell cycle activator E2F transcription factor 2 (E2F2) and reduced expression of cell cycle inhibitor E2F5 [189]. Taken together, although let-7 family members harbor the same seed sequence, they have specific individual function in macrophages (Fig. 2), indicating that non-seed sequences may be crucial for specific target recognition of miRNA family members.

miR-142-3p and miR-142-5p: a miRNA pair in macrophages

Like miR-155-5p and miR-223-3p, miR-142-3p expression driven by the transcription factor PU.1 is highly specific for hematopoietic cells [190, 191]. However, miR-155-5p can suppress miR-142-3p by targeting PU.1 [190]. In mice, the *Mir142* gene is embedded in the first intron of a long non-coding RNA with unknown function [192]. *Mir142* knockout mice are viable and do not show any gross abnormalities except an enlarged spleen due to an increase in B cells and myeloid cells, and a combined immunodeficiency [193]. In macrophages, miR-142-3p targets the transcription factor early growth response 2 (Egr2), which shifts the effect of PU.1 during myelopoiesis towards the macrophage cell fate [194, 195]. Conversely, M-CSF-induced Egr2 promotes macrophage proliferation by negatively regulating miR-142-3p expression [194]. Moreover, miR-142-3p mediates the anti-proliferative effect of miR-223-3p on hematopoietic cells, because miR-223-3p up-regulates miR-142-3p expression through targeting of C/EBP-β and LIM domain only 2 [196]. In addition, miR-142-3p limits IL-6 signalling by suppressing *Il6* mRNA and the IL-6 receptor protein IL6st in dendritic cells and macrophages, respectively [197, 198]. In mice, the mutual inhibition of miR-142-3p and C/EBP-β isoform LAP* expression may further amplify IL-6 signalling due to derepression of IL6st [197]. Whereas miR-142-3p targets Il6st through a canonical binding site in its 3′-UTR, isoform-specific suppression of C/EBP-β is mediated via a noncanonical site in the coding sequence of LAP* [197, 199]. Notably, miR-142-3p is up-regulated in tumor infiltrating macrophages through the CSF1-ETS2 pathway and overexpression of miR-142-3p in myeloid cells delays tumor growth following immunotherapy with tumor-specific T lymphocytes [143, 197]. This antitumor activity of miR-142-3p has been attributed to reduced IL-6-signaling in tumor macrophages, because increased IL-6 signalling induces the expression of M-CSFR and the IL-4 receptor and may, thus, skew the macrophage phenotype towards an M2 phenotype [197, 200, 201]. However, miR-142-3p also suppresses the pro-inflammatory cytokines TNF-α and IL-12 in inflammatory and alternatively activated macrophages, indicating a more complex role of miR-142-3p in macrophage polarization [105]. Moreover, miR-142-3p limits the phagocytic capability of macrophages by suppressing the Wiskott-Aldrich syndrome-like (Wasl) mRNA encoding for a protein that is a crucial regulator of Rho GTPases and thereby regulates cytoskeletal reorganization [106, 193, 202]. Accordingly, up-regulation of miR-142-3p in macrophages infected with mycobacteria reduces the phagocytic clearance of *M. tuberculosis*, indicating that regulation of this miRNA is a microbial strategy to prevent phagocytic clearance by macrophages [106].

In contrast to most miRNA genes, which give rise only to one mature miRNA strand, the processing of the primary miR-142 transcript results in the expression of miR-142-5p in addition to miR-142-3p in macrophages. Similar to the cooperative function of the two mature miR-126 strands in endothelial cells, miR-142-5p also plays an essential role in macrophages [203, 204]. IL-4 and IL-13 treatment induce miR-142-5p expression in macrophages, which in turn promotes the expression of M2 markers, such as CCL18, CCL17, and TGF-β1, and enhances the profibrogenic activity of macrophages by targeting SOCS1, a negative regulator of STAT6 phosphorylation [203].

miRNAs control macrophage cholesterol homeostasis

The cellular cholesterol content plays an essential role in macrophages by regulating PRRs and phagocytosis, and is regulated by the opposing actions of the transcription factors liver X receptor (LXR) and SREBP-2 [205]. LXR is activated by oxysterols, which are synthesized when cells accumulate excess cholesterol, in a negative feedback loop to reduce cellular cholesterol levels (e.g., by increasing cholesterol efflux), whereas low cholesterol levels activate SREBP-2 and thereby up-regulate genes that increase cholesterol uptake (e.g., LDL receptor) and synthesis (e.g., HMG-CoA reductase) [206]. Cholesterol efflux through ATP binding cassette (ABC) subfamily A member 1 (ABCA1) and subfamily G member 1 (ABCG1) plays a crucial role in the regulation of the cellular cholesterol content, because macrophages like many other cells cannot degrade cholesterol. During the phagocytosis of apoptotic cells, macrophages permanently face the challenge to tackle an enormous cholesterol load and to have enough cholesterol available to manage the massive membrane turnover [207]. Notably, the uptake of apoptotic cells greatly increases cholesterol efflux to the lipoprotein ApoA1 by LXR-mediated up-regulation of ABCA1 and thereby maintains their phagocytosis capacity and prevents apoptosis [208–211].

Free cholesterol complexed with sphingolipids or phospholipids is mainly localized in the plasma membrane and forms highly dynamic membrane microdomains, called lipid rafts, which provide a platform for the aggregation of surface receptors and intracellular signalling molecules, like CD14, TLR4, and Myd88 following LPS stimulation [212–216]. Moreover, plasma membrane cholesterol promotes pro-inflammatory CD40 signalling and plays an essential role in the phagocytosis of pathogens [217–219]. Conversely, TLR4-mediated inflammatory activation of

macrophages increases intracellular cholesterol levels by reducing cholesterol efflux through ABCA1 and ABCG1, which may in turn enhance TLR signalling by increasing the cholesterol content in lipid rafts [220–224]. However, increased cellular free cholesterol levels in the ER membrane can induce apoptosis through activation of the unfolded protein response [225]. To tightly adjust the potentially cytotoxic increased free cholesterol level, inflammatory macrophages esterify free cholesterol in the ER membrane via acetyl-CoA acetyltransferase 1 (ACAT1) to cholesteryl esters (CE), which are stored together with triglycerides in lipid droplets [221, 226, 227]. Excessive storage of CEs in lipid droplet may completely fill the cytoplasm of macrophages, which leads to the histologic picture of a foam cell. Foam cell formation suppresses inflammatory genes, up-regulates LXR target genes, and down-regulates SREBP2 target genes [228]. However, lipid droplet formation in macrophages can have pro-inflammatory effects, such as increased IL-1β secretion by activating the NLRP3 inflammasome [229]. The essential role of cellular cholesterol homeostasis in macrophage function dictates the handling of oxidatively modified LDL, which shares binding motifs with some pathogen-associated molecular patterns and is therefore recognized by TLR4 and macrophage scavenger receptors, such as CD36, but no longer by LDL receptors [230]. Oxidation of LDL occurs in the subendothelial space at arterial bifurcations, where the unrestricted uptake of oxLDL by macrophages leads to foam cell formation and atherosclerosis [204]. Taken together, intracellular cholesterol homeostasis is closely linked to macrophage functions, such as inflammatory activation and phagocytosis, through ABC transporters [231].

Intriguingly, numerous conserved miRNA binding sites (e.g., for miR-33-5p, miR-27-3p, miR-145, miR-19-3p, and miR-144-3p) were confirmed in the 3′-UTR of the ABCA1 mRNA (Fig. 4), suggesting that regulation of the cholesterol efflux by miRNAs has been an evolutionary advantage [232–236]. The role of miRNAs in cholesterol metabolism has been recently reviewed [237]. Here, we will focus on how miRNAs link cellular cholesterol metabolism with the innate immune response (Fig. 5).

The role of the highly conserved miRNA miR-33 in macrophage cholesterol metabolism has been extensively studied. The *MIR33A* gene (*MIR33-P1*) encoding miR-33a-5p has been acquired in the bilaterian ancestor of protostomes and deuterostomes, and a paralog of this gene *MIR33B* (*MIR33-P2*) occurred in vertebrates encoding miR-33b-5p, which differs in two nucleotides outside the seed sequence from miR-33a-5p [238]. However, several vertebrate species, including mice, have lost the *MIR33-P2* gene and retained only *MIR33-P1*, whereas the *MIR33* gene family is completely absent in the zebrafish genome. The MIR33-P1 gene is located in intron 16 of the human, mouse, cow and chicken *SREBP2* gene, which is a key transcription factor in cholesterol metabolism by inducing expression of the LDL receptor and cholesterol biosynthesis genes [239]. The expression of the *MIR33-P1* gene is co-regulated with its host gene by the cellular cholesterol content in macrophages [239, 240]. miR-33a-5p targets the 3′-UTR of several genes involved in cholesterol homeostasis including ABCA1 and thereby limits cholesterol

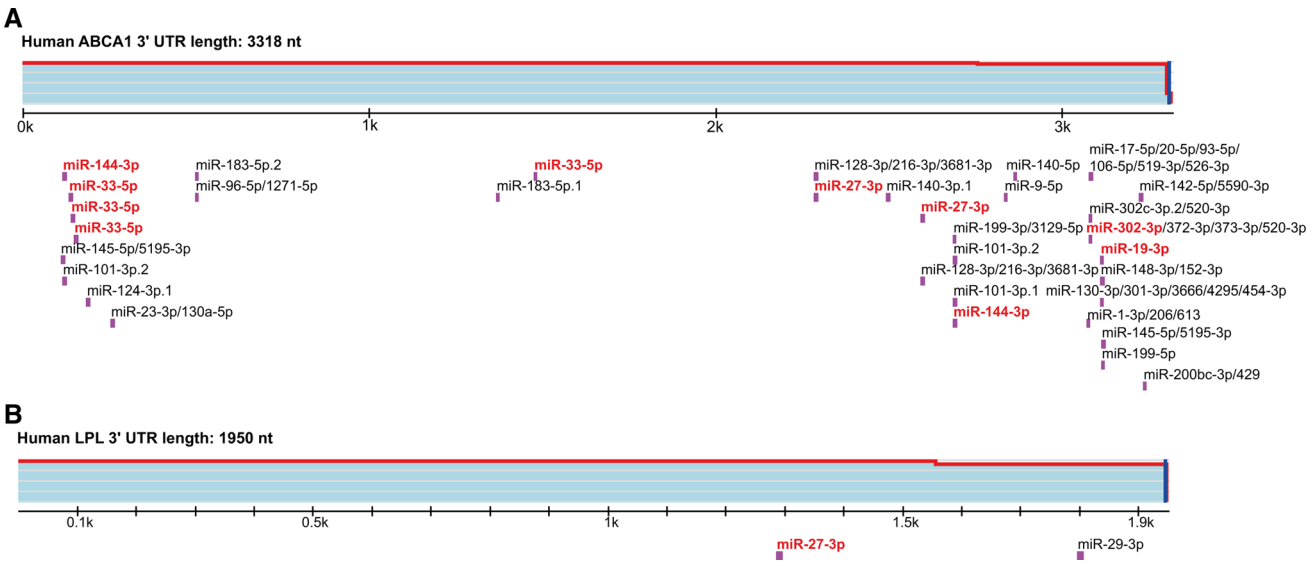

Fig. 4 The ABCA1 mRNA is a preferred miRNA target. Distribution of highly conserved miRNA binding sites in the 3′-UTR of human ABCA1 (**a**) and LPL (**b**) predicted by Targetscan (http://www.targetscan.org/). The miRNA target sites labeled in *red* have been confirmed experimentally in macrophages (see text for details)

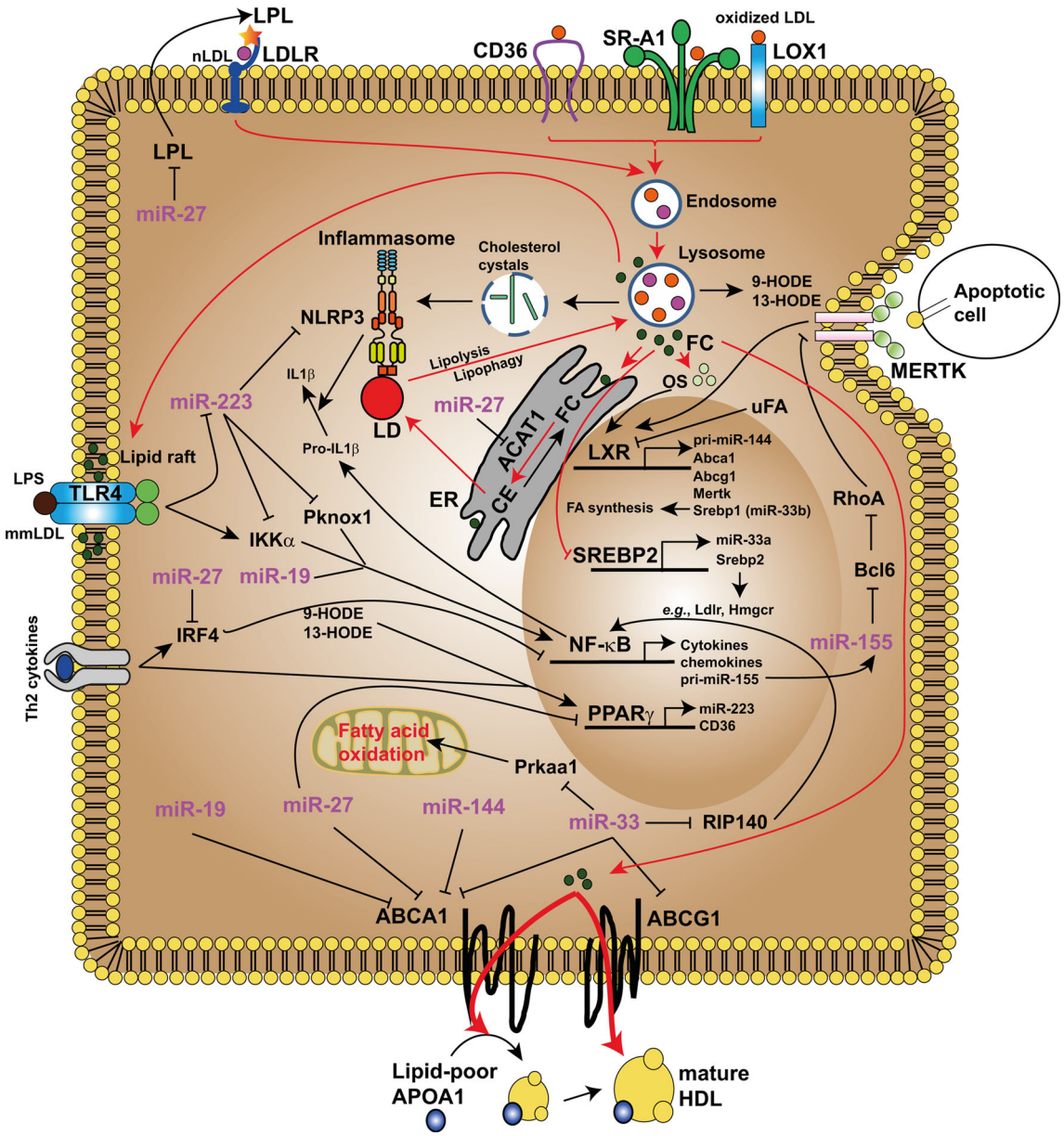

Fig. 5 miRNAs connect lipid metabolism and macrophage functions. LDL is taken up by macrophages via LDL receptor (LDLR) and lipoprotein lipase (LPL) enhances the uptake of LDL. However, modified LDL (e.g., oxidized LDL) cannot be recognized by LDLR, but by scavenger receptors (CD36, SR-A1 and LOX1). Lipoproteins engulfed by macrophages are transported to endosomes and then to lysosomes, where they are hydrolysed to generate free cholesterol (FC). In addition, 9- and 13-hydroxyoctadecadienoic acid (HODE) are formed upon peroxidation of the most abundant fatty acid in LDL, linoleic acid. 9- and 13-HODE activate PPARγ, which upregulates the expression of CD36. Free cholesterol is trafficked to the endoplasmic reticulum (ER) where excess cholesterol is re-esterificated to cholesteryl fatty acid esters (CE) by acyl-CoA acyltransferase-1 (ACAT1) and stored in cytoplasmic lipid droplet (LD). CEs in the lipid droplet can be transported back to lysosomes either via lipolysis or an autophagic process termed 'lipophagy'. Incorporation of FC into lipid rafts in the plasma membrane increases the activation of toll-like receptor 4 (TLR4) following stimulation with LPS or mildly modified LDL (mmLDL). In addition to the uptake of apoptotic cells, excess cholesterol metabolized into oxysterols (OS) activate LXR and thus induce the transcription of ABCA1 and ABCG1, which enhances the efflux of cholesterol efflux via apolipoprotein A1 (APOA1) and HDL particles. Moreover, cholesterol loading inhibits the uptake and synthesis of cholesterol by inhibiting SREBP2 activation. Excessive cholesterol uptake by scavenger receptors may lead to the formation of cholesterol crystals that activate the NLRP3 inflammasome and promote IL-1β production. The roles of miRNAs in cellular lipid homeostasis and macrophage functions are described in the text

efflux from macrophages to ApoA1 and increases macrophage apoptosis induced by free cholesterol loading [235, 240, 241]. Moreover, miR-33 targets PPARγ coactivator-1α in macrophages, which enhances mitochondrial ATP production required for the ATP-dependent cholesterol efflux via ABCA1 [242]. Thus, up-regulation of miR-33a-

5p and SREBP-2 by cholesterol depletion act synergistically to increase the macrophage cholesterol content [235, 240].

The *MIR33-P2* gene is located in the intron of the *SREBP1* gene and both genes are up-regulated through activation of LXR [243]. The *SREBP1* gene expression can give rise to two transcription factors SREBP1a and SREBP1c, which primarily promote fatty acid synthesis. Unsaturated fatty acids inhibit LXR activation and thereby reduce SREBP1c expression and fatty acid synthesis [244, 245]. miR-33b is co-expressed with its host gene, but suppresses SREBP1 mRNA by targeting its 3′-UTR and thereby counter-regulates the effect of SREBP1c on fatty acid metabolism [246]. Knockin of the *MIR33-P2* gene into the murine SRBF1 locus decreased the expression of ABCA1 and ABCG1 (only a target of miR-33 in mice) in macrophages and the cholesterol efflux to ApoA1 and HDL, indicating that expression of the MIR33 paralog enhances the effect of miR-33a on cholesterol efflux in humans [243].

miR-33 is up-regulated by LPS stimulation in M1 macrophages and promotes a pro-inflammatory phenotype [247, 248]; however, this effect of miR-33 is independent of ABCA1, but mediated through targeting of the protein kinase, AMP-activated, alpha 1 catalytic subunit (AMPK), which codes for a kinase that promotes ATP production via fatty acid oxidation [248]. In addition, AMPK mediates the effect of miR-33 on the expression of aldehyde dehydrogenase family 1, subfamily A2 (Aldh1a2) in macrophages by regulating the energy metabolism. Aldh1a2 is up-regulated in M2 macrophages and produces athero-protective retinoic acid. The expression of miR-33 in M1 macrophages has been linked to decreased secretion of retinoic acid, which promotes the generation of regulatory T cells [248]. Although these data would indicate that miR-33 expression in macrophages reduces atherosclerosis, the current evidence for this conclusion is contradictory. Several studies in animal models of atherosclerosis have demonstrated that systemic inhibition of miR-33 reduces lesion formation. However, it is difficult to attribute these effects clearly to the role of miR-33 in macrophages, because miR-33 affects many other tissues, such as liver and pancreas. For instance, *Mir33* knockout mice develop steatohepatitis, insulin resistance, and an increased body weight on a high fat diet [246]. Deletion of the miR-33 gene only in hematopoietic cells, however, does not substantially decrease atherosclerosis [241].

By contrast, feeding mice with a high fat diet down-regulates miR-33 in peritoneal macrophages and de-represses receptor-interacting protein-140 (RIP140), a coactivator of NF-κB, which increases inflammatory cytokine expression and may thereby contribute to the increased survival rate of mice fed a high fat diet in a sepsis model [249]. An anti-inflammatory role of miR-33 has also been reported in aged macrophages. Aging increases the expression of miR-33 in murine macrophages, which results in senescence-associated suppression of ABCA1 expression and cholesterol efflux [250]. Thus, macrophages from aged mice contain more cholesterol and accumulate more lipid droplets than those from younger mice [250]. Surprisingly, the aging-induced disturbance in cellular cholesterol homeostasis is associated with an anti-inflammatory M2 phenotype, characterized by increased IL-10 production. This M2 phenotype of aged macrophages also promotes endothelial cell proliferation and contributes to pathological angiogenesis related to age-related macular degeneration [250]. Moreover, inhibition of miR-33 up-regulated the expression of inflammatory genes like TNF-α and IL-1β in peritoneal macrophages from young and aged mice, indicating an anti-inflammatory role of this miRNA [250].

In addition to miR-33, miRNAs from the miR-19-3p member are predicted by Targetscan to bind the 3′-UTR of the ABCA1 mRNA via a highly conserved binding site (Fig. 4). The miR-19-3p family consists of three members transcribed from two different genetic loci. miR-19a-3p and miR-19b-3p, which differ in one nucleotide at position 11 of the miRNA, are embedded in the polycistronic primary pri-miR-17∼92 transcript that contains six mature miRNAs [251]. The miR-19b hairpin is also transcribed within the pri-miR-106∼363 cluster. Lv et al. confirmed the binding site for miR-19-3p in the ABCA1 3′-UTR using miR-19b-3p mimics and demonstrated that miR-19b-3p decreases cholesterol efflux in human macrophages and reverse cholesterol transport pathway in mice [236]. Moreover, miR-19-3p family members target several genes, such as A20 and its cofactor ring finger protein 11, and lysine-specific demethylase 2A (also known as FBXL11), which negatively regulate NF-κB activation and thereby inhibit inflammatory macrophage activation [109]. Although both miR-19a-3p and miR-19b-3p promote NF-κB activation, miR-19a-3p is more effective than its sister strand miR-19b-3p [109]. As expected from the effect of miR-19-3p on cholesterol efflux and NF-κB activation, systemic inhibition of miR-19b-3p reduced, whereas treatment with miR-19b-3p mimics enhanced atherosclerosis in mice, indicating that the effect of miR-19-3p on macrophages is pro-atherogenic [236]. However, HIF-1α up-regulates miR-19a-3p in atherosclerotic endothelial cells, which increases endothelial inflammation and atherosclerosis [252]. Therefore, further studies are needed to determine the role of the macrophage miR-19-3p family members in disease.

Another miRNA family that plays a central role in macrophage cholesterol metabolism is miR-27-3p. miR-27a-3p is transcribed together with miR-23 and miR-24

from the miR-23∼27∼24 gene cluster and miR-27b-3p (which differs from miR-27a-3p in one nucleotide at position 18) is included in a gene cluster with miR-23b. The ABCA1 3′-UTR contains two highly conserved binding sites for miR-27 family members (Fig. 4); however, only one binding site is targeted by miR-27a-3p and miR-27b-3p [232]. Akin to miR-33, miR-27a/b-3p decreases ABCA1 expression and cholesterol efflux in human macrophage cell line [232]. However, miR-27a/b-3p also limits the uptake of cholesterol via oxLDL partly by targeting lipoprotein lipase (LPL), which enhances the lipid uptake through a nonenzymatic bridging mechanism [232]. In contrast to the ABCA1 3′-UTR, only two conserved binding sites are predicted in the 1950-nt long LPL 3′-UTR by Targetscan including miR-27-3p and miR-29-3p (Fig. 4), indicating a less redundant control of the LPL expression by miRNAs. In addition, miR-27a/b-3p targets PPARγ in macrophages, which may contribute to the reduced oxLDL uptake by down-regulating CD36 expression [232, 253]. Moreover, miR-27a/b-3p targets human ACAT1 mRNA through a non-canonical seed match including a G:U wobble at position 1 of the miRNA, which results in reduced formation of cholesteryl ester [232]. In summary, different from miR-33, miR-27a/b-3p promotes a general inhibition of cellular cholesterol metabolism and a shift from cholesteryl esters to free cholesterol (by suppressing multiple targets) rather than a total increase of the cellular cholesterol content [232] (Fig. 5). Up- and down-regulation of miR-27a/b-3p expression by LPS treatment has been reported in human macrophages [111, 253], whereas IL-4 increases and LPS reduces miR-27-3p expression in mouse macrophages [171, 254, 255]. In addition to the targeting of PPARγ, miR-27a-3p may enhance inflammatory cytokine expression in LPS-treated macrophages by targeting the 3′-UTR of IL-10, which inhibits the inflammatory activation in an autocrine manner, and of interferon regulatory factor 4, a negative regulator of TLR signalling [254, 255]. In addition, miR-27b-3p may increase inflammatory macrophage activation by targeting the RNA-binding protein ZFP36 (also known as tristetraprolin), which limits the expression of various inflammatory genes by mediating their rapid decay through binding to AU-rich element motifs in their 3′-UTR [171, 256]. Thus, miR-27-3p may limit M2 polarization and decrease tumor growth promoted by the M2 phenotype of tumor-associated macrophages [255].

Activation of LXR in macrophages up-regulates the expression of ABCA1 and ABCG1 in response to cholesterol load and increases cholesterol efflux. Moreover, LXR also regulates the expression of miRNAs in macrophages, which are predicted to target lipid-related genes [234]. Notably, one of the miRNAs up-regulated by LXR activation is miR-144-3p, which targets ABCA1 at least via two highly conserved binding sites and reduces cholesterol efflux synergistically with miR-33 (Fig. 5), indicating that LXR activation induces a negative feedback regulation of ABCA1 through miR-144-3p [234, 257]. In addition, miR-144-3p increases inflammatory cytokine secretion in human macrophage foam cells. Systemic treatment with miR-144-3p increased the formation of atherosclerotic lesions in mice; however, it is unclear whether this effect is mediated by the role of miR-144-3p in macrophages, because HDL cholesterol levels and the reverse cholesterol transport were reduced most likely owing to the downregulation of ABCA1 in the liver [257].

Foam cell formation results in the differential regulation of several miRNAs in murine macrophages, which are predicted to target ABCA1, such as miR-302a-3p, miR-106b, and miR-216a [258]. Like miR-106b, cholesterol loading by oxLDL down-regulates the expression of miR-302a-3p in macrophages. The miR-302a hairpin is embedded in a polycistronic primary transcript containing also the miR-367, the miR-302d, the miR-302c, and the miR-302b hairpin, which is transcribed from an intron of its host gene La ribonucleoprotein domain family member 7 as an independent transcriptional unit [259]. All miR-302-3p family members have the same seed sequence and differ only in one nucleotide at position 18 or 19 of the miRNAs. miR-302a targets ABCA1 mRNA and limits cholesterol efflux to ApoA1 in macrophages. Moreover, inhibition of miR-302a reduces atherosclerosis and increases HDL cholesterol levels in mice, probably owing to increased ABCA1 expression in the liver [258].

Conclusions

miRNAs play crucial roles in many aspects of macrophages and thereby affect many pathological conditions, like infection, tumor growth, atherosclerosis, and macular degeneration by connecting inflammatory activation, cholesterol homeostasis, cell survival and proliferation, and phagocytosis. Targeting miRNAs through the application of modified oligonucleotides may become an effective therapeutic strategy for immune diseases [260, 261]. However, the function of miRNAs is highly context and cell type dependent, and can change during disease progression, like macrophage miR-155 in atherosclerosis, owing to changes in miRNA and target mRNA expression levels. Unfortunately, the miRNA-regulated context-specific network is still poorly understood, which hampers the drug development. Moreover, developing carriers to deliver miRNAs in a cell-type specific manner would greatly increase potential applications of miRNA-based therapies.

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