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MiRNA-Mediated Macrophage Polarization and Its Potential Role in the Regulation of Inflammatory Response

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Abstract

Monocytes and macrophages are important components of the immune system, specialized in either removing pathogens as part of innate immunity or contributing to adaptive immunity through antigen presentation. Essential to such functions is classical activation (M1) and alternative activation (M2) of macrophages. M1 polarization of macrophages is characterized by production of pro-inflammatory cytokines, antimicrobial and tumoricidal activity, whereas M2 polarization of macrophages is linked to immunosuppression, tumorigenesis, wound repair and elimination of parasites. MiRNAs are small non-coding RNAs with the ability to regulate gene expression and network of cellular processes. A number of studies have determined miRNA expression profiles in M1 and M2 polarized human and murine macrophages using microarray and RT-qPCR arrays techniques. More specifically, miR-9, miR-127, miR-155 and miR-125b have been shown to promote M1 polarization while miR-124, miR-223, miR-34a, let-7c, miR-132, miR-146a and miR-125a-5p induce M2 polarization in macrophages by targeting various transcription factors and adaptor proteins. Further, M1 and M2 phenotypes play distinctive roles in cell growth and progression of inflammation-related diseases such as sepsis, obesity, cancer and multiple sclerosis. Hence, miRNAs that modulate macrophage polarization may have therapeutic potential in the treatment of inflammation-related diseases. This review highlights recent findings in miRNA expression profiles in polarized macrophages from murine and human sources, and summarizes how these miRNAs regulate macrophage polarization. Lastly, therapeutic potential of miRNAs in inflammation-related diseases through modulation of macrophage polarization is also discussed.

Keywords

microRNAs; inflammation; monocytes; macrophage polarization; sepsis

1. Introduction

Monocytes are derived from common myeloid progenitor cells in bone marrow. From there, they enter the peripheral blood stream and continually migrate into all tissues, where they

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differentiate into macrophages, a mature form of monocytes (1). Macrophages are relatively long-lived cells with many important functions. One is to eliminate invading microorganisms via phagocytosis, which is a critical first defense in innate immunity (2). Another crucial role of macrophages is to modulate adaptive immune response to various pathogens through antigen processing and presentation (2). They would either induce inflammation or repair damaged tissues by secreting different signaling proteins. In addition to these functions in the immune system, macrophages can act as scavenger cells for clearing dead cells, cell debris, and other molecules such as cholesterol and fatty acids (2). Different functions of macrophages are associated with the type of receptor interaction and the presence of cytokines (2). In recent years, the concept of classical (M1) and alternative (M2) activation of macrophages has become increasingly recognized. The classical M1 macrophages are activated by "pro-inflammatory" cytokine profiles (i.e. IL-6 and TNF-α), but the alternative M2 macrophages appear to be involved in immunosuppression and tissue repair with low or null levels of pro-inflammatory cytokine secretion (3).

MicroRNAs (miRNAs) are small, endogenous, non-coding RNAs of ∼22 to 26 nucleotides in length that function primarily as post-transcriptional regulators. Accumulating evidences have shown that miRNAs can regulate many pathophysiological processes such as cell proliferation, metabolism, apoptosis, and organ development (4). Accordingly, miRNAs have been shown to regulate macrophage polarization and subsequent effects on inflammation (5). In this review, we will summarize recent findings in miRNA profiles in human and mouse polarized macrophages, and discuss the mechanisms underlying the miRNA-mediated regulation of macrophage polarization. We will also discuss the therapeutic potential of miRNA-mediated macrophage polarization in inflammation-related disease (*i.e.,* sepsis, obesity, cancer, multiple sclerosis).

2. MiRNA biogenesis and mechanism of action

The conventional miRNA biogenesis is initiated by the transcription of genes (mostly from the intron regions) by the type II RNA polymerase in the nucleus, generating primary miRNA (pri-miRNA), which will be cleaved by the endoribonuclease Drosha/DGCR8 complex and yield precursor miRNA (pre-miRNA) (6). Exportin 5 is the major transporter that exports pre-miRNA to the cytoplasm, where mature miRNA is formed via cleaving the loop end of the pre-miRNA by the Dicer complex. Together with other molecules such as Argonaute 2, mature miRNA is assembled into the miRNA-induced silencing complex, which would recognize and bind to the 3′ untranslated region (3′ UTR) of target mRNAs using the seed sequence through Watson-Crick base-pairing mechanism (6). MiRNA suppresses gene expression by causing target mRNA instability/degradation when perfect base-pairing occurs, or by inhibiting translation when it forms imperfect complementary base pairs (6). Recent evidence has indicated that the latter one is the primary mechanism by which miRNAs affect target gene expression (7). Interestingly, mature miRNA was previously denoted as the guide strand with inhibitory function, whereas another strand (miRNA*) was regarded as the passenger strand that had no cellular activity and would be degraded after formation. However, recent findings have demonstrated that the passenger strand is functional in regulating cellular process like the guide strand. For instance, miR-223 (guide strand, -3p) and miR-223* (passenger strand, -5p) were both dysregulated

in mouse hearts with moderate or severe sepsis and loss of miR-223 duplex (3p and 5p) exacerbated myocardial dysfunction and mortality caused by sepsis (8).

In addition to the activity within the cells in which they are transcribed, miRNAs can be secreted into extracellular compartments and delivered to distant target tissues. These miRNAs, along with other contents such as proteins or other RNAs (mRNAs, circRNAs, and lncRNAs), can be encapsulated into exosomes or microvesicles (4). Upon conventional uptake of exosomal content via direct fusion of cell membrane or endocytosis, miRNAs can regulate gene expression at post-transcriptional level and affect normal cellular activity in the recipient cell (4, 9). This novel mechanism of miRNA transfer constitutes an important element of intracellular communication. Very recently, our lab has reported that stem cell-derived exosomal miR-223 significantly suppressed the production of pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) by targeting Sema3A and STAT3 in macrophages of septic model (10). Similarly, another study showed that miR-155 and miR-146a, present in dendritic cell-derived exosomes, were passed between immune cells *in vivo* (11). Exosomal miR-146a inhibited while miR-155 promoted endotoxin-induced inflammation in mice (11). Currently, it is unclear whether exosomal miRNAs enter the nucleus of the target cell.

3. Mechanisms of macrophage polarization

3.1 M1 activation

Classical activation (M1) transforms macrophages into potent antimicrobial effector cells, and it requires two signals that can be fulfilled by the effector Th1 cells. One signal is the cytokine IFN-γ while the other signal, CD40 ligands, sensitizes macrophages to respond to IFN-γ (12, 13). Other cells, such as natural killer (NK) cells or macrophages themselves, have been shown to produce such cytokine as well. Once IFN-γ binds to the receptor (IFNGR), it recruits Janus kinase 1 (Jak1) and Jak2 adaptors, which activate factors (IRF1 or IRF8). This signaling pathway regulates specific gene expression of cytokine receptors (IL6R, IL2RA, or IL15 receptor α), cell activation markers (CD38, CD69 or CD97) and cell adhesion molecules (ICAM1, integrin α L, and mucin 1). Upon stimulation by these molecules, macrophage secretes TNF-α, which further stimulates macrophages through the TNFR1. Classical activation of macrophages would then increase the destruction of engulfed pathogens (14-16) (Fig. 1).

Other markers and M1 stimuli that induce prototypic inflammatory responses may originate from different sources and activate distinct signaling pathways. Two major examples are the bacterial lipopolysaccharide (LPS) and granulocyte macrophage colony stimulating factor (GM-CSF) (17). LPS is the well-known M1 macrophage signal that is propagated by the toll-like receptor 4 (TLR4) cascade (17). Similar to IFN-γ signaling pathway, LPS-mediated M1 activation is under the control of NF-κB, activator protein 1 (AP-1), IRFs, and STAT1. Typically, TLR4 activation leads to strong pro-inflammatory cytokine signaling (i.e. IL-1β, IL-6, IL-12, TNF, and IFN-β), high levels of chemokine secretion [i.e. Chemokine (C-C motif) ligand 2 (CCL2), chemokine (C-X-C motif) ligand 10 (CXCL10), and CXCL11], and antigen presenting molecules such as MHC family members (17) (Fig. 1).

GM-CSF is defined as a hematopoietic growth factor that originates from various cell types including T cells, macrophages, endothelial cells and fibroblasts upon different stimuli (18). The pro-inflammatory signal pathway of GM-CSF is very similar to IFN-γ and LPS. Activation of GM-CSF receptor (GMR) recruits JAK2, which activates STAT5, extracellular signal-regulated kinase (ERK), NF-κB and IRF5 (18). For the most part, secretion of pro-inflammatory cytokines (i.e. IL-1β, Il-6, TNF-α) are lower in GM-CSF-stimulated macrophages compared to LPS-induced macrophages, although fold expressions are not always consistent for the listed cytokines (18) (Fig. 1).

3.2 M2 activation

In contrast, alternative macrophage polarization is characterized by its ability to antagonize prototypic inflammatory responses by helping parasite clearance and promoting tissue remodeling (19). However, the mechanism of activation is more complicated and does not constitute a uniform population. Based on different stimuli, M2 macrophages can be further categorized into subsets: M2a, M2b, M2c, and M2d (19). Each category displays unique gene expression profile, but production of high levels of IL-10 and IL-1 receptor antagonist (IL-1RA) and low levels of IL-12 are observed in all subsets (19, 20). One key signature of all M2 subsets is the production of Arginase-1 enzyme that depletes L-arginine, which is the substrate of inducible nitric oxide synthase (iNOS), leading to suppression of T cell responses (19).

M2a is elicited by IL-4 (or in some cases, IL-13) that is produced by Th2 cells, eosinophils, basophils, and macrophages (21). Upon binding to receptors (IL-4Rα1, IL-13Rα1, or IL-13Rα2), JAK1 and JAK3 are activated, leading to activation of STAT6 and IRF4. IL-4-induced macrophage polarization would then decrease phagocytosis activity (Fig. 2A). Knocking out IL-4 does not affect the number and maturation of macrophages, but impairs immune response against nematodes and viral infections (21).

The type II-activated macrophages (M2b) are triggered by IL-1R ligands or exposure to immune complexes plus LPS (21). These ligands are recognized by Fc receptor family on the cell membrane, including activatory FcγR1 (or CD64) and inhibitory FCγRIIA (CD32). The signal is then passed down to the spleen tyrosine kinase (Syc) and phosphoinositide 3-kinase (PI3K), which increases IL-10 and TNF-α expression (21) (Fig. 2B).

Glucocorticoids (GC) and IL-10 are the main mediators that trigger M2c subtype phenotype (13). Glucocorticoids diffuse into the cell and bind to its receptor (GCR), followed by translocation of the complex to the nucleus. This complex can interact with NF-κB and AP-1, thereby interfering with inflammatory responses (22, 23). Moreover, it is reported that GCR impairs p38 MAPK activity by inducing MAP kinase phosphatase-1 (MKP-1), leading to improved survival outcome from LPS-induced mortality (24). The chemokine expression profile differs from other M2 subtypes with high expression of CCR2, which affect monocyte adherence, phagocytosis, and apoptosis (25, 26) (Fig. 2C).

Tumor-associated macrophages (TAMs) are the major inflammatory component of the tumor microenvironment (TME). Since they share the similar IL-10^{high}IL-12^{low} phenotype as M2 macrophages, TAMs are categorized as a novel M2 subset – M2d.(20, 27). TAMs can be

activated by IL-6 and macrophage colony stimulating factor (M-CSF). Upon Fra-1 binding to the IL-6 promoter in macrophages, increased production of IL-6 acts in an autocrine manner to induce M2d polarization (27). In addition, leukemia inhibitory factor (LIF) has been identified as another micro-environmental factor that promotes TAM transformation (28). Indeed, LIF and IL-6 can facilitate consumption of M-CSF by monocytes, leading to the differentiation of monocytes to TAM-like cells (28). Depletion of LIF, IL-6 and M-CSF suppresses such induction of TAM-like cells (28). TAMs share similar cytokine profiles with other M2 subtypes with augmented production of IL-10, TGF-β (29). However, the chemokine signatures of TAMs are distinct from other M2 subtypes by secretion of high levels of CCL5, CXCL10 and CXCL16 (29). Interestingly, TAMs also express M1 markers and can be transformed into M1-like phenotype with LPS or IFN-γ stimulation (30, 31).

4. MicroRNAs profiles in polarized macrophages of human and mice

A number of studies have profiled the expression of miRNAs in the various polarizing conditions in human- and murine-derived macrophages. Zhang et al. (32) used murine bone marrow-derived macrophages (BMDMs) to determine miRNA expression in M1 and M2 polarizing conditions. In this study, M1-polarized conditions in BMDMs were induced by LPS plus IFN-γ treatment whereas M2 polarization was stimulated by IL-4 (32). The miRNA-microarray results revealed that expression levels of 109 miRNAs were altered between M1 and M2 conditions (32). Using qRT-PCR, they confirmed that in M1 polarized macrophages, miR-181a, miR-155-5p, miR-204-5p and miR-451 were upregulated, whereas miR-125-5p, miR-146a-3p, miR-143-3p and miR-145-5p were downregulated in comparison to those levels in M2 polarized conditions (32). Although the qRT-PCR results of some miRNAs were inconsistent with the microarray results, the majority of miRNAs analyzed were congruent in both assays.

On the other hand, Cobos Jimenez et al. (33) performed miRNA profile assays in polarized monocytes derived from human peripheral blood mononuclear cells (PBMCs). They observed that 303 miRNAs were upregulated or downregulated in M1 (induced by combination of IFN-γ and TNF-α), M2a (induced by IL-4) or M2c (induced by IL-10) conditions compared to unstimulated conditions after miRNA arrays (33). They further confirmed, using RT-PCR, that miR-125b-5p, miR-181a- 5p, miR-193b-3p and miR-125a-5p were significantly upregulated in M1 macrophages compared to unstimulated conditions but these miRNAs were also dysregulated in M2 conditions (33). MiR-145-5p, miR-146a-5p, miR-193a-5p and miR-29b-3p were upregulated whereas miR-629-5p was downregulated solely in M1 macrophages (33). MiR-500a-5p and miR-502–3p were upregulated while miR-181b-5p was downregulated only in M2a macrophages (33). Also, miR-21- 5p, miR-22-3p and miR146b-5p were upregulated whereas miR-339-3p and miR-200a-3p were downregulated solely in M2c conditions (33).

Figure 3 summarizes a comparison of miRNA profiles in polarized macrophages from murine and human sources as presented by Zhang et al. (32) and Cobos Jimenez et al. (33). As showed in Figure 3, only miR-155-5p and miR-125b-5p showed similar expression patterns in murine and human polarized macrophages across both M1 and M2 polarized conditions. Some miRNAs displayed consistent expression profiles in human and murine

macrophages in a single polarized condition but had opposite profiles in the other. For instance, miR-221-5p was upregulated in both murine and human M1 macrophages but was downregulated in murine M2 macrophages, which contradicted with upregulation of levels in human M2a macrophages. Differences in origin of macrophages used in these studies may account for the contrasting expression profiles. Generally, human macrophages used in studies are isolated from blood peripheral mononuclear cells whereas tissue-associated or bone marrow-derived macrophages are utilized in murine studies (34). Thus, miRNA expression profiles may differ in polarized conditions depending on the location and type of macrophage. This idea expounds on recent reports that adult tissue-resident macrophages do not originate from bone marrow (35).

In the same light, Graff et al. (36) compared expression of miRNAs in polarized human monocyte-derived macrophages (MDM) versus polarized human monocytic THP-1 cell line. They observed that miR-125a-3p was upregulated in M1 polarized conditions, miR-193b in M2a conditions and miR-27a* in M2b conditions. Also, miR-155* was increased in both M1 and M2b conditions. Consistently, miR-125a-3p, miR-27a* and miR-155* had similar expression profiles in polarized THP-1 monocytes as in polarized MDMs. Luciferase assays also revealed that miR-29b and miR125a-5p targeted TNFAIP3, a negative regulator of NF-κB signaling (36).

In summary, profiling miRNA expression in polarized macrophages with techniques such as microarray and RT-qPCR arrays yields large amounts of dysregulated miRNAs. For the most part, the functional roles of such dysregulated miRNAs, in the studies discussed above, in regulating macrophage polarization were unexplored. Nonetheless, some studies, discussed below, have investigated the precise roles of miRNAs in the activation and polarization of macrophages.

5. Functional role of microRNAs in the regulation of macrophage polarization

Traditionally, the IRF/STAT signaling regulates either M1 or M2 polarization in macrophages (37-39). TLR ligands and IFN-γ stimulate STAT1 to activate IRF/STAT-mediated M1 responses, while IL-4/IL-10 stimulate STAT6 to induce IRF/STAT-mediated M2 responses (40). Given that miRNAs may target various transcription factors and adaptor proteins involved in IRF/STAT pathways, it is possible that these miRNAs could regulate either M1 or M2 polarization in macrophages. Indeed, as reviewed below, these miRNAs play distinctive roles in the regulation of macrophage polarization.

5.1 Controversial effects of miR-21 in modulating macrophage polarization

MiR-21 is one of the most highly studied miRNAs in mammalian cells due to its role in the pathophysiology of conditions such as solid tumors, cardiac injury and inflammation (41). However, there are contrasting views on the role of miR-21 in mediating macrophage polarization.

In a recent study performed by Caescu et al. (42), miR-21 was involved in the downstream effects of colony-stimulating factor 1 receptor (CSF-1R), which increased M2 macrophages

and suppressed the M1 phenotype. Profiling of the mRNA targets of the CSF-1/miR-21 regulatory axis revealed that 80% of targets were pro-inflammatory molecules (42). Accordingly, knockdown of miR-21 resulted in the reduction of M2 phenotype genes, arginase 1, mannose receptor 1, IL-4Ra, and FIZZ (42). When miRNA-21 inhibitor was injected into mice, there was increased recruitment of inflammatory monocytes and increased response to LPS by peritoneal macrophages, thus signifying the ability of miR-21 to initiate M2 polarization (42). Moreover, these authors demonstrated that miR-21 suppressed levels of *SIRPb1*, a MEK/ERK$_{1/2}$ pathway activator, further enhancing M2 responses (42).

In contrast to the findings by Caescu et al. (42), Wang et al. (43) reported that miR-21 promoted M1 polarization in peritoneal macrophages and inhibited M2 polarization. In miR-21-KO macrophages, expression levels of M1 markers (*Tnfa, Il12p40, Il1b, and Il6*) were lower while M2 markers (*Il10, Arg1, Retnla, and Chi3l3*) were increased remarkably compared to levels in wild type (WT) macrophages (43). Treatment of WT macrophages with prostaglandin E2 (PGE2), an endogenous lipid mediator produced in inflammation and infection, inhibited miR-21 expression (43). Further studies demonstrated that PGE2 decreased the expression of M1 markers and enhanced the expression of M2 markers (43). These data suggest that miR-21 acts as an endogenous brake to block PGE2-mediated generation of M2 macrophages. Mechanistically, there was an increase of STAT3 expression in miR-21-KO macrophages, which was further enhanced by PGE2 treatment (43). Luciferase assays also validated that miR-21 targeted STAT3 and thereby, promoted M1 polarization in macrophages (43).

At this moment, the reasons behind the conflicting effects of miR-21 on macrophage polarization remain unclear. However, there are some differences between the two studies described above. First, Caescu et al. (42) used bone marrow-derived macrophages, while Wang et al. (43) utilized peritoneal macrophages. Besides, Caescu et al. (42) elucidated effects on miR-21 expression in LPS-stimulated macrophages, while Wang et al. (43) investigated the role of miR-21 on macrophage polarization in miR-21-/- macrophages at basal expressions of different cytokines. Indeed, a recent study by Lu et al. (44) partially demonstrated such controversial effects of miR-21 on macrophage polarization by observing elevated miR-21 levels in both LPS-treated RAW 264.7 macrophages and IL-4 transgenic mouse lungs. Furthermore, according to Sheedy et al. (41), pri-miR-21 may be involved in the early inflammatory stage, while mature-miR-21 plays a role in the resolution phase. This observation may explain the contradictory observations in the findings described above. In the very early stage of inflammation, pri-miR-21 predominates and presents pro-inflammation activity and polarizes macrophages to the M1 phenotype. By contrast, at the resolution phase, mature-miR-21 regulates the anti-inflammatory responses and polarizes macrophages to the M2 phenotype (41). The authenticity of this theory, however, remains to be confirmed in future investigations.

5.2 MiRNAs in the regulation of M1 polarization

A recent study by Thulin et al. (45) showed that miR-9 enhanced M1 polarization by targeting peroxisome proliferator-activated receptor δ (PPARδ). The relative expression

levels of miR-9 were increased, while PPARδ levels were decreased in human primary monocytes after LPS exposure (45). PPARδ is well known to regulate lipid and glucose homeostasis, as well as inflammation by activating the protein B cell lymphoma-6 (BCL-6), an anti-inflammatory transcriptional suppressor. Thus, increased expression of miR-9 resulted in suppression of PPARδ activity, thereby preventing the BCL-6-mediated anti-inflammatory effects. Consistently, transfection of anti-miR-9 in LPS-treated primary monocytes caused the increased expression of PPARδ (45). Hence, miR-9 may act to keep macrophages in the M1 polarized state.

Another miRNA reported to augment classical activation of macrophages is miR-127 (46). Inhibition of miR-127 expression by antagonist suppressed the expression of M1 signature genes and promoted transcription of M2 marker genes (46). Mechanistically, LPS-stimulated elevation of miR-127 inhibited the expression of Bcl6, which in turn suppressed the phosphatase Dusp1. Reduction of Dusp1 would lead to increased phosphorylation of JNK, which promoted the inflammatory response and M1 phenotype (46).

O'Connell et al. (47) recently observed that stimulation of macrophages with TLR2, TLR3, TLR4 and TLR9 ligands remarkably upregulated the expression of miR-155. Such an elevation of miR-155 was hampered by the knockdown of MyD88 and TRIF, two factors known to activate the NF-κB signaling. Likewise, the use of an JNK inhibitor diminished the upregulation of miR-155 and production of TNF-α, suggesting that miR-155-mediated activation of M1 polarization are due to induction of the JNK pathway (47). In addition, miR-155 can target the IL-13 receptor α1 (IL13Rα1), thereby inhibiting STAT6 activation and promoting M1 polarization (48). What's more, there were decreased levels of miR-155 in Akt2-/- macrophages, which coincided with increased levels of C/EBPβ, a bona fide target of miR-155 which has anti-inflammatory capacity (49). By contrast, higher expression of miR-155 was observed in Akt1-/- macrophages, in which the expression levels of M1 phenotype genes (TNF-α, IL-6 and iNOS), but not M2-associated genes (Arg1, Fiz1, and Ym1), were elevated (50). A luciferase reporter assay revealed that miR-155 interacted with the 3′UTR of suppressor of cytokine signaling 1 (SOCS1) (50). Therefore, these observations suggest that Akt signaling is also involved in the miR-155-mediated promotion of M1 phenotype through targeting C/EBPβ and SOCS1.

Finally, miR-125b has been demonstrated to positively regulate M1-phenotype in macrophages (51). Overexpression of miR-125b in macrophages was found to enhance responses of macrophages to M1 inducer IFN-γ through targeting of IRF4 (51). Accordingly, knockdown of IRF4 enhanced M1 activation and pro-inflammatory responses in macrophages (51).

Taken together, miR-9, miR-127, miR-155 and miR-125b have been shown to promote classical activation of macrophages (M1) and pro-inflammatory responses through inhibiting various factors, as summarized in Figure 4 and Table 1.

5.3 MiRNAs in the regulation of M2 polarization

Sun et al. (52) identified that miR-124 promoted cholinergic agonist-elicited anti-inflammatory effects. They observed that miR-124 was upregulated by cholinergic agonists

in LPS-stimulated macrophages as well as in mice. Transfection of miR-124 mimics in RAW 264.7 cells resulted in a reduction of LPS-induced production of TNF-α and IL-6 (52). The authors further identified that miR-124 directly targeted STAT3 and TNF-α converting enzyme (TACE) to reduce IL-6 and TNF-α release, respectively (52). Similarly, Veremeyko et al. (53) observed that miR-124 expression was upregulated in both IL-4 and IL-13-treated macrophages, and knockdown of miR-124 suppressed the expression of M2 markers (i.e., CD206 and Ym1) and enhanced the expression of M1 markers (i.e., CD86, iNOS, TNF) in M2-polarized macrophages (53).

MiR-223 has also been shown to initiate M2 polarization in macrophages (54). MiR-223 is highly expressed in myeloid cells of the bone marrow, where it is mainly resident in the myeloid, granulocytic and monocytic compartments (55). Overexpression of miR-223 in RAW 264.7 macrophages inhibited LPS-stimulated release of IL-6 and IL-1β by targeting STAT3 (54). In addition, Jiang et al. (56) reported that miR-34a blocked pro-inflammatory responses in LPS-stimulated macrophages. MiR-34a levels were reduced in LPS-treated RAW 264.7 macrophages, but transfection of miR-34a mimics diminished pro-inflammatory responses, evidenced by lower levels of M1 cytokines TNF-α and IL-6 (56). Mechanistically, miR-34a targeted *Notch1*, which is needed for LPS-mediated production of pro-inflammatory cytokines in macrophages (56).

Let-7c has been reported to promote M2 phenotype through targeting of p21-activated kinase 1 (PAK1), a serine/threonine kinase that is upregulated in M1 polarized macrophages (57). This was evidenced by: 1) Levels of M1-associated genes such as TNF-α, IL-6, IL-12a, IL-12b, MCP-1, and Nos2 were decreased in LPS-treated PAK-/- BMDMs; 2) the introduction of let-7c mimics in LPS-treated RAW 264.7 macrophages diminished levels of PAK mRNA; and 3) enhancer of zeste homolog 2 (EZH2) suppressed levels of let-7c in M1 polarized macrophages, thereby preventing the inhibitory effects of let-7c on PAK1 (57). Furthermore, Banerjee et al. (58) observed that let-7c was highly expressed in M2 macrophages compared to M1 macrophages. Accordingly, overexpression of let-7c reduced the expression of M1 genes (i.e., IL-12 and iNOS), and increased levels of M2 marker (i.e., FR-β) in M1 macrophages *via* targeting C/EBP-δ (58). By contrast, knockdown of let-7c in M2 macrophages resulted in downregulation of M2 markers and upregulation of in M1 markers (58).

Additionally, miR-132 has been shown to elicit anti-inflammatory effects in alveolar macrophages (59). Treatment of rat alveolar macrophages with LPS resulted in an increase in miR-132 levels together with high levels of TNF-α, IL-1β, and IL-6 (59). Coinciding with the elevation of miR-132 was an initial increase in AChE protein levels but AChE levels declined at 24 h post-LPS treatment (59). Transfection of miR-132 mimics in ACh-pretreated macrophages repressed the pro-inflammatory responses upon LPS challenge through inhibition of the NF-κB and STAT3 pathways (59). Conversely, transfection of miR-132 inhibitor in ACh-pretreated macrophages enhanced production of TNF-α, IL-1β, and IL-6 (59). Similar to miR-132, miR-146a has the capacity to dampen the inflammatory response in alveolar macrophages (60). Transfection of peritoneal macrophages with miR-146a reduced the expression of M1-associated proteins (i.e., iNOS), and increased the expression of M2-associated genes after LPS treatment (60). Mechanistically, miR-146a

may target NF-κB signaling mediators such as IRAK1 and TRAF6, thereby preventing pro-inflammatory responses (61). Lastly, miR-125a-5p targeted Kruppel-like factor 4 (KLF4), thereby inhibiting M1 polarization and promoting M2 polarization in bone marrow macrophages (62).

Collectively, miR-124, miR-223, miR-34a, let-7c, miR-132, miR-146a and miR-125a-5p have been identified thus far to promote anti-inflammatory responses and M2 polarization in macrophages, as summarized in Figure 4 and Table 1.

6. Therapeutic potential of miR-induced macrophage polarization in inflammation-related disease

The plasticity and diversity of macrophages in response to different pathophysiological conditions means that they could enhance or diminish progression of various inflammation-related disorders (63-66). Typically, classical activation (M1) is characterized by microbicidal and tumoricidal activity, whereas alternative activation (M2) is characterized with tumor progression and tissue remodeling (63-66). As such, miRNAs that regulate macrophage polarization may affect inflammation-related diseases such as sepsis, obesity, cancer and multiple sclerosis.

6.1 Macrophage polarization in sepsis

Sepsis is a severe inflammatory response syndrome that is a leading cause of death in the intensive care units of hospitals (67-70). Sepsis pathophysiology is complex, with the current consensus recognizing concurrent activation of pro-inflammatory and anti-inflammatory responses (71). However, it is generally believed that excessive production of pro-inflammatory cytokines in the initial stages of sepsis contributes to multiple organ damage observed in sepsis patients (67-71). This initial dominance of pro-inflammatory responses (systemic inflammatory response syndrome; SIRS) leads to a late resolution phase of higher anti-inflammatory cytokine production (compensatory anti-inflammatory response syndrome: CARS), which may counteract the earlier pro-inflammatory response (71). This resolution phase may lead to immunodepression and 'persistent inflammation – immunosuppression catabolism syndrome (PICS) which is characterized by reduction in lean body mass, poor wound healing, susceptibility to secondary infection and eventual death (71). Given the complicated balance between pro-inflammatory and anti-inflammatory responses, the role of macrophage polarization in sepsis is puzzled. Nevertheless, miRNAs that are involved in the macrophage polarization may contribute to the sepsis pathogenesis.

Indeed, numerous studies have implicated the beneficial effects of blocking TLR-NF-κB signaling in sepsis (72-78). Considering that TLR-NF-κB is essential for M1 activation (37), inhibiting those miRNAs that promote M1 polarization or activating the M2 polarization-associated miRNAs may have therapeutic potential in sepsis. For instance, serum concentrations of M2 phenotype-associated miRNAs (i.e., miR-146a and miR-223) were remarkably decreased in septic patients compared with SIRS patients and healthy controls (79). Accordingly, the beneficial effects of miR-146a in sepsis has been reported, where overexpression of miR-146a inhibited the expression of TLR4-NF-κB pathway proteins,

IRAK1 and TRAF6, in cardiac monocytes, thereby blocking NF-κB activation and attenuating sepsis-induced cardiac dysfunction, inflammatory cell infiltration and inflammatory cytokine production (80). Although the mouse survival rate was increased, the authors did not investigate the effects of miR-146a overexpression on bacterial loads. Regarding the effects of circulating miR-223 in sepsis, Wang et al. (79) showed the correlation between reduced miR-223 levels and increased sepsis severity. However, a recent study by Benz et al. (81) suggested that serum levels of miR-223 did not predict sepsis prognosis or survival in patients with critical illness. Benz et al. (81) used samples from murine models of sepsis and human septic patients to conduct their studies. In the mouse model of cecal ligation and puncture (CLP) surgery, levels of circulating miR-223 remained unchanged at different time points after induction of sepsis compared to controls (81). In humans, serum levels of miR-223 were slightly lower in critically-ill patients compared with healthy controls (81). Importantly, no significant differences in miR-223 levels between septic and non-septic patients were evident, which is contradictory to findings by Wang et al. (79), where miR-223 was significantly decreased in septic patients compared to healthy individuals. The conflicting results between these two studies may be partially explained by differences in experimental procedures. The size and characteristics of the patient cohorts analyzed in these two studies may also influence the differences observed.

Currently, the definitive role of miRNA-induced macrophage polarization in sepsis is still unclear, although studies characterizing changes in miRNA expression in sepsis are extensive (72). As discussed above, changes in miR-146a and miR-223, which are confirmed modulators of macrophage polarization, seem to contribute to clinical outcomes in sepsis patients. Moving forward, miR-146a and miR-223 represent ideal miRNAs which could be utilized to investigate the role of miRNA-induced polarization in sepsis.

6.2 miR-induced macrophage polarization in other inflammation-related diseases

Polarized macrophages interact with various tissues and organs in diseases such as obesity (82-87), cancer (63-66) and multiple sclerosis (88,89), thus affecting pathophysiology of such disorders. MiRNAs that regulate activation or alternative activation of macrophages in these disease states may have potential for therapy. Overexpression of M2-related miRNAs (i.e., miR-223) (90, 91) and inhibition of M1-associated miRNAs (i.e., miR-33 and miR-155) (92, 93) have been shown to generate beneficial effects on obesity. Similarly, increased expression of pro-inflammatory miR-155 (88) and downregulation of anti-inflammatory miR-124 (89) has been linked to aggravation of multiple sclerosis. Finally, increased levels of miR-19a-3p (94), miR-16 (95), miR-155 (96,97) and miR-511-3p (98) promoted activation of tumor-associated macrophages (TAMs) and inhibition of tumor growth. On the other hand, overexpression of miR-146a (99), an M2 phenotype inducer, in TAMs was characterized with tumor progression.

7. Summary and future directions

Modulating inflammatory response has always been a hot topic for both basic and clinical research. Given that miRNAs epigenetically fine-tune hundreds of gene expression, there is increased attention on the regulatory roles of miRNAs in macrophage polarization.

Especially, some miRNAs have been demonstrated to regulate the expression of various adaptor proteins and transcriptions factors, which are known to participate in macrophage polarization. Thus, alteration of such miRNA levels in macrophages should affect the switch between M1 and M2 phenotypes. As discussed above, some miRNAs (i.e., miR-9, miR-127, miR-155 and miR-125b) can promote M1 polarization, while other miRNAs (miR-124, miR-223, miR-34a, let-7c, miR-132, miR-146a and miR-125a-5p) can induce M2 polarization in both circulatory monocytes and tissue-resident macrophages. Future studies will be greatly needed to test whether such miRNAs can be utilized in clinical inflammation-related diseases. In addition, it would be significant to investigate whether dysregulation of polarization-associated miRNAs serves as biomarkers for prognosis and diagnosis of diseases.

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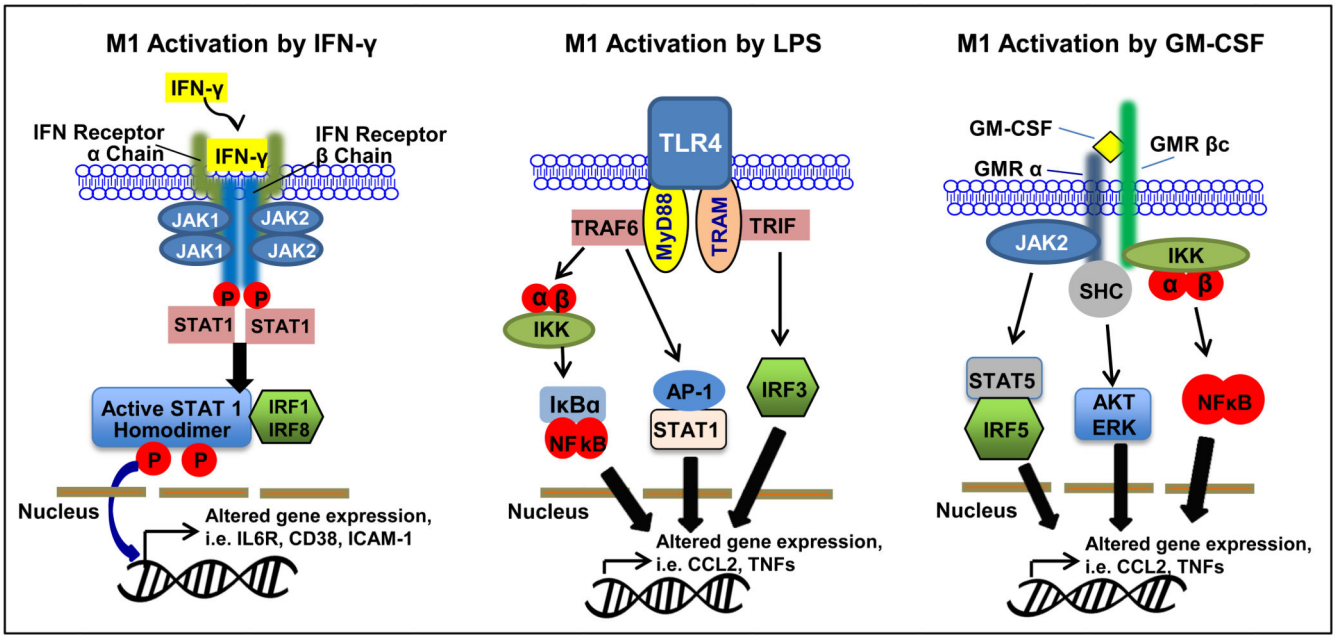

Figure 1. Classical activation of macrophages (M1)

Ligands IFN-γ, LPS and GM-CSF activate STAT1, NF-κB, AP-1, IRFs to stimulate transcription of M1 associated genes.

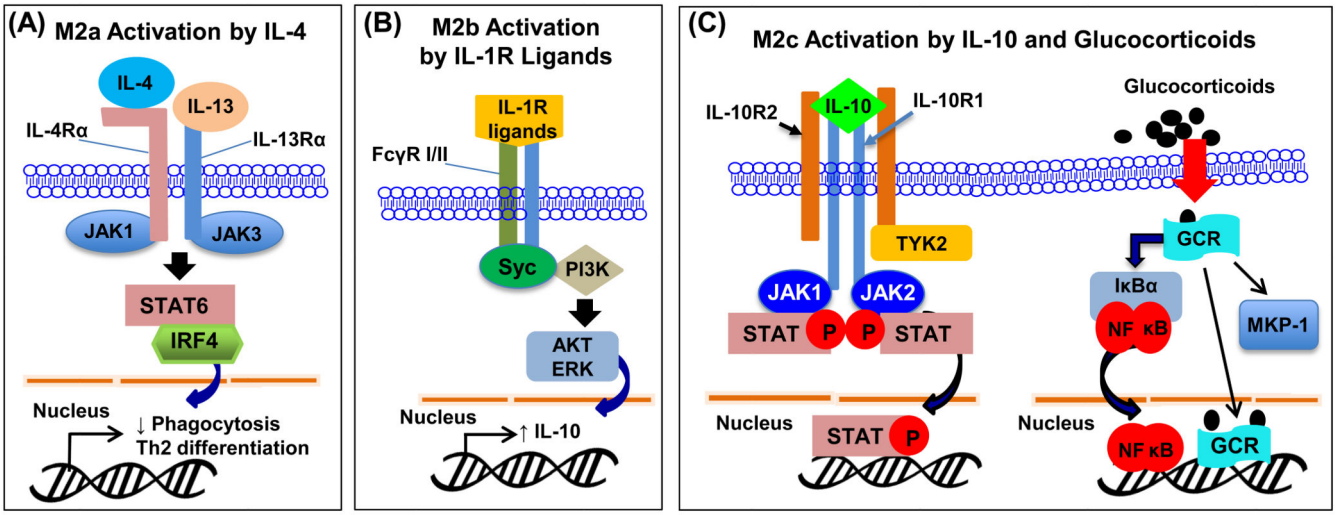

Figure 2.

(A) M2a polarization. Ligands IL-4 and IL-13 activate STAT6/IRF4 signaling pathway to induce M2a phenotype in macrophages. **(B) M2b polarization**. IL-1R ligands or immune complexes in combination with LPS activate Syc and PI3K to induce transcription of IL-10 and M2b phenotype. **(C) M2c polarization**. IL-10 and glucocorticoids activate M2c phenotype through STAT, NF-κB and MKP-1 activation.

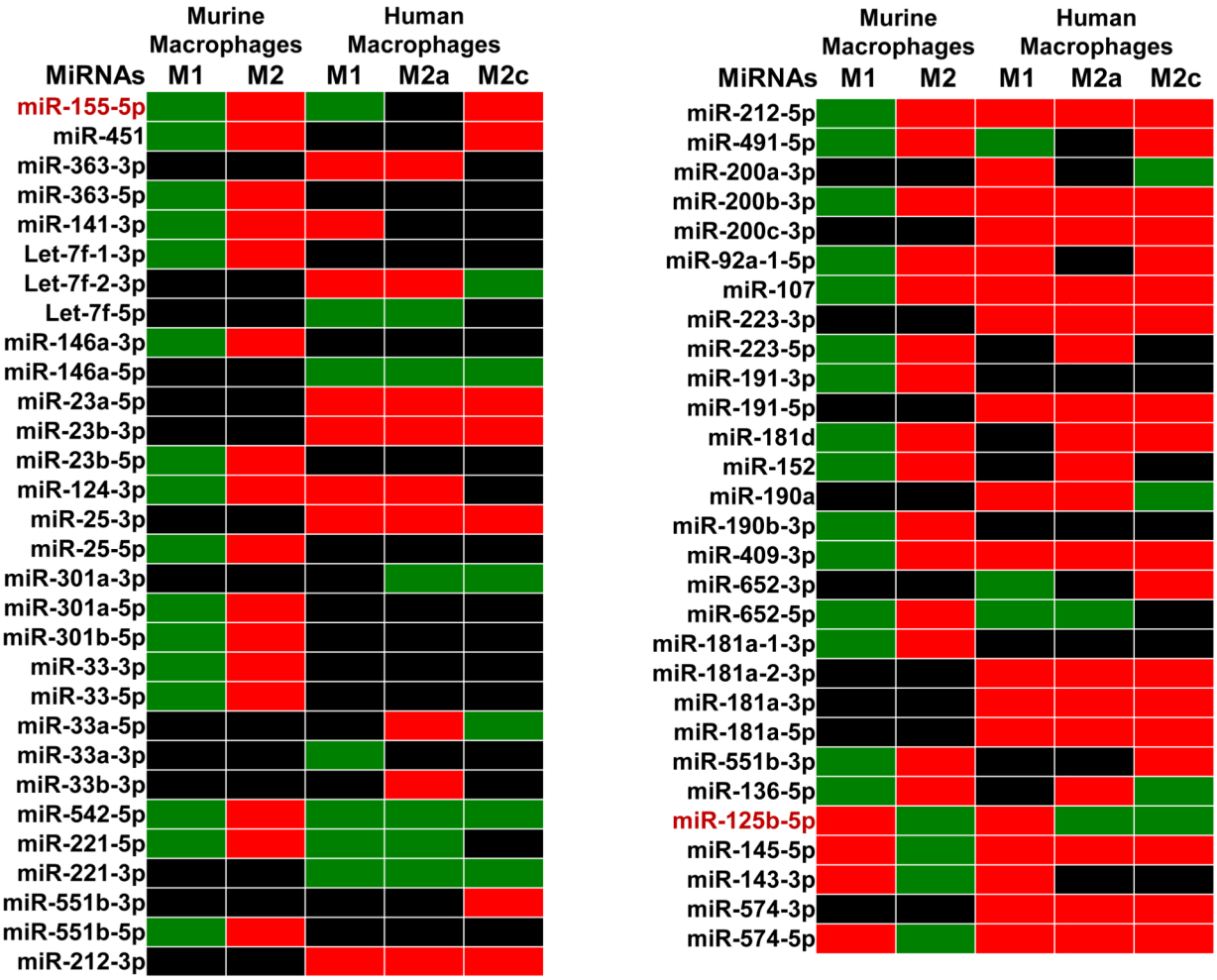

Figure 3.

miRNA expression profile in polarized macrophages, based on the papers by Zhang *et al.* (28) and by Cobos Jimenez *et al.* (29). Green: Upregulated miRNAs, Red: Downregulated miRNAs, Black: unknown or unchanged miRNAs.

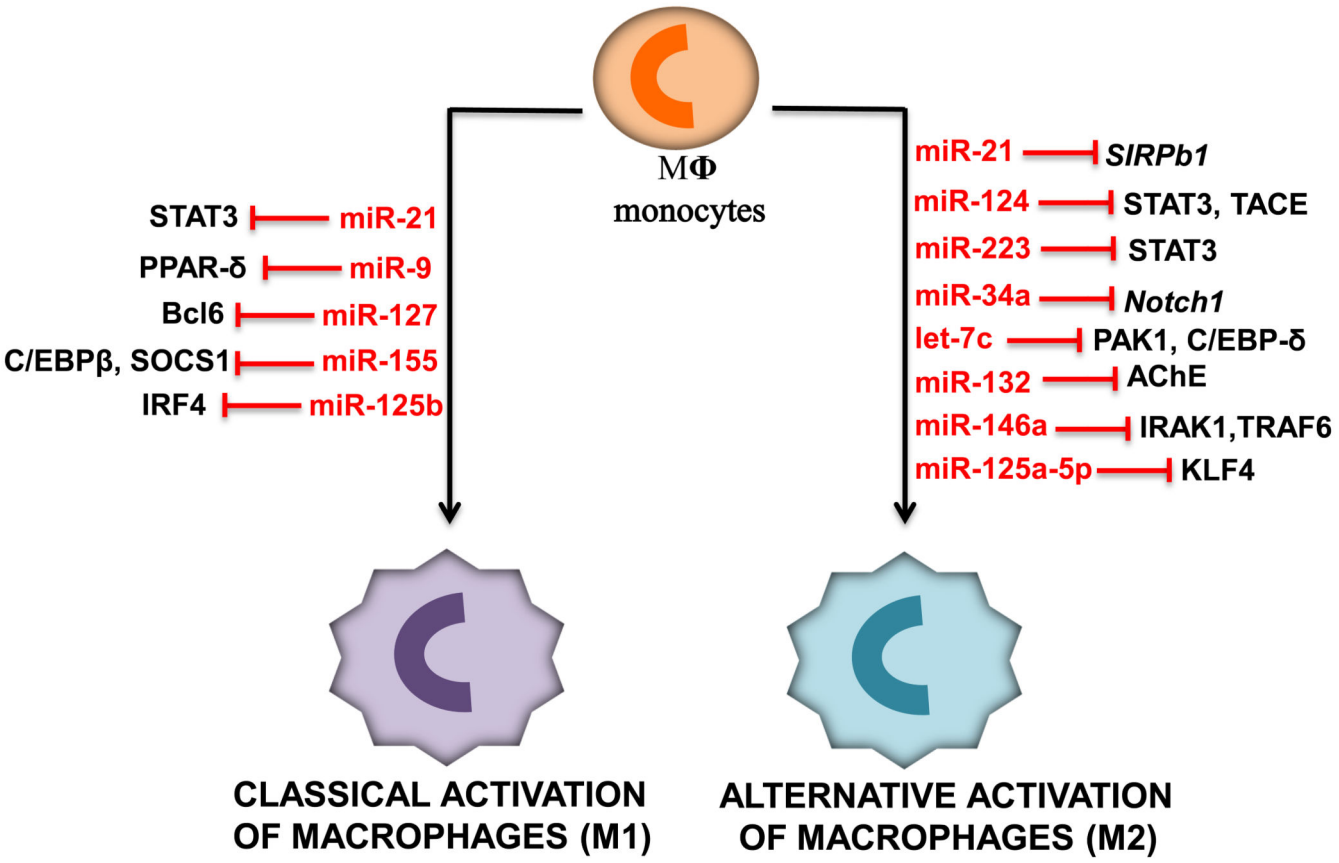

Figure 4.
Diagram illustrates miRNAs that induce either classical activation or alternative activation of macrophages and their corresponding targets.

Table 1

miRNAs regulate M1 and M2 polarization through targeting various adaptor proteins and transcription factors

MiRNAs	Phenotype promoted	Targets	Function	Ref.
miR-21	M2	*SIRPb1*	Promotes M2 polarization, downstream of CSF-1R	[42]
miR-21	M1	STAT3	Prevents PGE2-Mediated M2 polarization	[43]
miR-9	M1	PPAR-δ	Suppresses anti-inflammatory response	[45]
miR-127	M1	Bcl6	Promotes M1 phenotype through activation of JNK pathway	[46]
miR-155	M1	????	Promotes pro-inflammatory responses via JNK pathway	[47]
		C/EBPβ	Inhibits M2 polarization	[49]
		SOCS1	Promotes pro-inflammatory responses via P13K/Akt1 pathway	[50]
miR-125b	M1	IRF4	Enhances pro-inflammatory responses	[51]
miR-124	M2	STAT3, TACE	Inhibits production of pro-inflammatory cytokines	[52]
miR-223	M2	STAT3	Promotes anti-inflammatory response	[54]
miR-34a	M2	*Notch1*	Inhibits production of pro-inflammatory cytokines	[56]
Let-7c	M2	PAK1	Inhibits activation of NF-κB pathway	[57]
		C/EBP-δ	Promotes M2 phenotype	[58]
miR-132	M2	AChE	Promotes cholinergic anti-inflammatory response	[59]
miR-146a	M2	IRAK1,TRAF6	Prevents activation of NF-κB	[61]
miR-125a-5p	M2	KLF4	Promotes M2 polarization	[62]