FORUM REVIEW ARTICLE

miRNA in Macrophage Development and Function

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Abstract

Significance: MicroRNAs (miRNAs) control cellular gene expression *via* primarily binding to 3' or 5' untranslated region of the target transcript leading to translational repression or mRNA degradation. In most cases, miRNAs have been observed to fine-tune the cellular responses and, therefore, act as a rheostat rather than an on/off switch. Transcription factor PU.1 is a master switch that controls monocyte/macrophage development from hematopoietic stem cells. **Recent Advances:** PU.1 induces a specific set of miRNAs while suppressing the miR17-92 cluster to regulate monocyte/macrophage development. In addition to development, miRNAs tightly control the macrophage polarization continuum from proinflammatory M1 or proreparative M2 by regulating expression of key transcription factors involved in the process of polarization. **Critical Issues:** miRNAs are intricately involved with fine-tuning fundamental macrophage functions such as phagocytosis, efferocytosis, inflammation, tissue repair, and tumor promotion. Macrophages are secretory cells that participate in intercellular communication by releasing regulatory molecules and microvesicles (MVs). MVs are bilayered lipid membranes packaging a hydrophilic cargo, including proteins and nucleic acids. Macrophage-derived MVs carry functionally active miRNAs that suppress gene expression in target cells *via* post-transcriptional gene silencing, thus regulating cell function. In summary, miRNAs fine-tune several major facets of macrophage development and function. Such fine-tuning is critical in preventing exaggerated macrophage response to endogenous or exogenous stimuli. **Future Directions:** A critical role of miRNAs in the regulation of innate immune response and macrophage biology, including development, differentiation, and activation, has emerged. A clear understanding of such regulation on macrophage function remains to be elucidated. *Antioxid. Redox Signal.* 25, 795–804.

Keywords: miRNA, macrophages, wound healing, inflammation, resolution of inflammation, chronic wounds

Introduction

HIGH-THROUGHPUT RNA sequencing has unveiled the existence of a large number of noncoding (nc) RNAs in the human genome (57). MicroRNAs (miRNAs), first discovered in 1993 in *Caenorhabditis elegans*, are evolutionarily conserved 19–25-nt-long ncRNAs of extraordinary functional significance in human health (44). miRNAs are centrally involved in modifying the cellular gene expression response. Gene expression, comprising transcription and translation, is a tightly regulated sophisticated process. Extensive posttranscriptional processing of pre-mRNA is a hallmark of eukaryotic gene expression. This processing provides an additional checkpoint in gene regulation governing the expression of protein, which in turn influences cell function (9). Posttranscriptional control of the 3' untranslated region (3'-UTR) is a key step that determines the rate of translation of mRNA to protein (9).

miRNAs control posttranscriptional gene silencing (PTGS) primarily *via* binding to 3' or 5' UTR of the target transcripts causing translational repression or mRNA degradation. The first evidence of miRNA binding to the 3'-UTR of target mRNA came with the discovery of lin-4 miRNA in *C. elegans*. Lin-4 was noted to contain sequences that were complementary to 3'-UTR of lin-14 gene, suggesting an antisense RNA-RNA interaction (44). miRNAs are evolutionary conserved across even distant related species, suggesting probable uniformity in the molecular evolution of cellular miRNA gene activity (57). Studies indicate that posttranscriptional regulation of genes by miRNAs is a prevalent phenomenon. According to bioinformatics-based predictions, almost one third of all protein coding genes are posttranscriptionally regulated by miRNAs (21). Recently, a miRNA reporter library study identified functional “miRNAome” of a cell, which represents about 40% of the total miRNAs with any

detectable activity (54). This study raised a significant issue with miRNA profiling, where the number of miRNAs differentially expressed in a cell will be much larger than the actual “functional miRNAome” (54).

Studies during the past two decades have identified and highlighted the significance of miRNAs in a multitude of physiological processes and pathological functions, including development, cell proliferation, hematopoiesis, and cell death. Emerging studies indicate that tissue repair and injury, immune and inflammatory responses are substantially regulated by miRNAs (16, 76, 78). Cells of the monocyte/macrophage lineage are critical for mounting and resolving the inflammatory response during tissue injury and repair (17). In this context, this review focuses on miRNAs that control the development, differentiation, and function of monocytes/macrophages by targeting molecules critical in each of these processes.

miRNAs Regulating Development of Myeloid Cells

Circulating monocytes that originate from adult hematopoietic stem cells (HSCs) are primary precursors of macrophages (88). During homeostasis, HSCs are located in bone marrow (BM) niches. Upon injury, HSCs get mobilized to the spleen and liver (91). Microenvironmental niches in BM dictate the maintenance and differentiation of HSCs (91). miRNAs have been identified as key regulators of HSC renewal and fate (27). Initial evidences pointing toward the significance of miRNAs in HSC regulation came from studies performed on Arsenic resistance protein 2 (Ars2/Asr2) null mice (31). Ars2 is a RNA binding protein that contributes to the delivery of stable miRNA transcripts to the pri-miRNA microprocessor complex, Drosha and DGCR (31). Ars2 null mice suffered from BM failure, suggesting a critical role of miRNAs in HSC renewal and fate (31). Conditional ablation of

Dicer, an RNase III enzyme required for miRNA biogenesis, from the HSC compartment resulted in hematopoietic stem/progenitor cell apoptosis (32). A subset of miRNAs, including miR-29a, miR-126, miR-155, and miR-125a/b that were specifically enriched in the HSC compartment compared to other cells in the BM (58). Elaborate activities of miR-126, miR-130a, and miR-196b were observed in the most primitive HSCs; a loss in such activities was noted with differentiation of HSCs to more mature progenies (27). miR-125a alone was sufficient to augment the stem cell pool specifically targeting proapoptotic protein BAK1 (32). miR-126, abundantly expressed in HSCs, controls expansion of stem cells *via* targeting the PI3K/AKT/GSK3 β pathway (43). Forced expression of miR-126 in HSC impaired cycle progression of HSC, while knockdown of miR-126 resulted in increased HSC proliferation without exhaustion (43). Homozygous ablation of the miR-29a/b-1 in mice indicated a critical role of miR-29a/b-1 in HSC function by silencing Dnmt3a (33). These studies recognize miR-29a, miR-126, miR-130a, miR-155, and miR-125a/b as key miRNAs that are capable of controlling HSC biology (58) (Fig. 1).

The origin of monocytes from HSC involves differentiation from monoblasts to promonocytes, followed by maturation to monocytes (29). The transcription factor PU.1 facilitates commitment of HSC to lymphoid–myeloid progenitor (LMP) fate *via* suppression of GATA1 activity (24). The CCAAT/enhancer binding protein (C/EBP) α promotes differentiation of LMP to the granulocyte–monocyte progenitor (GMP) stage (24). Increased PU.1 activity favors differentiation of GMP to monocytic lineage (24). miRNAs control multiple steps in the monocyte/macrophage maturation process. PU.1 induces the expression of a subset of four miRs (miR-146a, miR-342, miR-338, and miR-155). This constitutes the initial step in the myeloid cell differentiation/maturation process (28). Forced expression of miR-146a, a negative

FIG. 1. Involvement of microRNAs (miRNAs) in monocyte/macrophage development. Circulating monocytes are primarily considered the precursors of macrophages. Monocytes originate from adult hematopoietic stem cells (HSCs) that under favorable conditions differentiate to → lymphoid–myeloid progenitor (LMP) → granulocyte–monocyte progenitor (GMP) and *via* multiple steps mature to monocytes. In blood, two populations of monocytes namely Ly6c⁺ or Ly6c⁻ have been identified in mice. The monocytes then enter to tissues and differentiate to macrophages.

regulator of innate immune response, was sufficient to drive maturation of stem cells to monocyte/macrophages during adult hematopoiesis (28). In addition to promoting expression of certain miRNA, PU.1 suppresses miR-17p-92 during myeloid differentiation by targeting *Egr2* (64). *EGR2*, in turn, recruits histone demethylase *Jarid1b* resulting in demethylation of CpG island located at the miR-17–92 promoter (64). The miR17-92 cluster comprises the following six miRNAs: miR-17, miR18a, miR-19a, miR-20a, miR-19b-1, and miR-92a. This cluster is present in high levels in early stem and progenitor cells. Expression of the cluster is downregulated upon inception of differentiation to myeloid lineage (64). PU.1 controls a regulatory circuitry involving transcription activation of miR-424 that leads to monocyte differentiation *via* translational repression of NFI-A (69). A comprehensive list of miRNAs involved in myeloid cell development has been provided by El Gazzar and McCall (20). The studies described above uphold the extraordinary significance of miRNAs in controlling monocyte/macrophage development (20, 28).

The relative expression levels of PU.1 and C/EBP α dictate the fate of myeloid progenitor cells to monocytic *versus* granulocytic lineages. Low levels of C/EBP α prevent GMP formation, whereas higher levels promote granulopoiesis over monopoiesis (25). C/EBP α induces miR-223 resulting in degradation of NFI-A mRNA to promote granulopoiesis (25). Both miR-21 and miR-196b promote the formation of monocytes while suppressing granulopoiesis (89). Interaction of colony stimulating factor-1 (CSF-1) with its CSF-1R promotes differentiation and maturation to monocytic lineage. CSF-1R expression is controlled by Runt-related transcription factor-1 (RUNX1, also known as AML1). miRNAs 17-5p, 20a, and 106a target the 3'-UTR of *Runx1* and suppress its translation (22). These studies point toward a central role of miRNAs in controlling major stages of monocyte/macrophage development from HSCs. Studies using appropriate conditional knockout/transgenic mice are required to understand specific regulation of these miRNAs in myeloid cell lineage commitment and maturation.

Control of Macrophage Polarization and Activation

Macrophages are dynamic cells and have the ability to undergo transition through a continuum of phenotypes and

activation states primarily based on the microenvironmental *milieu*. The outstanding significance of microenvironmental cues to direct macrophage polarization at the tissue injury/repair site has been recognized (17). Although contentious, macrophages are broadly classified as M1 and M2 polarization states (48, 53, 55). M1 macrophages, also known as “classically activated,” are induced by cytokines primarily secreted by Th1 cells, including IFN- γ and TNF α . In addition, granulocyte-macrophage colony-stimulating factor (GM-CSF), lipopolysaccharide (LPS), and other Toll-like receptor (TLR) ligands are also known to induce M1 polarization in macrophages. The M1 polarized macrophages support Th1 responses and produce copious amounts of reactive oxygen species (ROS) to help in killing pathogen (48). Conversely, activation of macrophages with Th2 cell factors results in M2 macrophages or “alternatively activated” macrophages. M2 macrophages influence the processes of infection, tissue repair, vascularization, and tumor promotion/invasion. These macrophages are characterized by their high expression of scavenger receptors in addition to high IL-10, VEGF, and MMPs. (48). The microenvironmental signals elicit a transcriptional response that regulates the phenotype and function of the macrophages (42). The signal transducer and activator of transcription 1 (STAT1) and interferon-regulatory factor 5 (IRF5) play a major role in dictating M1 macrophage phenotype (42), whereas, STAT6, peroxisome proliferator-activated receptor- γ (PPAR γ), and IRF4 direct M2 macrophage polarization (42) (Fig. 2). These transcription factors are directly controlled by specific miRNA (52, 86, 92, 94) (Fig. 2). PU.1, on the contrary, irrespective of polarization, serves as the master transcription factor for maintenance of macrophage identity and differentiation by acting on macrophage-specific enhancers (42).

The JAK/STAT pathway is under miRNA regulation (96). miR-146b suppress the antiviral function in T cells *via* directly targeting STAT-1 (90). It is not clear if miR-146a also targets STAT-1 in macrophages to control polarization. In a comprehensive study to decipher the role of miRNAs in macrophage polarization, macrophages were subjected to stimuli inducing four different patterns of macrophage activation/polarization (M1, M2a, M2b, and M2c). The changes in miRNA expression profile were elucidated using TaqMan low-density array human miRNA assays (version 2.0; Applied Biosystems) (30). Specific subsets of miRNAs uniquely expressed under four

FIG. 2. Transcriptional control of macrophage polarization: role of miRNA. The microenvironmental signals elicit a transcriptional response that regulates the phenotype and function of the macrophages. The signal transducer and activator of transcription 1 (STAT1) and interferon regulatory factor 5 (IRF5) play a major role in dictating M1 macrophage phenotype, whereas, STAT3/STAT6, peroxisome proliferator-activated receptor- γ (PPAR γ) and IRF4 direct M2 macrophage polarization. Major reported miRNAs that control these major transcription factors involved in macrophage polarization have been presented.

conditions of polarization were noted. Specifically, miR-125a-3p and miR-26a-2* in M1 macrophages, while miR-193b, miR-27a*, miR-29b-1*, miR-132*, and miR-222* were uniquely expressed in M2b phenotypes (30) (Fig. 3). M2c phenotype did not display any specific change in miRNA expression. Recent studies, including those from our laboratory, demonstrate a critical role of miR-21 in macrophage polarization (8, 16). We reported that miR-21 is central in efferocytosis (engulfment of apoptotic cells)-mediated change in macrophage from a proinflammatory (M1) to proresolving (M2) phenotype (16). In line with this observation, activation of CSF-1R by its ligands CSF-1 and IL-34 plays a major role in macrophage development and differentiation. The molecular signaling elicited by CSF-1R pathway has been shown to regulate macrophage polarization *via* PI3K $\rightarrow$ ERK $\rightarrow$ NF κ B and activation miR-21. Inhibition of miR-21 directed macrophage toward the M1 phenotype, suggesting that sufficient miR-21 in macrophages is essential for their M2 polarization (8). A central role of miR-33 in regulation of cellular lipid metabolism by repressing genes involved in cholesterol efflux, and fatty acid oxidation, has been recognized (39). A shift of macrophage metabolism toward glycolysis promotes M1 or proinflammatory phenotype (26). miR-33 was recently reported to promote macrophages toward M2 phenotype *via* targeting AMP-activated protein kinase, a key player in intracellular energy equilibrium (61).

Regulation of Monocyte/Macrophage Function

Macrophages/monocytes are implicated in regulation of a multitude of biological processes, including inflammation and phagocytosis (53, 55). To maintain focus, we limit discussion to the roles of miRNAs in regulation of key functions of monocyte/macrophages related to inflammation and tissue repair (Fig. 4).

Inflammation

Inflammation is a dynamic process that is elicited following injury/infection and other external stimuli with the objective to repair and restore homeostasis (68). Macrophages

are key cells of the innate immune system, an evolutionarily conserved form of host defense against infection (36). miRNAs control multiple regulatory checkpoints to fine-tune macrophage inflammatory response (71, 72). TLR and related downstream signaling cascade pathways are important detection mechanisms that enable the initial steps of the inflammatory cascade. The first evidence recognizing inflammation-sensitive miRNAs in macrophages was provided using miRNA expression profiling in endotoxin-treated murine macrophages (84). Of the 200 miRNAs investigated, miR-146a/b, miR-132, and miR-155 were identified as LPS-responsive miRNAs (84). Promoter analysis studies recognized miR-146a as a NF κ B-inducible miRNA (84). In addition, the study also identified binding sites for miR-146a/b at the 3'-UTRs of the TNF receptor-associated factor 6 (TRAF6) and IL-1 receptor-associated kinase 1 (IRAK1) genes, suggesting these targets may be subjected to PTGS by miR146a/b (84). A key function of miR-146 controlling TLR and cytokine signaling *via* a negative feedback regulation loop was proposed (84). This study was instrumental in highlighting the significance of inflammation-sensitive miRNAs in macrophages. An induction of miR-155 in mouse macrophages exposed to inflammatory mediators polyribinosinic:polyribocytidylic acid or the cytokine IFN- β was identified (59). miR-155 tightly controlled the expression of SHIP1 and SOCS1, both critical regulators of the inflammatory response in macrophages (59). Bala *et al.* reported that increased miR-155 levels contribute to alcohol-induced elevation in TNF- α production (4). miRNAs let-7i, miR-145, and miR-346 were shown to regulate TLR signaling. Of these, only let-7i inversely correlated with TLR signaling and immune response (60, 74). NF κ B is a master transcription factor that regulates the expression of proinflammatory cytokines such as TNF- α , IL-1 β , IL-6, and IL-8 in macrophages (37, 38). miR-146, one of the first miRNA identified to be involved in the NF κ B activation pathway, constitutes a negative feedback loop where NF κ B activates miR-146, which in turn suppresses NF κ B signaling by directly targeting TRAF6 and IRAK1 (84). Conversely, miR-181b indirectly regulated NF κ B in a positive feedback

FIG. 3. Involvement of miRNAs in macrophage polarization. In tissues based on microenvironment, the macrophages get polarized to M1 or M2 (M2a, M2b, or M2c subtypes). Major miRNAs involved/expressed in macrophage polarization have been shown.

FIG. 4. miRNAs in macrophage function and intercellular communication. Macrophages are closely involved with a number of biological functions. Major miRNA that has been identified with each of the macrophage-mediated key process has been presented.

loop that included $\text{NF}\kappa\text{B} \rightarrow \text{IL-6} \rightarrow \text{STAT3} \rightarrow \text{miR-181b} \rightarrow \text{CYLD} \rightarrow \text{NF}\kappa\text{B}$ (46). $\text{NF}\kappa\text{B}$ -dependent reporter assay screening of several hundred miRNA genes identified miR-301a as one of the most effective activators of $\text{NF}\kappa\text{B}$ by repressing $\text{NF}\kappa\text{B}$ repressing factor (46) (Fig. 5). miR-9 has been linked with innate immune response; the expression of this miRNA contributes to a negative feedback loop in the $\text{NF}\kappa\text{B}$ pathway, where miR-9 is activated by $\text{TLR4} \rightarrow \text{NF}\kappa\text{B}$

in human PMNs and monocytes target $\text{NF}\kappa\text{B}$ subunit p50 (6). The studies discussed above clearly indicate that miRNAs fine-tune the inflammatory response by controlling major pathways such as TLR and $\text{NF}\kappa\text{B}$. miRNA control of other major transcription factors implicated with inflammatory response has been highlighted (5, 10, 14, 34, 45, 56, 62) (Fig. 5). Such control by miRNA is likely to prevent an exaggerated inflammatory response.

FIG. 5. miRNAs control the expression of major transcription factors that govern the inflammation response. Gene expression in inflammation response is under tight control of a number of transcription factors, including $\text{NF}\kappa\text{B}$, activator protein-1 (AP-1), hypoxia inducible factor (HIF-1 α), and JAK/STAT. The expression of these transcription factors is regulated by miRNA, often in a regulatory feedback loop. In addition, shown are the miRNA, the expression of which is regulated by these transcription factors.

ROS production and function

In response to inflammation, phagocytosis, or environmental stimuli, macrophages produce copious amounts of ROS by a process known as “respiratory burst” (2, 63). NADPH oxidases are primarily responsible for the “respiratory burst” action in cells of myeloid origin (3). NADPH oxidase is a multisubunit complex that comprises membrane-bound cytochrome component, that is, gp91phox (phagocyte oxidase) and cytoplasmic components, including p47phox, p67phox, and p40phox, and rac (23, 63, 75). Upon activation, the cytosolic subunits translocate to the membrane-bound gp91. In 2008, our laboratory reported first evidence demonstrating that the rate-limiting p47phox subunit of NADPH oxidase is a target for miRNA (80). Global lowering of cellular miRNAs by knocking down Dicer, a key enzyme of miRNA biogenesis, resulted in decreased production of inducible ROS (80). Recently, a cross talk between ROS and miR-451 was identified using BM-derived macrophages from p47phox null mice (65). Furthermore, miR-21 *via* targeting PDCD4-AP-1 pathway was observed to regulate H₂O₂-dependent responses in smooth muscle vascular cell injury model. Using a global profiling approach, miRNA signature in response to oxidative stress such as ionizing radiation and H₂O₂ was determined (81). The pathways underlying oxidant-induced miRNA expression remain to be characterized.

Efferocytosis

Macrophages are responsible for cleaning up host tissue of invading pathogens as well as dead/dying cell debris. The process of engulfment of dead/dying cells by macrophages is referred to as “efferocytosis” (19). Efferocytosis is well known to play a central role in the resolution of inflammation (70). Despite the resemblance between the processes of efferocytosis and phagocytosis, the two processes are clearly distinct with respect to receptors and ligands involved (67, 87). Efferocytosis initiates signaling events involving two main complexes, (i) CrkII/ELMO/Dock180 or (ii) ABCA1/GULP (40). Activation of either complex leads to activation of Rac1, which initiates cytoskeletal rearrangement and subsequent engulfment of apoptotic cells (40). miRNAs have been implicated in the signaling cascade involving efferocytosis-induced resolution of inflammation, more specifically the change in macrophage phenotype following efferocytosis (16). Engulfment of apoptotic cells by macrophages dampened proinflammatory response and promoted an anti-inflammatory *milieu* by secretion of cytokines such as IL-10. Studies from our laboratory identified that in macrophages miR-21 can be induced by efferocytosis. Elevated macrophage miR-21 supported efferocytosis and silenced target genes PTEN and PDCD4, which in turn upregulated IL-10 and suppressed TNF α production, respectively, accounting for a net anti-inflammatory M2-like phenotype observed in post-efferocytotic macrophages (16). CrkII, a protein of the efferocytosis engulfment complex, has also been proposed to be a direct target of miR-126, indicating a potential regulation of miRNA at an upstream efferocytosis-mediated signaling pathway (15).

Wound healing

Wound healing is a well-orchestrated reparative event that occurs in response to injury and its microenvironment (49).

Macrophages infiltrating the wound site postinjury are often termed as wound macrophages (17). Wound macrophages are essential for repair (17, 50, 51). Work from our laboratory has focused on developing the significance of miRNAs in controlling molecular mechanisms that regulates wound healing (7, 11–13, 76, 77, 79, 80). Here, we limited discussion to those miRNAs that directly regulate macrophage function in wound healing. miR-21 was identified by us recently as a major negative regulator of LPS-induced inflammatory response in wound macrophages (16). miR-146a, an inflammation-sensitive miRNA, was significantly low in diabetic wounds (93). Such decreased miR-146a levels caused upregulation of proinflammatory target genes (93). The study did not, however, identify a role of wound macrophages in miR-146 dysfunction in diabetic ulcers. The studies to explore the significance of miRNAs in wound macrophage activation and function have just begun. Further studies investigating significance of miRNAs in wound macrophage activation and function are required to understand the mechanisms underlying the critical role of macrophages in wound healing.

Tumor-associated macrophages

Macrophages located in tumor stroma are referred to as tumor-associated macrophages (TAMs) (47). These macrophages primarily display M2 phenotype and modify inflammation, promote angiogenesis, matrix remodeling, and tumor cell migration/invasion, thereby suppressing antitumor immunity (47, 82). The presence of a higher number of TAMs is associated with tumor progression and poor patient prognosis (47). Cancer-related studies suggest the involvement of miRNAs in modulating response to TAM by directly acting on these macrophages and the precursors of these cells, including HSCs (82). In mouse models, a specific subset of TAMs that displays high expression of the mannose receptor (MRC1/CD206) and attenuated CD11c expression has been identified with high proangiogenic and tumorigenic activities (83). miR-511-3p, an intronic miRNA, is encoded by both mouse and human MRC1 genes (83). Using reporter vector bioassay, miR-511-3p was noted to show high expression and activity in CD206⁺ TAMs (83). By targeting CD206 (MRC1), miRNA-511-3p plays a critical role as gatekeepers of the protumorigenic activity of TAMs. Detailed studies investigating relevance of miRNAs in TAM subset phenotype and their influence on the activity of TAM are warranted.

Macrophage Intercellular Communications Via Extravesicular Release

Macrophages are secretory cells that control specific cellular processes *via* intercellular communication. Extracellular membrane vesicles (EVs) function as shuttles between cells for delivery of cargo such as proteins; mRNAs and miRNAs have emerged as major mediators of intercellular communication (66). Exosomes, microvesicle (MVs), and apoptotic bodies are three broad categories of EVs that are released by a number of cells, including macrophages (95). Exosomes are vesicles of endosomal origin that originate by inward budding and are between 40 and 100 nm in diameter (85). However, the MVs are released from plasma membranes as a result of outward budding and fission (85, 95). Macrophages secrete both MVs and exosomes that carry cargo, including proteins; mRNA and miRNA are for intercellular communication (41).

In peripheral blood, the majority of circulating microvesicles originate from platelets, while monocytes/macrophages contribute the second most abundant population of circulating MVs in blood (35). Macrophage-derived MVs have been demonstrated to carry functionally active miR-223 that subsequently induces macrophage differentiation and is an important contributor to the innate immune response (35). Another example of transferring functionally active miRNAs was provided by an observation where human macrophages, *via* gap junctions, transferred miR-142 and miR-223 to hepatocarcinoma cells (HCCs) (1). Such transfer of miRNAs resulted in PTGS of target proteins, that is, stathmin-1 and insulin-like growth factor-1 receptor in HCC, suggesting that such transfer is not just a passive process, rather it actively regulates functions in the acceptor cell. Another example of active transfer has been recently reported where monocytes exposed to alcohol communicated with naive monocyte *via* miR-27a cargo transferred from EVs (73). Transfer of miRNA resulted in naive macrophages to polarize to M2 macrophages (73). Taken together, macrophage-derived exosome and MV-mediated transfer of miRNAs add a novel dimension to how macrophages modify inflammatory responses and other cellular activities.

Conclusion

During the last decade, we have witnessed an unprecedented growth in understanding of miRNAs in health and disease. In most cases, miRNAs have been observed to fine-tune cellular responses and, therefore, act as a rheostat rather than an on/off switch. A critical role of miRNAs in the regulation of innate immune response and macrophage biology, including development, differentiation, and activation, has emerged. miRNA expression and activity are evolutionarily conserved and highly cell specific. Increased macrophage-specific knockout/transgenic studies will clarify the significance of these tiny molecular rheostats on development, differentiation, and activity of these cells in regulation of biological responses. Such information will be critical in developing miRNA-directed therapies. In summary, miRNAs fine-tune numerous facets of macrophage development and function. Such fine-tuning is critical in preventing exaggerated macrophage response to endogenous or exogenous stimuli.

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Abbreviations Used

3'-UTR = 3'-untranslated region
 BM = bone marrow
 C/EBP = CCAAT/enhancer binding protein
 CSF-1 = colony stimulating factor-1
 EVs = extracellular membrane vesicles
 GMP = granulocyte-monocyte progenitor
 HCC = hepatocarcinoma cell
 HSC = hematopoietic stem cell
 IRAK1 = IL-1 receptor-associated kinase 1
 IRF5 = interferon regulatory factor 5
 LMP = lymphoid-myeloid progenitor
 LPS = lipopolysaccharide
 miRNAs = microRNAs
 PPAR γ = proliferator-activated receptor- γ
 PTGS = posttranscriptional gene silencing
 ROS = reactive oxygen species
 RUNX1 = Runt-related transcription factor-1
 STAT1 = signal transducer and activator of transcription 1
 TAM = tumor-associated macrophage
 TLR = Toll-like receptors
 TRAF6 = TNF receptor-associated factor 6