miR-125a-5p Regulates Differential Activation of Macrophages and Inflammation*

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Background: The role of miRNAs in macrophage polarization has not been well defined.

Results: miR-125a-5p promotes M2, while it suppresses M1 macrophage polarization.

Conclusion: miR-125a-5p is an important regulator of the differential activation of macrophages.

Significance: Delineation of the role of miR-125a-5p in macrophage polarization provides insights into miRNA regulation of inflammation, tissue repair and tumor progression.

Macrophage activation is a central event in immune responses. Macrophages undergoing classical activation (M1 macrophages) are proinflammatory, whereas alternatively activated macrophages (M2 macrophages) are generally anti-inflammatory. miRNAs play important regulatory roles in inflammatory response. However, the manner in which miRNAs regulate macrophage activation in response to different environmental cues has not been well defined. In this study, we found that M-BMM macrophages (M2) express greater levels of miR-125a-5p than do GM-BMM macrophages (M1). Stimulation of macrophages through TLR2 and TLR4 but not through TLR3 enhanced miR-125a-5p expression. Up-regulation of miR-125a-5p after TLR2/4 activation requires the adaptor MYD88 but not TRIF. Overexpression of miR-125a-5p diminished M1 phenotype expression induced by LPS but promoted M2 marker expression induced by IL-4. In contrast, knockdown of miR-125a-5p promoted M1 polarization and diminished IL-4-induced M2 marker expression. We found that miR-125a-5p targets KLF13, a transcriptional factor that has an important role in T lymphocyte activation and inflammation. KLF13 knockdown had similar effects on M1 activation as did miR-125a-5p overexpression. In addition, miR-125a-5p regulates phagocytic and bactericidal activities of macrophages. Our data suggest that miR-125a-5p has an important role in suppressing classical activation of macrophages while promoting alternative activation.

Differentially activated macrophages display distinct functional phenotypes (1–3). Macrophages stimulated with Toll-like receptor (TLR)2 ligands such as LPS and/or IFN-γ are termed as M1 macrophages (1, 2, 4), whereas activation by Th2 cytokines such as IL-4 and IL-13, immune complexes plus TLR ligands, IL-10, or glucocorticoids generates M2 macrophages (1–4). Culture of bone marrow cells with GM-CSF or M-CSF produces M1 or M2 macrophages (5–11). M1 macrophages are characterized by high capacity to produce proinflammatory cytokines, including TNF-α and IL-12, and to eliminate bacterial, viral, and fungal infections (1, 2, 4). M2 macrophages have high expression of markers of alternative activation such as arginase-1 (Arg1) and have important roles in regulation of parasite infection, tissue remodeling, immune modulation, and tumor progression (1, 2, 12).

The polarized activation of macrophages has been extensively studied at the transcriptional level (1). It has been shown that NF-κB, AP-1, PU.1, CCAAT/enhancer-binding protein α, and IFN-regulatory factor 5 mediate M1 activation by TLR ligands, whereas IRF4, CCAAT/enhancer-binding protein-β, Kruppel-like factor 4, STA16 (signal transducer and activator of transcription 6), and peroxisome proliferator-activated receptor-γ promote differentiation into M2 phenotype (1). Epigenetic mechanisms are also implicated in macrophage polarization. For example, JMJD3 (Jumonji domain containing 3) and histone deacetylase 3 have been shown to play pivotal roles in M2 macrophage polarization (13, 14).

miRNAs play an important role in epigenomic regulation of gene expression and have critical roles in many cellular processes (15). There are a number of studies showing that miRNAs participate in immune response (16). However, it is less clear how miRNAs regulate differential activation of macrophages (3).

In this study, we found that miR-125a-5p is expressed at a higher level in M-BMM (M2) than in GM-BMM (M1) macrophages. miR-125a-5p was up-regulated in response to cellular activation through TLR2 or TLR4 but not by TLR3 activation. We found that miR-125a-5p suppresses classical activation, whereas it enhances alternative, activation of macrophages.

MATERIALS AND METHODS

Establishment of Mouse GM-BMM and M-BMM—GM-BMM and M-BMM were established from bone marrow cells of...
C57BL/6 mice (17). Briefly, after red blood cells were lysed, bone marrow cells were cultured in DMEM supplemented with 10% FBS and 20 ng/ml GM-CSF (R&D Systems) or 50 ng/ml M-CSF (R&D Systems) for 5 days to establish GM-BMM or M-BMM macrophages. C57BL/6 mice were from the NCI Frederick Laboratory and The Jackson Laboratory. MYD88+/− and TRIF−/− mice were from The Jackson Laboratory. The animal protocol was approved by the University of Alabama at Birmingham Institutional Animal Care and Use Committee.

miRNA Array—Total RNAs were isolated from GM-BMM and M-BMM with the mirNeasy mini kit (Qiagen). The miRNA array was performed by Exiqon using mirCURY LNA™ microRNA Array (Exiqon). The data were submitted to Gene Expression Omnibus with accession no. GSE46085 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE46085).

Quantitative Real-time PCR—Probe Master Mix kit (Roche Applied Science) and miScript SYBR Green PCR kit (Qiagen) were used for amplification of miR-125a-5p, miR-125b-5p, or internal control sno135 or U6. Taqman probes for miR-125a-5p and sno135 were purchased from Applied Biosystems. SYBR Green Master Mix kit (Roche Applied Science) was used for the following genes. Primer sequences were as follows: mouse GAPDH, 5'-CGACTTCAACGACAACTCCACTTCC-3' (sense) and 5'-TGGGTGTTCCAGGGTTTCTTACCTCTT-3' (antisense); mouse TNF-α, 5'-AGGCTCAAGAGGATCAACCAGCAGC-3' (sense) and 5'-TCAGATTACGGTGCACTTCCAT-3' (antisense); mouse IL-12 p40, 5'-TGACTGAAGTAGACAAGCTGGGGAT-3' (antisense) and 5'-GGACTAGTAGAAGTTCAGCTGCCCCATC-3' (sense); mouse IL-10, 5'-TTC-3' (antisense) and 5'-CAGTTAATCCTCC-3' (sense) and 5'-TGGCTGTGCTGACGAG-3' (antisense); mouse CCL-5, 5'-GGTCAACTTCACAT-3' (antisense); and mouse TNF-α, 5'-TTCC-3' (antisense) and 5'-TGGTTTCTATTCCTGCTGTC-3' (sense).

Western Blotting—Western blotting was performed as described previously (18). Rabbit anti-GAPDH, anti-iNOS, and anti-Arg1 antibodies were from Santa Cruz Biotechnology.

 Luciferase Assay—A 1-kb fragment containing the predicted miR-125a-5p binding element in the KLF13 3' UTR was obtained by PCR amplification using human genomic DNA as template and cloned into SpeI and HindIII sites of pMIR-Report luciferase vector (Applied Biosystems). The sequences of the primers used for the PCR amplification were as follows: 5'-GGAAGCGGACATTAAGTGTCACCGGATTCC-3' (sense) and 5'-CTGGTGTGCTGACGAG-3' (antisense). The resulting construct was designated as pMIR-Report-KLF13. HEK-293T cells were transfected with 5 ng of miR-125a-5p mimics and 20 nm control mimics or 20 nm miR-125a-5p mimics using Lipofectamine 2000 reagent (Invitrogen). 24 h after transfection, luciferase activity was determined by a Luciferase Assay System (Promega).

Generation of Flag-KLF13-expressing Plasmid Containing WT or Mutant KLF13 3' UTR—The 3' UTR of the KLF13 gene was obtained by PCR amplification. Nucleotides in the 3' UTR of the KLF13 gene that match the miR-125a-5p seed sequence were mutated by site mutagenesis. FLAG-KLF13 cDNA was generated by addition of a FLAG tag sequence at the 5' of the full-length KLF13 cDNA (a gift from Dr. Alan M. Krenskey, NIH). The WT or the mutant KLF13 3' UTR was ligated downstream the FLAG-KLF13 and cloned into FG-12 expressing vector.

Bacterial Killing Assay—Bacterial killing assay was performed as described previously (17, 19). Briefly, 0.1 × 10⁶ cfu/ml Escherichia coli (BL21DE3plysS) were added to macrophages in 96-well plates and incubated at 37 °C for 1 h. Supernatants were diluted 100 times, and 100 μl of the diluted supernatants was plated on Luria broth-agar plates. The plates were incubated over night at 37 °C, and bacterial colonies were counted. Data were presented as cfu/ml. cfu/ml indicates the numbers of bacterial colonies × dilution factor/volume of the plated diluted supernatants.

Phagocytosis Assay—Phagocytosis assay was performed as described previously (17, 20). Briefly, apoptotic thymocytes were first labeled with PKH26. 0.5 × 10⁶ labeled apoptotic cells suspended in 500 μl of media were added to macrophages and followed by incubation at 37 °C for 30 min. Macrophages were then washed with cold PBS, resuspended in PBS containing 1% BSA, anti-CD90.2 antibody (APC, allophycocyanin) (thymocyte marker), and anti-CD11b (FITC) (macrophage marker) antibody, and analyzed by flow cytometry. The phagocytic index was calculated as the ratio of FITC+PKH26+APC- cells to all cells gated.

Flow Cytometry Assay—Macrophages were trypsinized and incubated in PBS containing 1% BSA and 1 μg/ml Fc γ block (BD Pharmingen) for 30 min, followed by incubation with 1 μg/ml FITC-conjugated mouse anti-MHCII and phycoerythrin-conjugated anti-CD40 monoclonal antibody for 30 min. Flow cytometry was performed.

Transfection of miRNAs and siRNAs—Macrophages were transfected with miRNA mimics, miRNA inhibitors, or siRNAs using HiperFect transfection reagent (Qiagen). Control and miR-125a-5p mimics were from Invitrogen. Control and miR-125a-5p inhibitors were from Exiqon. Control and KLF13 siRNA were from Dharmacon.

Statistical Analysis—One-way analysis of variance followed by the Bonferroni test was performed for multiple group comparisons. The Student’s t test was used for comparison between two groups. p < 0.05 was considered statistically significant.

RESULTS

miR-125a-5p Is Expressed at a Higher Level in M-BMM Than in GM-BMM—We performed a miRNA array assay on RNAs isolated from GM-BMM and M-BMM, macrophages representative of the two opposite polarized states (M1 versus M2, respectively) (5–11) and found that miR-125a-5p is one of the miRNAs that exhibit greater expression in M-BMM than in GM-BMM (Fig. 1A and GSE46085). miR-125a-5p was among miRNAs that had relatively higher basal levels in M-BMM (data not shown). Therefore, we selected miR-125a-5p for further characterization of its role in macrophage activation. To validate the miRNA array data, we performed real-time PCR and demonstrated that M-BMM exhibit substantially higher levels of miR-125a-5p than do GM-BMM (Fig. 1B). These data sug-
miR-125a-5p is up-regulated in response to TLR2/4 stimulation—Because LPS stimulation induces M1 activation and promotes the transition of M2 macrophages to the M1 state (4), we next determined whether TLR4 engagement affects miR-125a-5p levels in macrophages. Unexpectedly, we found that miR-125a-5p levels are significantly up-regulated in GM-BMM at 24 h after LPS treatment (Fig. 2A). LPS stimulation also increased miR-125a-5p expression in M-BMM (data not shown) consistent with previous studies (21). miR-125a-5p levels had little change at 4 h after LPS stimulation. However, TNF-α, an early response proinflammatory cytokine, was significantly induced in macrophages after 4 h of LPS treatment (Fig. 2B). These data suggest that up-regulation of miR-125a-5p is a late event after TLR4 stimulation. Given our finding that miR-125a-5p demonstrates greater expression in M-BMM than in GM-BMM, the up-regulation of miR-125a-5p in LPS treated macrophages suggests that miR-125a-5p may be a negative regulator of classical activation of macrophages.

**Figure 1.** miR-125a-5p is expressed at a higher level in M-BMM than in GM-BMM. A, M-BMM and GM-BMM were generated by culturing mouse bone marrow cells in 50 ng/ml M-CSF or 20 ng/ml GM-CSF for 7 days. RNA was isolated and miRNA array analysis was performed. Unsupervised hierarchical clustering is presented. B, levels of miR-125a-5p in M-BMM and GM-BMM were determined by real-time PCR. snRNA RNU6B was used as an internal control (n = 3); mean ± S.D.; ***, p < 0.001 compared with GM-BMM. mmu, Mus musculus.

**Figure 2.** miR-125a-5p is up-regulated in response to TLR2 and TLR4 stimulation. A, GM-BMM were treated with 100 ng/ml LPS for the indicated time. Levels of miR-125a-5p were determined by real-time PCR (n = 3); mean ± S.D.; **, p < 0.01 compared with 0 h. B, experiments were done as in A. Levels of TNF-α were determined by real-time PCR. C, GM-BMM were treated without or with 1 μg/ml Pam(3)CSK(4) or 2 μg/ml poly(I:C) for 24 h. Levels of miR-125a-5p were determined by real-time PCR (n = 3); mean ± S.D.; ***, p < 0.001 compared with control (con). D, peritoneal macrophages isolated from WT, MYD88−/− or TRIF−/− mice were treated without or with 100 ng/ml LPS. Levels of miR-125a-5p determined by real-time PCR (n = 3); mean ± S.D.; ***, p < 0.001 compared with the WT group without treatment. E, peritoneal macrophages isolated from MYD88−/− mice were treated without or with 1 μg/ml Pam(3)CSK(4) (PAM). RNA was isolated, and levels of miR-125a-5p were determined (n = 3); mean ± S.D. F, GM-BMM were treated with LPS for the indicated time. Levels of miR-125b-5p were determined (n = 3); mean ± S.D.; *, p < 0.05 compared with 0 h. The experiments were performed two to three times with similar results.
To determine whether miR-125a-5p expression is regulated by TLRs other than TLR4, we treated macrophages with Pam(3)CSK(4), a TLR2 ligand, or poly(I:C), a TLR3 ligand (22–24). As shown in Fig. 2C, stimulation of macrophages through TLR2 but not TLR3 up-regulated miR-125a-5p expression. Because engagement of TLR2 and TLR4 primarily leads to NF-κB activation, whereas stimulation through TLR3 largely results in IRF3 activation (22–24), up-regulation of miR-125a-5p by TLR2 and TLR4 but not by TLR3 suggests that its up-regulation may be mediated by NF-κB.

LPS-induced NF-κB activation primarily requires the adaptor MYD88 but not TRIF. To determine whether up-regulation of miR-125a-5p by LPS is dependent on NF-κB, we treated macrophages from WT, MYD88<sup>−/−</sup>, and TRIF<sup>−/−</sup> mice with LPS and found that LPS-induced expression of miR-125a-5p in MYD88<sup>−/−</sup> macrophages is significantly diminished (Fig. 2D). Furthermore, there was no miR-125a-5p induction in Pam(3)CSK(4)-treated MYD88<sup>−/−</sup> macrophages (Fig. 2E). These data indicate that miR-125a-5p induction requires NF-κB. miR-125b-5p, which shares the same seed sequence as miR-125a-5p, also demonstrated greater expression in M-BMM than in GM-BMM (Fig. 1A). However, in contrast to miR-125a-5p, miR-125b-5p was down-regulated by LPS stimulation (Fig. 2F). These data suggest that the two miRNAs may have distinct roles in immune response.

**miR-125a-5p Suppresses Expression of the M1 Phenotype**—Macrophages activated by TLR ligands are termed as classically activated macrophages or M1 macrophages, which are characterized by elevated expression of proinflammatory cytokines (4, 7). To determine whether miR-125a-5p regulates proinflam-
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miR-125a-5p Promotes Expression of the M2 Phenotype

We next determined whether knocking down miR-125a-5p in M-BMM in M-BMM achieves an effect opposite to what observed in GM-BMM that overexpress miR-125a-5p. miR-125a-5p knockdown by anti miR-125a-5p-5 inhibitors enhanced LPS-induced expression of TNF-α, IL-12, and iNOS. These data suggest that miR-125a-5p is a negative regulator of TLR4 induced proinflammatory responses.

miR-125a-5p Down-regulates KLF13—Several computational algorithms predict that the transcriptional factor KLF13 could be a relevant mediator to the effects of miR-125a-5p. KLF13 has been shown to regulate T cell activation and promote T cell-associated inflammation (27, 28). To determine whether miR-125a-5p regulates KLF13, GM-BMM were transfected with control miRNAs or miR-125a-5p mimics. Levels of KLF13 were determined by real-time PCR. G, GM-BMM were transfected with control siRNAs or KLF13 siRNAs. Levels of KLF13 were determined by real-time PCR. H, GM-BMM were transfected with control siRNAs or KLF13 siRNAs, followed by LPS treatment for 6 h. Levels of TNF-α and IL-12 were determined by ELISA (n = 3); mean ± S.D.; **, p < 0.01 compared with the control group treated with LPS. Representative results of two experiments are shown.

miR-125a-5p Promotes Expression of the M2 Phenotype—We have shown that overexpression of miR-125a-5p in GM-BMM diminishes LPS-induced M1 phenotypes, we next determined whether miR-125a-5p also regulates the M2 phenotype. To do this, GM-BMM that were transfected with control mimics or miR-125a-5p mimics were treated with IL-4, a classical Th2 cytokine that induces M2 macrophage polarization (4). We found that the expression of Arg1 in GM-BMM transfected with miR-125a-5p after IL-4 exposure is significantly greater than that in those transfected with control mimics (Figs. 5, A and B). In contrast, knockdown of miR-125a-5p in M-BMM diminished IL-4 induced expression of Arg1 (Fig. 5C). Taken together, these data suggest that miR-125a-5p promotes macrophage polarization toward the M2 phenotype.

FIGURE 5. miR-125a-5p promotes expression of the M2 phenotype. A, GM-BMM were transfected with 20 nm control (con) mimics or miR-125a-5p mimics. After transfection, the cells were treated without or with 10 ng/ml IL-4 for 24 h. Levels of Arg1 were determined by real-time PCR (n = 3); mean ± S.D.; **, p < 0.01 compared with the control groups. B, GM-BMM were transfected with control mimics or miR-125a-5p mimics. The cells were then treated without or with IL-4 for 24 h. Levels of Arg1 and actin were determined by Western blotting. C, M-BMM were transfected with 20 nm control inhibitors or miR-125a-5p inhibitors. After transfection, the cells were treated without or with IL-4 for 24 h. Levels of Arg1 and actin were determined by Western blotting. The experiments were performed twice with similar results.

FIGURE 6. miR-125a-5p down-regulates KLF13. A, GM-BMM were transfected with 20 nm control (con) mimics or miR-125a-5p mimics. Levels of KLF13 were determined by real-time PCR (n = 3); mean ± S.D.; **, p < 0.01 compared with the control group. B, GM-BMM were transfected with control inhibitors or miR-125a-5p inhibitors. After transfection, the cells were stimulated with LPS for 24 h. Levels of KLF13 in the cells were determined by real-time PCR (n = 3); mean ± S.D.; **, p < 0.01 compared with the control group. C, 5 ng of PMIR-Report-KLF13 were co-transfected with 20 nm control mimics or miR-125a-5p mimics into HEK-293T cells. 24 h after transfection, luciferase activity in the cells was determined (n = 3); mean ± S.D.; **, p < 0.01 compared with the control group. D, mimics or miR-125a-5p mimics were transfected into HEK-293 cells. 6 h after the transfection, FLAG-KLF13 expressing vector that contains WT or mutant 3′-UTR of the KLF13 gene was transfected into the same HEK-293 cells. Two days after transfection, FLAG-KLF13 levels were determined. E, GM-BMM were transfected with LPS for the indicated time. Levels of miR-125a-5p and KLF13 were determined by real-time PCR. F, levels of KLF13 in GM-BMM and M-BMM were determined by real-time PCR. G, GM-BMM were transfected with control siRNAs or KLF13 siRNAs. Levels of KLF13 were determined by real-time PCR. H, GM-BMM were transfected with control siRNAs or KLF13 siRNAs, followed by LPS treatment for 6 h. Levels of TNF-α and IL-12 were determined by ELISA (n = 3); mean ± S.D.; **, p < 0.01; ***, p < 0.001 compared with the control group treated with LPS. Representative results of two experiments are shown.
KLF13 by miR-125a-5p (Fig. 6D). Furthermore, the kinetics of LPS induced miR-125a-5p was consistent with that of KLF13 down-regulation (Fig. 6E). The expression of KLF13 in M-BMM and GM-BMM was also inversely correlated to that of miR-125a-5p in these two types of macrophages (Fig. 6F). To determine whether KLF13 knockdown reproduces the inhibitory effect of miR-125a-5p on the expression of the M1 phenotype, GM-BMM were transfected with control siRNA or KLF13 siRNA (Fig. 6G). We found that KLF13 knockdown attenuates LPS-induced TNF-α and IL-12 (Fig. 6H). These data suggest that KLF13 may mediate the effect of miR-125a-5p on macrophage activation.

**miR-125a-5p Regulates Cellular Functions Associated with the M1 and M2 Phenotypes**

M2 macrophages are associated with greater ability to engulf apoptotic cells than do M1 macrophages (29–32). We found that miR-125a-5p overexpression significantly enhanced the ability of GM-BMM to ingest apoptotic cells (Fig. 7C). In contrast, miR-125a-5p knockdown diminished the capability of M-BMM of phagocytosing apoptotic thymocytes (Fig. 7D). These findings are congruent with the general effects of miR-125a-5p on the expression of the M1 and M2 phenotypes. Furthermore, KLF13 knockdown also enhanced the ability of GM-BMM to engulf apoptotic thymocytes (Fig. 7E).

**DISCUSSION**

We found that miR-125a-5p is up-regulated after macrophage activation through TLR2 and TLR4 but not TLR3. We also demonstrated that up-regulation of miR-125a-5p requires MYD88 but not TRIF. These data suggest that miR-125a-5p expression may be mediated by NF-κB. However, unlike TNF-α, miR-125a-5p was induced only at late time points in LPS-treated macrophages. These data suggest that, in contrast to early proinflammatory responses in TLR4-stimulated macrophages, additional transcriptional or epigenetic regulators are involved in LPS-induced expression of miR-125a-5p.

In contrast to miR-125a-5p, miR-125b-5p, which has the same seed sequence as miR-125a-5p does, was shown to be down-regulated by inflammatory stimuli, including LPS (33–35). miR-125b targets TNF-α and has been implicated in nega-
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tive regulation of inflammatory responses (34–39). However, a recent study showed that miR-125b potentiates proinflammatory activity of macrophages (25). Enforced expression of miR-125b drives macrophages to adapt an activated morphology that is accompanied by increased co-stimulatory factor expression and elevated responsiveness to IFN-γ, whereas anti-miR-125b treatment decreases CD80 surface expression in Raw264.7 macrophages (25). In our study, we found that miR-125a-5p suppresses classical activation of macrophages. We found that miR-125a-5p diminishes not only LPS-induced expression of TNF-α but also other LPS-induced proinflammatory mediators such as iNOS and IL-12. These data suggest that although TNF-α may be a target of miR-125a-5p, the anti-inflammatory effect of miR-125a-5p may be mediated by more general regulators. As we and others (40, 41) demonstrated that the transcriptional factor, KLF13, which has been shown to negatively regulate inflammation associated with T cell activation, is a target of miR-125a-5p, KLF13 could participate in modulating the effects of miR-125a-5p. In a recent study, Graff et al. (42) found that overexpression of miR-125a-5p appears to cause inflammatory activation of THP-1 cells, although the effect was not robust. Another study showed that miR-125a-5p activates NF-κB activity in diffuse large B-cell lymphoma cells (43). The discrepancy between these studies and ours could be explained by the different cell systems (primary macrophages versus tumor cell lines) that were employed. We found that miR-125a-5p expression is markedly increased in LPS-treated mouse bone marrow-derived macrophages. However, it was shown by Graff et al. (42) that miR-125a-5p expression is only marginally increased in LPS-treated THP-1 cells.

KLF13 was initially found to regulate RANTES expression in T cells and thus play an important role in T cell activation (27, 28). However, there is currently little information about the role of KLF13 in regulating TLR signaling events or macrophage activation. We found that KLF13 knockdown suppresses the expression of the M1 phenotypic but promotes basal expression of M2 markers (data not shown). These findings are consistent in general with those observed with miR-125a-5p overexpression in macrophages, suggesting that the effects of miR-125a-5p may be mediated, at least partially, by KLF13. However, it remains to be determined how KLF13 participates in this process. We found that KLF13 does not regulate signaling events immediately downstream of TLR4 engagement such as IκB-α degradation (data not shown) (44). Given that KLF13 is a Krüppel-like transcriptional factor (27, 28), it is likely that it may act at the promoters of genes that are associated with macrophage polarization. Indeed, Krüppel-like transcriptional factors have been implicated in differential activation of macrophages. For example, Kruppel-like factor 4 was shown to promote M2 macrophage polarization and to suppress expression of the M1 phenotype (45, 46). We showed that miR-125a-5p not only regulates the expression of M1 and M2 phenotypic markers but is also in control of M1/M2 associated macrophage functions. Increasing miR-125a-5p diminished the bactericidal activity of macrophages, a typical property associated with M1 macrophages (1, 2, 4), whereas it enhanced the phagocytic activity to ingest apoptotic cells, an ability found to be greater with M2 macrophages (29–32). In contrast, knockdown of miR-125a-5p in M-BMM decreased their ability to phagocytose apoptotic cells. Our data suggest that the functional effects of miR-125a-5p are likely mediated by KLF13 because KLF13 knockdown diminished the activity of macrophages to kill bacteria, whereas it enhanced the ability of macrophages to engulf apoptotic cells, similar to the effects of miR-125a-5p overexpression in macrophages. However, the effects of miR-125a-5p on macrophage activation may not be solely mediated by KLF13 because KLF13 knockdown did not reproduce all of the phenomena observed with miR-125a-5p overexpression.

In these studies, we found that miR-125a-5p is up-regulated after macrophage activation through TLR2 or TLR4 and serves as a negative regulator of macrophage-associated inflammatory responses. Furthermore, we demonstrated that miR-125a-5p enhances alternative activation of macrophages. Our data are in agreement with a number of recent studies showing that negative regulators of inflammatory response generally promote M2 polarization of macrophages (1). One of the best examples is SOC3, which has been demonstrated to be capable of dampening TLR signaling as well as enhancing alternative activation of macrophages (47–49). Therefore, our present results demonstrate that macrophage polarization is modulated not only by protein regulators but also by small non-coding RNAs such as miR-125a-5p and likely other miRNAs as well (26, 50, 51).

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