

ATVB in Focus

MicroRNAs: From Basic Mechanisms to Clinical Application in Cardiovascular Medicine

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MicroRNAs in Immune Response and Macrophage Polarization

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Abstract—Inflammation is essential to combat invading microbial pathogens. In this process, the involvement of multiple immune cell populations is crucial in mounting an optimum immune response. In the past decade, a new class of noncoding small RNAs, called microRNAs (miRNAs), has emerged as important regulators in biological processes. The important role of miRNAs in inflammation and immune response is highlighted by studies in which deregulation of miRNAs was demonstrated to accompany diseases associated with excessive or uncontrolled inflammation. In this brief review, we summarize the roles of miRNAs that have been characterized in innate and adaptive immune responses. We discuss the role of miRNAs in macrophage polarization, a molecular event that has clear effect on inflammation. (*Arterioscler Thromb Vasc Biol.* 2013;33:170-177.)

Key Words: immune response ■ macrophage polarization ■ microRNA

Inflammation plays a crucial role in host defense to invading microbial pathogens and is also essential for the successful repair of tissue damage.¹⁻⁴ Myeloid-derived cell populations, such as monocytes, macrophages, dendritic cells, and granulocytes, can recognize pathogen- and damage-associated molecular patterns present in microbial pathogens or as cellular molecules released by damaged tissues.¹ Binding of pathogen- or damage-associated molecular patterns to specific cell surface or intracellular receptors, such as Toll-like receptors (TLRs), Nod-like receptors, and the receptor for advanced glycation endproducts, in immune cells initiates a cascade of molecular events that leads to the production of proinflammatory cytokines, chemokines, reactive oxygen and nitrogen species, and antimicrobial peptides, as well as enhanced phagocytic activity.¹⁻⁴ These signaling events are essential for the eradication of infection and rapid clearance of cell debris in damaged tissues.¹⁻⁴ An appropriately orchestrated inflammatory response is also crucial for the subsequent activation of T lymphocytes and development of specific adaptive immunity.⁵

Despite the benefits of inflammation in the protection of host from exogenous and endogenous insults, untimely and unnecessarily high degrees of inflammation can cause host tissue damage.⁶ Therefore, the molecular networks that control the initiation, magnitude, and resolution of inflammation must be properly tuned for maintenance of homeostasis and optimization of host response.⁶ It is clear that every step of the inflammatory pathway is subject to both positive and negative regulatory events to achieve an optimal immune response.^{3,7}

In the past decade, a new class of small noncoding RNAs, called microRNAs (miRNAs), has emerged as important regulators in the development of immune and inflammatory responses.^{6,8-12} The importance of miRNAs in the modulation of normal and pathological immune function has been shown in multiple studies in which deregulation of miRNAs was demonstrated to accompany diseases associated with excessive or uncontrolled inflammation.^{6,8-11} This brief review aims to summarize the role of miRNAs in the regulation of innate immune response and adaptive immunity. It also discusses the role of miRNAs in macrophage polarization, a molecular event that has clear effect on inflammation, wound repair, and tumor progression.¹³

Overview of miRNA Biogenesis and Mode of Action

Like protein-coding genes, miRNAs are generally transcribed by type II RNA polymerase.¹⁴ This feature subjects miRNA expression to positive and negative regulation at the transcriptional level, similar to protein-coding genes. Perhaps less well appreciated is the regulation of miRNA biogenesis at the posttranscriptional level. The canonical miRNA biogenesis pathway starts with transcription of the miRNA gene, leading to formation of primary miRNA. The primary miRNA transcript is processed by the endoribonucleases Droscha/DGCR8 in the nucleus before transport into the cytoplasm.¹⁴ The resulting pre-miRNA is further processed by the endoribonuclease Dicer that cleaves the looped end of the pre-miRNA to produce mature miRNA that is 20 to 25 base-pairs in length.¹⁴ Mature

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miRNA is then assembled into the miRNA-induced silencing complex. Individual miRNA-induced silencing complex binds to the 3' untranslated region of target mRNAs using the seed sequence in the miRNA through Watson-Crick base-pairing mechanisms.¹⁴ It was previously shown that miRNAs function by inducing target mRNA instability and inhibiting mRNA translation.¹⁴ However, recent evidence indicates that induction of target mRNA instability is the primary mechanism by which miRNAs suppress target expression.¹⁵ Given that miRNAs are suppressors of protein expression, the conventional model of miRNA participation in inflammatory regulation could be simplified into a mechanism in which miRNAs target important regulators in inflammatory pathways.

In addition to their activity in the cytoplasm, miRNAs can be secreted into the extracellular environment by immune cells, with such extracellular miRNA typically being enclosed in exosomes or microvesicles.¹⁶ These extracellular miRNAs present in exosomes can be transferred from cell to cell and regulate gene expression in cells after transfer through canonical binding to their target transcripts. This mechanism of intercellular miRNA transfer is thought to be an important component of communication between cells.¹⁶ Recent studies have described an additional mechanism by which miRNAs can regulate inflammation. In these experiments, miR-21 and miR-29a enclosed in exosomes, as well as extracellular synthetic let-7b, were found to activate microglia, macrophages, and neurons through interactions with TLR7 (TLR8 in humans) in the endosomes of these cells.^{17,18} Interestingly, binding of miR-21, miR-29a, and let-7b to TLR7 is not incidental because some other miRNAs, such as miR-16, as well as mutant forms of miR-21, miR-29a, or let-7b, were unable to bind to TLR7 or stimulate immune response.^{17,18} miR-21, miR-29a, and let-7b all have a GU-rich element in their sequence, which has a GU content similar to that of ssRNA40, a known TLR7 ligand.^{17,18} The GU-rich element was shown to be crucial for binding of these miRNAs to TLR7.^{17,18} Conceivably, miRNAs and other small RNAs that have this GU-rich element can also activate immune response by binding to TLR7. These surprising findings demonstrate that extracellular miRNAs are more than biomarkers and can act as paracrine agonists of TLRs.

miRNAs and Innate Immunity

miRNAs constitute an integrated part of the regulatory networks in innate immunity. The modulation of inflammatory responses by miRNAs is primarily through altered expression of specific miRNAs in stimulated immune cells. More than a dozen miRNAs have been shown to be upregulated or downregulated in activated immune cells (Table 1). One of the first examples is from Baltimore's group.²⁰ They found that miR-146a is rapidly upregulated in human monocytic cells stimulated with lipopolysaccharide (LPS), a TLR4 ligand, and acts as a negative feedback regulator of TLR signaling, presumably by targeting tumor necrosis factor receptor-associated factor 6 and interleukin (IL)-1 receptor-associated kinase 1.¹⁹ miR-146a targeting of tumor necrosis factor receptor-associated factor 6 and IL-1 receptor-associated kinase 1 has been confirmed in a more recent study, although done in T cells, from the same group.²⁰ miR-146a upregulation may be an essential component of

endotoxin tolerance in innate immune response.²¹ These findings suggest that an insufficient induction of miR-146a could lead to hyperactivated or prolonged inflammation. In addition, miR-146a is a negative regulator of vesicular stomatitis virus-induced type 1 interferon (IFN) response in macrophages.²² Thus, miR-146a seems to play important roles in the inflammatory response to both bacterial and viral infections. The crucial involvement of miR-146a in inflammation was also highlighted in mice with either global miR-146a deficiency or miR-146a deficiency in regulatory T (Treg) cells.^{23,24} These mice develop autoimmune disorders, and their macrophages are hypersensitive to LPS stimulation.^{23,24}

miR-21 is induced in monocytic cells by LPS stimulation.²⁵ Upregulated miR-21 was found to dampen LPS-induced nuclear factor- κ B activation and IL-6 expression but to enhance IL-10 expression.²⁵ The anti-inflammatory effect of miR-21 lies in targeting tumor suppressor programmed cell death protein 4, a proinflammatory protein.²⁵ miR-21 can also trigger inflammatory responses by binding to TLR7 and TLR8.¹⁷ Therefore, the functional consequence of miR-21 upregulation in inflammatory conditions may be determined by many factors, such as the local concentration of extracellular miR-21 and the kinetics of mRNA targeting mediated by intracellular miR-21. It is certainly possible that at early time points miR-21 upregulation is proinflammatory through binding to TLR7/8. However, downregulation of programmed cell death protein 4, at later time points by miR-21 may play a role in returning inflammatory responses to baseline levels, thereby maintaining homeostasis of immune responses.

miR-155 is induced in macrophages in response to both bacterial and viral-derived antigens that activate TLR4, TLR2, TLR3, or TLR9.^{19,26–28} An early study found that miR-155 is a negative regulator of inflammatory response to LPS in dendritic cells. miR-155 was shown to target transforming growth factor- β -activated kinase 1-binding protein 2 and Pellino-1, adaptors in the signaling complex that activates I κ B kinase β . These results suggest that miR-155 is part of a negative feedback loop to dampen inflammatory responses.²⁹ However, most evidence indicates that miR-155 is proinflammatory. This proinflammatory effect of miR-155 likely occurs via repression of negative regulators of inflammation, including suppressor of cytokine signaling 1 and phosphatidylinositol-3,4,5-trisphosphate 5-phosphatase 1, which are both direct targets of miR-155.^{9,26,30} Through the downregulation of these anti-inflammatory pathways, miR-155 is able to potentiate inflammation. miR-155 can also increase the half-life of tumor necrosis factor- α transcripts, although the mechanism for this effect is presently unknown.^{28,31,32} miR-155-deficient mice have decreased immune responses, whereas miR-155-overexpressing mice develop a myeloproliferative disorder resembling chronic inflammation and also suffer from hematopoietic cancers.^{6,33–36} Given the evidence that miR-155 plays both positive and negative regulatory roles in immune responses, it would appear that the effects of this miRNA are dependent on the cellular context.

Different miRNAs seem to show synergies that result in fine-tuning of inflammatory responses. An example is the induction of both miR-155 and miR-21 in TLR ligand-

Table 1. miRNAs in Innate Immunity

miRNA	Targets	Effect
miR-9	NF- κ B1	Negative regulator of TLR4 signaling
miR-19	TLR2	Decreases TLR2-mediated inflammation
miR-21	PDCD4, IL-12 p35	Negative regulator of TLR4 signaling
miR-27b	PPAR- γ	Enhances response to LPS
miR-105	TLR2	Decreases TLR2-mediated inflammation
miR-106a	IL-10	Decreases IL-10
miR-125b	TNF- α ; IRF4	Diminishes inflammation; enhances macrophage activation
miR-145	TIRAP	Inhibits TLR signaling
miR-146a	TRAF6, IRAK1, IRAK2	Negative regulator of TLR signaling
miR-155	AID; MyD88; TAB2; Pellino-1; IKK ϵ ; SHIP-1; SOCS1; C/EBP- β	Enhances inflammation; negative feedback regulation
miR-223	IKK α ; Pknox1	Proinflammatory activation of macrophages
Let-7i, let-7e	TLR4	Downregulate inflammatory signaling

miRNAs indicate microRNAs; NF- κ B, nuclear factor- κ B; TLR, Toll-like receptor; IL, interleukin; PPAR- γ , peroxisome proliferator-activated receptor; LPS, lipopolysaccharide; TNF, tumor necrosis factor; IRF4, interferon-regulatory factor 4; TRAF6, tumor necrosis factor receptor-associated factor 6; IRAK1, IL-1 receptor-associated kinase 1; AID, activation-induced cytidine deaminase; TAB2, transforming growth factor- β -activated kinase 1-binding protein 2; IKK, κ B kinase; SHIP-1, phosphatidylinositol-3,4,5-trisphosphate 5-phosphatase 1; SOCS, suppressor of cytokine signaling; C/EBP, CCAAT/enhancer-binding protein; PDCD4, programmed cell death protein 4; and TIRAP, Toll-interleukin 1 receptor domain containing adaptor protein.

stimulated immune cells. In these settings, miR-155 may have early effects in promoting inflammatory response by targeting suppressor of cytokine signaling 1 and phosphatidylinositol-3,4,5-trisphosphate 5-phosphatase 1.^{9,26,30} This action is later dampened by miR-21-induced IL-10 because IL-10 can inhibit miR-155, allowing phosphatidylinositol-3,4,5-trisphosphate 5-phosphatase 1 to be derepressed with resultant inhibition of TLR signaling.³⁷

Several miRNAs regulate aspects of innate immune responses by targeting signaling components in inflammatory pathways. For example, miR-223, miR-105, miR-19, and let-7 family members directly target TLR2, TLR3, or TLR4.^{11,26,38–40} Thus, these miRNAs can impact the ability of immune cells to engage with TLR-specific extracellular ligands. miR-147 is induced after the activation of TLR2, TLR3, or TLR4 in both an MyD88- and TIR-domain-containing adapter-inducing interferon- β -dependent manner and acts as a negative regulator to prevent excessive inflammatory responses in macrophages.⁴¹ miR-9 is induced by LPS in both neutrophils and monocytes and negatively regulates nuclear factor- κ B-dependent inflammatory responses by suppressing the expression of nuclear factor- κ B1 transcripts.⁴² Some miRNAs, such as Let-7i and miR-125b, are downregulated in response to LPS and microbial infections. The reduced expression of these miRNAs was shown to lead to enhanced immune responses.^{28,32,40,43} Therefore, miRNAs are important components of feed-forward networks in inflammatory amplification. Ectopic expression of miR-125b has also been shown to promote macrophage activation, as reflected by findings that overexpression of miR-125b in macrophages increases costimulatory factor expression and responsiveness to IFN- γ .⁴⁴ IFN-regulatory factor 4, a direct target of miR-125b, could mediate the effect of miR-125b to promote macrophage activation because IFN-regulatory factor 4 knockdown in macrophages mimics the miR-125b overexpression phenotype.⁴⁴

miRNA and Adaptive Immunity

miRNAs Regulate T-Cell Development, Differentiation, and Activation

T cells directly carry out specific types of inflammatory responses, and their activation is dependent on presentation of specific antigens in the context of major histocompatibility complex by antigen-presenting cells, a function performed predominately by innate immune cells that are the first to come into contact with infectious agents.^{6,9}

The overall significance of miRNAs in T-cell development and activation was primarily established by using global and specific miRNA-deficient mice. Early studies observed that disruption of miRNA biogenesis by conditional removal of Dicer in the early stages of T-lymphocyte development results in reduced T-cell numbers in the thymus and peripheral lymphoid organs and causes aberrant T-helper (Th) cell differentiation and cytokine production.^{45,46} Recent studies have confirmed the crucial involvement of specific miRNAs in T-lymphocyte biology and in the pathogenesis of autoimmune diseases associated with T-cell perturbation. T cells deficient in miR-155, an miRNA expressed in activated CD4+ T cells, exhibit a Th2 bias under neutral conditions *in vitro*,^{47,48} indicating that miR-155 is normally required for a Th1-type response and for the polarization of T cells toward a proinflammatory phenotype. miR-155^{-/-} mice are defective in Th1 and Th17 cell production during autoimmune inflammation.^{47,48} These observations may help explain the increased resistance of miR-155^{-/-} mice to experimental autoimmune encephalomyelitis, colitis, and collagen-induced arthritis.^{35,49–51} The inhibitory effect of miR-155 on Th2 differentiation is thought to be mediated through effects on c-Maf, which is expressed in Th2 cells and promotes the development of this lineage.⁴⁸

miR-155 not only regulates effector T-cell functions but is also involved in the development of Treg cells. Although

miR-155^{-/-} mice have a reduced number of Tregs in the thymus and peripheral lymphoid tissues, the suppressive activity of miR-155^{-/-} Tregs remains intact.⁵² This suggests that miR-155 is important for Treg development but is not essential for the suppressive functions of Tregs. However, miR-146a, one of the major miRNAs expressed in Treg cells, is crucial for their suppressive functions. Deficiency of miR-146a in Treg cells resulted in breakdown of immunologic tolerance manifested by fatal IFN- γ -dependent immune-mediated lesions in a variety of organs.²³ This was likely a result of augmented expression and activation of signal transducer and activator transcription 1, a direct target of miR-146a.²³

miRNAs have been well characterized in the regulation of T-cell differentiation and clonal expansion. miR-181a has a significant influence on positive selection by heightening T-cell receptor signaling during thymic development.⁵³ These effects are achieved in part by the downregulation of multiple phosphatases, leading to elevated steady-state levels of phosphorylated intermediates and reduction of the T-cell receptor signaling threshold.⁵³ Inhibition of miR-181a led to excessive reactions to self-peptides that are normally sufficient only for positive selection.⁵⁴ Thus, miR-181a contributes to clonal selection and to preventing autoreactive T-cell clones from reaching the periphery and potentially causing autoimmune disorders.⁵³ miR-17-92 cluster miRNAs are involved in cell survival by repressing Bcl-2 interacting mediator and phosphatase and tensin homolog, both of which potentiate cell death.⁵⁵ Overexpression of the miR-17-92 cluster in transgenic mice results in T-cell populations that show more proliferation and less activation-induced cell death and is associated with lymphoproliferative disease.⁵⁵ miR-326 was shown to target Ets, a transcription factor that negatively regulates Th17 development, and overexpression of miR-326 led to the promotion of Th17 differentiation and IL-17 production.⁵⁶

miRNAs Regulate B-Cell Development, Differentiation, and Activation

B-cell development and differentiation start with the proliferative expansion of progenitor cells that undergo sequential rearrangements of their antigen receptor genes to produce a diverse, clonally selected receptor repertoire.⁵⁷ Cells expressing functional, nonself-reactive receptors are positively selected to become the peripheral pool of mature B cells. Major subsets of mature B cells are B2 B cells, including follicular B cells that are responsible for T cell-dependent antibody responses and marginal zone B cells that can respond rapidly to infectious agents in the blood, and B1 B cells that are home to the peritoneal and pleural cavities and represent an important source of natural antibodies in the blood as an evolutionarily selected first line of defense against pathogens.⁵⁷

Many cytokines and transcription factors play crucial regulatory roles in B lymphopoiesis. In recent years, the role of miRNAs in B-cell development and differentiation has been increasingly appreciated. The first report of miRNA regulation of B-cell development came in a seminal study showing that miR-181 was highly expressed in B cells and that ectopic expression of miR-181 guided B-cell development.⁵⁸

With the establishment of additional miRNA-deficient mice, there has been further understanding of the regulatory

role of miRNAs at different stages of B-cell development. miR-150 was found to be highly expressed in mature and resting B lymphocytes, a pattern that is inversely correlated to the transcriptional factor c-Myb.⁵⁷ miR-150 deficiency leads to B1-cell expansion and enhanced humoral immune responses, a phenomenon that is opposite to that observed in mice with B cell-specific deletion of c-Myb, which is accompanied by severe block of B-cell development at the pro- to pre-B transition and disappearance of B1 B cells.⁵⁹ Of note, c-Myb was found to be a target of miR-150, and ectopic expression of miR-150 in mice mirrored the observations in c-Myb^{-/-} mice.⁶⁰ These findings have established a central role for miR-150 in B-cell development.⁵⁷

miR-155 plays a crucial role in B-cell activation. B cells lacking miR-155 generate reduced extrafollicular and germinal center responses and fail to produce high-affinity IgG1 antibodies.⁴⁸ These phenomena are thought to be mediated by derepressing of miR-155 targets in B cells, such as PU.1, phosphatidylinositol-3,4,5-trisphosphate 5-phosphatase 1, and activation-induced cytidine deaminase.^{48,61} However, it should be noted that none of the above targets are solely responsible for the perturbation in B-cell activation in miR-155^{-/-} cells. This concept is supported by an elegant study that used transgenic mice carrying a mutation in the putative miR-155 binding site in the 3' untranslated region of activation-induced cytidine deaminase (called *Aicda155* mice).⁶² Activation-induced cytidine deaminase is required for immunoglobulin gene diversification in B lymphocytes and also promotes chromosomal translocations.⁶² *Aicda155* caused an increase in steady-state *Aicda* mRNA and protein amounts by increasing the half-life of this mRNA, resulting in a high degree of Myc-Igh translocations.⁶² Compared with *Aicda155* mice, a similar but more pronounced translocation phenotype was also found in miR-155-deficient mice.⁶²

Specific miRNAs are clearly essential for the development, differentiation, activation, and function of T and B

Table 2. miRNAs in Adaptive Immunity

miRNA	Targets	Effect
miR-10	Ncor2; Bcl6	Treg \uparrow
miR-17-92	Bim; PTEN	CD4 T-cell proliferation \uparrow
miR-29b	T-bet; IFN- γ	Th1 \downarrow
miR-146a	IRAK1; TRAF6; Stat1	TCR signaling \downarrow ; Treg \uparrow
miR-155	c-Maf	Th1 \uparrow ; Th17 \uparrow ; Treg survival \uparrow ; Th2 \downarrow
miR-181a	SHP1, 2; DUSP5, 6	TCR signaling \uparrow
miR-182	Foxo1	CD4 T-cell expansion \uparrow
miR-301a	PIAS3	Th17 \uparrow
miR-326	Ets	Th17 \uparrow
miR-150	c-Myb	B1 cell expansion \downarrow
miR-155	PU.1; SHIP-1; AID	antibody secretion \uparrow ; class-switch recombination \uparrow
miR-181		B-cell expansion \uparrow

miRNAs indicate microRNAs; IFN, interferon; IRAK1, interleukin-1 receptor-associated kinase 1; TRAF6, tumor necrosis factor receptor-associated factor 6; TCR, T-cell receptor; SHIP-1, phosphatidylinositol-3,4,5-trisphosphate 5-phosphatase 1; AID, activation-induced cytidine deaminase; Th, T helper; Treg, regulatory T; Bim, Bcl-2 interacting mediator; PTEN, phosphatase and tensin homolog; and PIAS3, protein inhibitor of activated STAT, 3.

lymphocytes (Table 2). The temporal and spatial expression of miRNAs must be tightly controlled in the development of adaptive immune responses. Dysregulation of specific miRNAs involved in these T and B cell-related events is likely to lead to chronic inflammation and autoimmune diseases.

miRNA in Macrophage Polarization

Macrophages display remarkable plasticity, with the ability to undergo dynamic transition between different functional phenotypes.^{63,64} Macrophages activated by TLR ligands and IFN- γ are called M1 macrophages (also referred to as classically activated macrophages).⁶³⁻⁶⁵ Conversely, stimulation of macrophages with Th2 cytokines, such as IL-4 or IL-13, immune complexes plus TLR ligands, IL-10, transforming growth factor- β , or glucocorticoids induces the generation of M2-type macrophages (also called alternatively activated macrophages).⁶³⁻⁶⁵ M1 macrophages produce high amounts of proinflammatory cytokines and NO by expressing inducible NO synthase and are important for eradicating bacterial, viral, and fungal infections.⁶³⁻⁶⁵ M2 macrophages are characterized by their high expression of markers of alternative activation, such as arginase-1, Chitinase 3-like 3 (also called YM-1), and found in inflammatory zone 1 (FIZZ1), and regulate responses to parasite infection, tissue remodeling, angiogenesis, and tumor progression.^{63,64,66} The classical M2 macrophage markers in mice, such as arginase-1, YM-1, and FIZZ1, are not induced in human macrophages even in the presence of IL-4.⁶⁷ Therefore, one should be cautious when applying findings obtained with murine M2 macrophages into pathophysiological states in humans where M2 macrophages play an important role.

The polarization of macrophages has been the focus of recent studies, particularly with regard to transcriptional regulation.⁶³ Transcriptional factors nuclear factor- κ B, activator protein-1, CCAAT/enhancer-binding protein α (C/EBP- α), PU.1, and IFN-regulatory factor 5 participate in TLR ligand-induced M1 activation, whereas signal transducer and activator transcription 6, peroxisome proliferator-activated receptor- γ , IFN-regulatory factor 4, C/EBP- β , and Kruppel-like factor 4 are involved in the polarization of M2 macrophages.⁶³ Enzymes involved in epigenetic regulation, such as Jumonji domain containing 3 and histone deacetylase 3, also play important roles in M2 macrophage polarization.⁶⁸

As discussed earlier, many miRNAs are involved in inflammation by either diminishing or potentiating inflammatory response through targeting important regulators in signaling networks. Given that TLR stimulation induces M1 macrophage polarization, one could arbitrarily consider the inflammation modulating miRNAs as those that can regulate M1 polarization. However, it remains unclear whether these miRNAs also participate in M2 macrophage polarization and, if so, what are the potential regulatory targets for such an action. There was an effort to systemically identify miRNAs that have altered expression in differentially polarized human monocyte-derived macrophages.⁶⁹ However, only a few miRNAs were found to have >2-fold change of expression in M1 macrophages or 1 of the 3 M2 subtypes.⁶⁹ Among these miRNAs, all but 2 are low abundant passenger strands.⁶⁹ Although

there are examples that passenger strands do have regulatory roles,⁷⁰ they are generally excluded from miRNA-induced silencing complex.⁷¹ Thus, the functional significance of these miRNAs in polarized macrophages remains to be determined. Furthermore, previously identified miRNAs with altered expression in inflamed immune cells, such as miR-21 and miR-146a, were not identified in this study, possibly because of a great abundance of these miRNAs under basal conditions in human monocyte-derived macrophages.⁶⁹ Nevertheless, this was the first systemic attempt to uncover M1- and M2-related miRNAs in a model with definitive induction of macrophage polarization.

Efforts to delineate the role of miRNAs in macrophage activation in inflammatory diseases have been fruitful. One of the best examples is miR-124, a brain-specific miRNA.⁷² miR-124 is highly expressed in microglia but not in other tissue macrophages. miR-124 is downregulated in activated microglia from the central nerve system of mice with experimental autoimmune encephalomyelitis, a mouse model of the human disease multiple sclerosis.⁷² Experimental autoimmune encephalomyelitis is characterized by inflammation of the central nerve system and is associated with microglia activation and infiltration of T cells and leukocytes. Overexpression of miR-124 diminished M1 polarization and enhanced M2 polarization in bone marrow derived macrophages (BMDMs), as reflected by reduced expression of the surface markers CD45, CD11b, F4/80, major histocompatibility complex class II, and CD86, but increased expression of the M2 phenotypic markers FIZZ1 and arginase-1.⁷² In contrast, knockdown of miR-124 enhanced the expression of the surface markers CD45 and major histocompatibility complex class II in BMDMs cocultured with neural and astroglial cells.⁷² Furthermore, systemic administration of miR-124 in vivo inhibited the development of experimental autoimmune encephalomyelitis

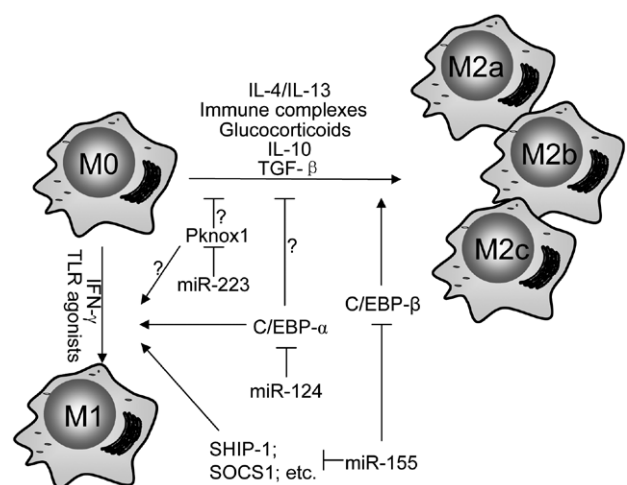


Figure. A few microRNAs (miRNAs), such as miR-124, miR-155, and miR-223, have been identified to regulate both M1 and M2 macrophage polarization. However, it is unknown whether the large number of miRNAs that regulate inflammatory response also modulate macrophage plasticity. IL indicates interleukin; TGF, transforming growth factor; TLR, Toll-like receptor; IFN, interferon; SHIP-1, phosphatidylinositol-3,4,5-trisphosphate 5-phosphatase 1; SOCS1, suppressor of cytokine signaling 1; and C/EBP, CCAAT/enhancer-binding protein.

and reduced central nerve system inflammation.⁷² This study identified C/EBP- α as the mediator of the miR-124 effect on macrophage polarization.⁷² Although it remains unclear how C/EBP- α suppresses M2 polarization, it is one of the few cases in which a specific miRNA has been shown to regulate macrophage plasticity.

Another elegant example of miRNA participation in macrophage polarization is the discovery that miR-223 regulates adipose tissue inflammation and insulin resistance.⁷³ In that study, miR-223 was found to be differentially expressed during macrophage polarization, with upregulation in LPS-treated macrophages but downregulation in IL-4-treated BMDMs.⁷³ miR-223-deficient macrophages were hypersensitive to LPS stimulation, whereas such macrophages exhibited delayed responses to IL-4 compared with controls.⁷³ Furthermore, miR-223-deficient mice exhibited an increase in adipose tissue inflammatory responses and decreased adipose tissue insulin signaling.⁷³ The importance of macrophage miR-223 was also confirmed by increased adipose tissue inflammation and insulin resistance in mice with transplantation of bone marrow from miR-223-deficient mice.⁷³ Pknox1 was identified in these studies as a genuine target of miR-223. The expression of Pknox1 is inversely correlated with miR-223 levels in activated BMDMs and adipose tissue. The function of Pknox1 as a target of miR-223 in regulating macrophage polarization was validated in gain-of-function and loss-of-function analyses in BMDMs.⁷³ However, a key question of how Pknox1 regulates macrophage polarization has not been answered.

Akt kinase has been shown to be both pro- and anti-inflammatory in immune cells, including in macrophages.^{74,75} Recent studies addressed the role of Akt isoforms in differentially contributing to macrophage polarization.⁷⁶ Akt1 ablation in macrophages gave rise to an M1 macrophage phenotype, and Akt2 ablation resulted in an M2 phenotype.⁷⁶ Akt2^{-/-} mice were more resistant to LPS-induced endotoxin shock and to dextran sulfate sodium-induced colitis than were wild-type mice, whereas Akt1^{-/-} mice were more sensitive.⁷⁶ These phenomena converge on miR-155, whose expression was repressed in naive and LPS-stimulated Akt2^{-/-} macrophages. The miR-155 target, C/EBP- β , seems to play a key role in this process.⁷⁶ C/EBP- β , a hallmark of M2 macrophages that regulates arginase-1, was upregulated upon Akt2 ablation or silencing. Overexpression or silencing of miR-155 confirmed its central role in Akt isoform-dependent M1/M2 polarization of macrophages.⁷⁶ Furthermore, knocking down miR-155 had a direct effect on IL-13-induced expression of M2 phenotypic markers by derepressing IL-13R α 1, leading to increased signal transducer and activator transcription 6 phosphorylation.⁷⁷ These studies support the hypothesis that miR-155 has a key role in determining macrophage plasticity, with the ability to skew macrophages toward the M1 phenotype.

In summary, although many miRNAs can regulate inflammatory response in macrophages, only a few miRNAs are shown to participate in both M1 and M2 macrophage polarization. This certainly reflects the fact that only a limited number of protein regulators are known to be involved in both processes (Figure). Although additional miRNAs that can modulate macrophage plasticity await identification,

there is more effort required to reveal the mechanism by which the targets of those miRNAs regulate this important event.

Concluding Remarks

In just a few short years, miRNA research in the immune system has produced a wealth of knowledge firmly supporting the concept that miRNAs have a central role in modulating inflammatory responses. With the identification of specific miRNAs that play key roles in negative or positive regulatory pathways in innate and adaptive immunity and with the demonstration that dysregulation of specific miRNAs is associated with inflammatory diseases, the field has opened new avenues for uncovering novel targets in treating pathological inflammatory conditions.

There is no doubt that additional miRNAs will be shown to participate in a variety of inflammatory responses. However, there are several intriguing questions that remain inadequately answered. First, it is clear that every stage of the inflammatory response involves >1 regulatory miRNA. However, an improved understanding of how multiple miRNAs can collaborate to properly balance inflammatory responses is needed. This will provide a better understanding of how miRNA networks can work together to regulate inflammation and other immune responses. Second, as even 1 miRNA is capable of targeting a large number of mRNAs, it remains challenging to identify the specific targets of any single miRNA. Most studies primarily rely on computational algorithms to predict target transcripts and often select to study 1 or 2 targets known to be involved in inflammatory response. This approach can certainly introduce bias and runs the risk of missing key targets with presently unknown but important regulatory functions. To overcome these limitations, several techniques, such as RNA immunoprecipitation-microarray, RIP sequencing, photoactivatable-ribonucleoside-enhanced crosslinking and immunoprecipitation, have been developed to capture bona fide miRNA targets. These advanced techniques to identify miRNA targets have been thoroughly reviewed elsewhere.⁷⁸ Third, better understanding of how modest suppression or fine-tuning of multiple targets achieves optimal immune responses will provide complete insights into how miRNAs modulate central events in inflammation, including immune cell development and activation.

The development of atherosclerotic lesions is shaped by immune responses and their regulation.⁷⁹ Given that miRNAs are centrally involved in innate and adaptive immune responses, miRNAs that participate in such processes have been shown to participate in the regulation of atherosclerosis.⁷⁹ Thus, delineation of the mechanisms by which miRNAs modulate immune response is likely to lead to therapeutic targeting of specific miRNAs that may be efficacious in preventing or treating atherosclerosis.

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Disclosures

None.

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