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MicroRNA-101 Targets MAPK Phosphatase-1 To Regulate the Activation of MAPKs in Macrophages

Qing-Yuan Zhu,*‡ Qin Liu, ‡ Jian-Xia Chen,* Ke Lan,* and Bao-Xue Ge*‡

MAPK phosphatase-1 (MKP-1) is an archetypical member of the dual-specificity phosphatase family that deactivates MAPKs. Induction of MKP-1 has been implicated in attenuating the LPS- or peptidoglycan-induced biosynthesis of proinflammatory cytokines, but the role of noncoding RNA in the expression of the MKP-1 is still poorly understood. In this study, we show that MKP-1 is a direct target of microRNA-101 (miR-101). Transfection of miR-101 attenuates induction of MKP-1 by LPS as well as prolonged activation of p38 and JNK/stress-activated protein kinase, whereas inhibition of miR-101 enhances the expression of MKP-1 and shortens p38 and JNK activation. We also found that expression of miR-101 is induced by multiple TLR ligands, including LPS, peptidoglycan, or polyinosinic-polycytidylic acid, and that inhibition of PI3K/Akt by LY294002 or Akt RNA interference blocks the induction of miR-101 by LPS in RAW264.7 macrophage cells. Moreover, treatment of cells with dexamethasone, a widely used anti-inflammatory agent, markedly inhibits miR-101 expression and enhances the expression of MKP-1 in LPS-stimulated macrophages. Together, these results indicate that miR-101 regulates the innate immune responses of macrophages to LPS through targeting MKP-1. The Journal of Immunology, 2010, 185: 7435–7442.

Innate immune response to microbial infections occurs primarily through TLRs, which recognize the pathogen-associated molecular patterns and elicit the biosynthesis of proinflammatory cytokines from macrophages and other cells (1–3). LPS, a cell wall component of Gram-negative bacteria, can be recognized by a TLR4 receptor complex (4). Stimulation of TLR4 by LPS triggers the recruitment of adaptor protein MyD88, which in turn transmits a series of signaling cascades that lead to the activation of MAPKs (5–8). The MAPKs are a group of highly conserved serine/threonine protein kinases, including ERKs, JNK/stress-activated protein kinase, and p38 MAPKs. Once activated, MAPKs phosphorylate downstream protein kinases and transcription factors, leading to the production of proinflammatory cytokines, such as TNF-α, IL-1, IL-6, and IL-8.

Deactivation of MAPKs is regulated mainly by a family of MAPK phosphatases (MKPs) (9, 10). MKP-1, an archetypical member of this family, functions as a vital negative regulator in the innate immune system (9, 11). It localizes to the nucleus through its N terminus (12) and preferentially dephosphorylates activated p38 and JNK relative to ERK in vitro (13). Upon stimulation with LPS, MKP-1-deficient macrophage cells exhibit prolonged p38 and JNK/stress-activated protein kinase activation as well as enhanced production of TNF-α and IL-6 as compared with that of wild-type cells (11, 14–17). Moreover, MKP-1 knockout mice produce substantially greater quantities of inflammatory cytokines and exhibit increased mortality from endotoxic shock (11, 17).

MKP-1 expression itself is induced rapidly by stimulation with LPS and other microbial components, leading to feedback control of deactivation of MAPKs (17). Induction of MKP-1 by LPS is regulated differentially by multiple signaling pathways in different cell types. Both MyD88 and TRIF, two adaptor proteins of the TLR4 signaling pathway, have been found to be involved in the induction of MKP-1 by LPS (17). In bone marrow macrophages, Raf-1, protein kinase C, and JNK have been found to regulate LPS-induced production of MKP-1 (18–20). Moreover, p38 and its downstream kinase MK2 also play a significant role in the induction of MKP-1 in RAW264.7 macrophages and peritoneal primary macrophages (21).

MicroRNAs (miRNAs) play a central role in the regulation of gene expression at the posttranscriptional level via an RNA interference (RNAi) mechanism (22). Partial complementary pairing of miRNAs with target mRNAs in the 3′ untranslated region (UTR) causes the translation repression and/or degradation of the mRNAs and thus the silencing of the target gene (23–26). It is believed that up to 30% of human genes are more or less mediated by miRNAs (27), and miRNAs have been found to play important roles in many biological processes ranging from cellular development and differentiation to tumor growth (22). Recently, miRNAs have been shown to be involved in innate immune responses. In response to stimulation by LPS or other microbial components, a rapid increase in the expression of selected miRNAs, namely, let-7e (28), miR-146 (29), miR-21 (30), miR-155 (31), and miR-181c (28), has been observed in monocytes, macrophages, and mouse macrophages. These miRNAs target the different components of TLR signaling pathways, including TLR4, IL-1R–associated kinase 1/TNF receptor–associated factor 6, PDCD4, SCOS1/SHP1, and TNFSF11 (RANKL), thereby regulating the inflammatory response. LPS stimulation also suppresses the expression of miR-125b, which targets TNF-α (32).

In this study, we identify MKP-1 as a target of microRNA-101 (miR-101), a tumor-related miRNA. miR-101 is induced after cellular activation through multiple TLRs. Induction of miR-101

Abbreviations used in this paper: GR, glucocorticoid; DEX, dexamethasone; miR-101, microRNA-101; miRNA, microRNA; MKP, MAPK phosphatase; PGN, peptidoglycan; poly-IC, polyinosinic-polycytidylic acid; qRT-PCR, quantitative real-time PCR; RNAi, RNA interference; RT-PCR, reverse transcription PCR; siRNA, small interfering RNA; UTR, untranslated region.

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by LPS is dependent on the PI3K/Akt pathway. We also demonstrate that miR-101 regulates the LPS-induced production of MKP-1 as well as subsequent activation of MAPKs in macrophages. Our studies thus identify a previously unrecognized feedback loop in which TLR-induced miR-101 expression is capable of upregulating innate immune responses.

Materials and Methods

Reagents, inhibitors, and Abs

RPMI 1640 medium, DMEM, FBS, penicillin, and streptomycin were purchased from Invitrogen (Shanghai, China). PI3K inhibitor (LY294002), p-ERK inhibitor (PD98059), p-JNK inhibitor (SP600125), p-p38 inhibitor (SB203580), and RU-486 were obtained from Calbiochem (San Diego, CA). Abs against p-p65, p38, p-p38, p-ERK, p-JNK, and p-AKT were obtained from Cell Signaling Technology (Beverly, MA). Abs against MKP-1 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). LPS, peptidoglycan (PGN), polyinosinic-polycytidylic acid (poly-IC), PMA, ionomycin and dexamethasone (DEX) were obtained from Sigma-Aldrich (St. Louis, MO). Cell Counting Kit-8 was purchased from Beyotime (Shanghai, China).

Cell culture

The murine macrophage cell line RAW264.7 (American Type Culture Collection, Manassas, VA) was cultured in RPMI 1640, supplemented with 10% FBS and antibiotics (50 U/ml penicillin and 50 μg/ml streptomycin). The human embryonic kidney cell line 293 (American Type Culture Collection) was cultured in DMEM with high glucose. Peritoneal macrophages were isolated from 8-week-old specific pathogen-free male C57BL/6 mice as described previously (33). Briefly, mice were injected i.p. with 2 ml of 4% thioglycollate medium (Sigma-Aldrich). After 3 d, cells harvested by peritoneal lavage with cold PBS were allowed to adhere for 2 h and the RPMI 1640 medium was changed to remove nonadherent cells. The remaining adherent monolayer cells were used as primary peritoneal macrophages after at least 24 h. Before LPS stimulation, cells were cultured in serum-free RPMI 1640 with antibiotics for 12 h to minimize the influence of FBS. All of the cells were cultured at 37°C in a humidified incubator with 5% CO2.

Bioinformatics analysis

The bioinformatics calculation and analysis were done using miRanda miRBase (34) and TargetScan online (35) following online instructions.

RNA preparation, reverse transcription PCR, and quantitative real-time PCR

For reverse transcription PCR (RT-PCR), total RNA was isolated using TRIzol reagent (Invitrogen) following the manufacturer’s instructions. Reverse transcription was done with the MMLV Reverse Transcription System (Invitrogen). PCR was run on an Eppendorf Gradient master with Premix Taq Mix (Takara, Dalian, China). Reverse transcription and PCR reverse primers for mouse MKP-1 were 5’-CTCTTCAGCAGCTTGAGAGA-3’ and 5’-GGCCATGGTGATGGAGG-3’. PCR sense primer was 5’-GGGATCTGTTGATGGAGG-3’ and antisense primer was 5’-GCCGTCATGCCGAGAAG-3’. Primers for quantitative real-time PCR (qRT-PCR) of MKP-1 were sense 5’-GGACTCTGGACGAAACGTC-3’ and antisense 5’-CTTCCAGAGGGTGAAGGAGG-3’. Primers target the whole sequence of Mus musculus MKP-1 coding region (NM_013642). For qRT-PCR, total RNA with miRNA was prepared using a mirVana miRNA Isolation Kit (Ambion, Tokyo, Japan) following the manufacturer’s protocol. First-strand cDNA was synthesized using a TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). Real-time PCR was run on an HT7900 with the TaqMan Universal Real-Time PCR System (Applied Biosystems). The reverse transcription stem-loop primer, real-time PCR primer, and primers for MKP-1 and sno135 (control) were purchased from Applied Biosystems. All of the PCR experiments were done in triplicate within each experiment, and experiments were replicated at least three times.

Dual-luciferase reporter assay

A whole fragment of 3’ UTR Mus musculus mkp-1 mRNA and a mutant form were cloned into pGL-3-Lac. The 293 cells were seeded in 12-well plates and cotransfected with pGL-3-MKP-1 wild-type or mutant portion and pRL-TK (Promega, Madison, WI) using Lipofectamine 2000 (Invitrogen). After 48 h of incubation, cells were collected for application in the Dual-Luciferase Reporter System (Promega, Madison, WI) following the manufacturer’s instructions. All of the dual-luciferase reporter assays were done in triplicate within each experiment, and three independent experiments were conducted.

Results

Bioinformatics identification of mkp-1 as a putative target for miR-101

It is accepted generally that miRNAs exert their function through regulation of the expression of their downstream target gene(s). To identify miRNAs that regulate the expression of MKP-1, we searched the putative miRNA binding sites at the 3’ UTR of mkp-1 mRNA using miRNA target prediction programs (miRanda and TargetScan). Data collection and analysis reveal that the 3’ UTR of mkp-1 mRNA contains a complementary site for the seed region of miR-101, an miRNA widely known as a regulator in the molecular etiology of cancer (36). Homo sapiens hsa-miR-101 (MIMAT0000099) and Mus musculus mmu-miR-101a (MIMAT000133) target a region from 441 to 461 and 469 to 489 at the 3’ UTR of human and mouse mkp-1, respectively (Fig. 1A). According to the accredited miRNA sequence registry at http://microrna.sanger.ac.uk/sequences/, hsa-miR-101 receives −13.5 KCal/Mol energy and a score of 151, whereas mmu-miR-101 receives −14.1 KCal/Mol energy and 156 score under miRanda calculation. Also, as shown in Fig. 1B, the predicted 8-mer seed binding region (gray sequence) of miR-101 is highly conserved in different mammalian species, thus revealing a possibly critical role for miRNA–mRNA interaction (37).

miR-101 represses MKP-1 expression through 3’ UTR interactions

To further examine the effect of miR-101 on MKP-1 expression, RAW264.7 macrophage cells were transfected with miR-101 mimic or control dsRNA and then stimulated with LPS. The mRNA level of MKP-1 was evaluated by RT-PCR (Fig. 2A) or qRT-PCR (Fig. 2B). As shown in Fig. 2A and 2B, transfection with miR-101 mimic did not significantly change the mRNA level of MKP-1 as com-
pared with control dsRNA. We also have analyzed the effect of miR-101 on the protein level of MKP-1 using Western blot analyses. The results reveal that transfection of miR-101 mimic but not control dsRNA markedly inhibits the production of MKP-1 protein (Fig. 2C). These results suggest that miR-101 may function at the translational level.

To confirm whether mkp-1 is a direct target of miR-101, we generated luciferase reporter constructs by cloning either the wild-type or a mutated portion of the 3′ UTR of mkp-1 into the 3′ UTR of a pGL-3M-Luc vector. The mutated 3′ UTR of mkp-1 had three bases changed, from GUACUGUA to GGUACGGGU, at the putative miR-101 binding sites (Fig. 2D). We transfected these vectors with miR-101 mimics into 293 cells and analyzed the lysates 48 h later. Transfection with miR-101 mimics markedly inhibited the luciferase activity for the wild-type 3′ UTR of mkp-1 but showed no repression effect for the mutated 3′ UTR of mkp-1 when compared with that for the control dsRNA (Fig. 2E), suggesting that miR-101 may repress MKP-1 expression by binding to the 3′ UTR of mkp-1 in a direct and sequence-specific manner.

Because miR-101 negatively regulates the expression of MKP-1, which is known as a phosphatase of MAPKs, we next examined whether miR-101 plays a role in the activation of MAPKs. RAW264.7 macrophage cells were transfected with miR-101 mimics or mi-R101 ssRNA inhibitor and then stimulated with LPS. Activation of MAPKs was measured by Western blot analysis using anti-phospho Abs. LPS stimulation induced the expression of MKP-1 protein with a peak at ~60 min, but transfection with miR-101 mimic markedly inhibited MKP-1 expression and prolonged the phosphorylation of JNK and p38 apparently to 60, 90, or 120 min time points (Fig. 3A). By contrast, treatment of cells with miR-101 inhibitor enhanced the expression of MKP-1 and attenuated the phosphorylation of JNK and p38 (Fig. 3B). These results suggest that miR-101 may regulate the LPS-induced activation of JNK or p38 through targeting MKP-1.

miR-101 regulates the LPS-induced production of TNF-α
Regulation of MAPKs by MKP-1 has been implicated in the production of proinflammatory cytokines, such as TNF-α, during the innate immune response (38). Because miR-101 has been shown to regulate the expression of MKP-1 and subsequent activation of MAPKs, we next examined whether miR-101 regulates the production of TNF-α. RAW264.7 cells were transfected with miR-101 mimic and then stimulated with LPS. The level of TNF-α was measured using ELISA. As shown in Fig. 3C, miR-101 dsRNA
mimics increased the production of TNF-\(\alpha\) up to \(\sim 1.7\)-fold in RAW264.7 cells as compared with dsRNA control treatment.

**Induction of miR-101 by LPS**

To examine whether miR-101 can be induced by microbial components, peritoneal macrophage cells were stimulated with different TLR ligands, and the expression level of miR-101 was evaluated using qRT-PCR. Treatment of cells with LPS enhanced miR-101 expression in a time-dependent manner that reached a peak in \(\sim 90\) min (Fig. 4A). Stimulation with PGN, a TLR2 ligand, or poly-IC, a TLR3 ligand, also induced the expression of miR-101 (Fig. 4B, 4C) in macrophages. However, treatment of cells with PMA/ionomycin did not cause a significant change in miR-101 production (Fig. 4D). These results indicate that miR-101 can be induced by different TLR ligands and may play a role in the innate immune responses to microbial infection through targeting MKP-1.

The PI3K/Akt pathway regulates the induction of miR-101 by LPS

Stimulation of TLR4 by LPS triggers the activation of multiple signaling pathways. We next examined which pathway regulates the LPS-stimulated induction of miR-101 by using different kinase inhibitors. PI3K/Akt inhibitor LY294002, p38 inhibitor SB203580, JNK inhibitor SP600125, or ERK inhibitor PD98059 showed specific inhibition of the corresponding kinases (Fig. 5A) and no significant toxicity (Table I). RAW264.7 cells were pretreated with these inhibitors and then stimulated with LPS. The expression of miR-101 was measured by qRT-PCR. Only inhibition of PI3K/Akt by LY294002 markedly attenuated the LPS-induced expression of miR-101 (Fig. 5B). Treatment of cells with SB203580, SP600125, or PD98059 did not have a significant effect on the induction of miR-101 by LPS (Fig. 5C–E). Furthermore, upon LPS stimulation, treatment of cells with LY294002 increased the production of MKP-1 and shortened the activation of JNK and p38 (Fig. 5F–H).
Specific inhibition of Akt by RNAi also showed a similar result (Fig. 5I–K). These results suggest that TLR4 stimulation activates the P3IK/Akt pathway, thus inducing the expression of miR-101, which inhibits MKP-1 to prolong the activation of the MAPKs.

**DEX inhibits miR-101 expression**

Glucocorticoids (GRs) are some of the most effective anti-inflammatory drugs that have been shown to inhibit the activation of MAPKs (39). In many cell types, MKP-1 also is upregulated profoundly by GRs. To examine whether GRs have any effect on miR-101, RAW264.7 cells were treated with LPS, LPS plus DEX, or DEX only for different time periods. Upon LPS stimulation, treatment of cells with DEX markedly increased the expression of MKP-1 and attenuated the activation of JNK and p38 (Fig. 6A). Also, treatment of cells with DEX inhibited the LPS-stimulated activation of P3IK/Akt and the LPS-induced expression of miR-101 (Fig. 6A, 6B). These results suggest that upregulation of MKP-1 by DEX may correlate with the downregulation of miR-101 as a result of Akt inhibition. We next examined the effect of miR-101 mimic on the upregulation of MKP-1 by DEX in LPS-stimulated RAW264.7 cells. As shown in Fig. 6C, the treatment of cells with miR-101 mimic together with DEX inhibited the induction of MKP-1 in LPS-stimulated RAW264.7 cells. We also have found that the treatment of cells with RU-486 (mifepristone), a GR antagonist, reversed the inhibition of Akt by DEX and attenuated the induction of MKP-1 by DEX in LPS-stimulated RAW264.7 cells (Fig. 6D). These results suggest that

**Table I. Toxicity assay of MAPK and P3IK inhibitor**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration (µM)</th>
<th>Cell Viability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSO</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>LY294002</td>
<td>25</td>
<td>97 ± 6</td>
</tr>
<tr>
<td>SB203580</td>
<td>10</td>
<td>94 ± 6</td>
</tr>
<tr>
<td>SP600125</td>
<td>20</td>
<td>95 ± 5</td>
</tr>
<tr>
<td>PD98059</td>
<td>20</td>
<td>92 ± 8</td>
</tr>
</tbody>
</table>

RAW264.7 cells were incubated with each inhibitor for 3 h, and cell viability was analyzed using the Cell Counting Kit-8.
part of the anti-inflammatory effect of DEX may occur through inhibition of the LPS-induced activation of PI3K/Akt, thus reducing the expression of miR-101 and the subsequent induction of MKP-1 to deactivate the MAPKs (Fig. 7).

**Discussion**

MAPKs are critical mediators of innate immune responses to microbial infection. In response to the stimulation of pathogen-associated molecular patterns, MAPKs are activated rapidly, leading to the production of proinflammatory cytokines. Although the adequate production of proinflammatory cytokines is essential for host defense, uncontrolled inflammatory reactions may lead to detrimental systemic effects, including septic shock and autoimmune diseases (40). Therefore, the threshold and magnitude of MAPK activation need to be tightly controlled to modulate the inflammatory responses. MKP-1 has been shown to be a critical negative regulator of proinflammatory cytokine production via inactivation of MAPKs. A previous study by Kuwano et al. (41) has demonstrated that RNA-binding proteins HuR and NF90 regulate the stabilization of MKP-1 mRNA. In this study, we demonstrate for the first time, to our knowledge, that MKP-1 is a direct target of miR-101. miR-101 showed no significant effect on the level of MKP-1 mRNA but may attenuate the translation of MKP-1. Moreover, stimulation of the LPS-activated PI3K/Akt pathway to induce the expression of miR-101 and expression of miR-101 repressed the expression of MKP-1 to prolong the activation of MAPKs. Together, our data suggest an essential role for miR-101 in regulating innate immune responses to LPS stimulation. Because the expression of miR-101 also is enhanced by PGN and poly-IC, repression of MKP-1 by miR-101 may be a general mechanism by which the production of proinflammatory cytokines are regulated by different microbial stimuli.

The miR-101–coding genes, MIRN101-1 and MIRN101-2, are located in genomic loci with a high frequency of allelic losses in several types of cancer (42, 43). The aberrant expression profile of miR-101 also is shown in the majority of cancer cell lines and cancer tissues examined (44), suggesting that it may act as a cancer-related miRNA. To date, five genes, EZH2/ENX-1, cyclooxygenase-2 (COX-2), PTGS2, MYCN, Mcl-1, and mTOR, have been identified as targets of miR-101 (44–47). All of these genes have been found to be involved in tumorigenesis and implicate miR-101 as a potent tumor suppressor in cancer therapy. Our present studies also have identified MKP-1 as a target of miR-101. Expression of MKP-1 has been found to correlate with cancer development and progression. Upregulation of MKP-1 in the early phase of cancer helps the tumor to evade JNK1-induced apoptosis, whereas downregulation of MKP-1 allows for proliferation and increased tumor mass in the more advanced stages of tumorigenesis (48). However, whether the expression of miR-101 correlates with MKP-1 activity and how the expression of miR-101 is regulated during tumorigenesis remain to be investigated.

Previous studies have shown that PI3K and its downstream target, Akt, are activated by TLR4 signals and that they may function as negative regulators of innate immune responses (49). PI3K-deficient cells exhibit enhanced p38 activation as well as IL-12 production (50). One reasonable explanation is that PI3K/Akt suppresses p38 activation through inhibition of their upstream regulators, apoptosis signal-regulating kinase 1 and MEK kinase 3 (51, 52). In addition, inhibition of PI3K/Akt enhances LPS-induced activation of NF-κB through prevention of Akt-dependent inactivation of glycogen synthase kinase-β in human monocytic cell line THP-1 (53, 54). However, Okugawa et al. (55) have reported that treatment of cells with PI3K inhibitor LY294002 inhibits the LPS-induced activation of JNK in a murine macrophage-like cell line RAW264.7. Therefore, the threshold and magnitude of MAPK activation need to be tightly controlled to modulate the inflammatory responses. MKP-1 has been shown to be a critical negative regulator of proinflammatory cytokine production via inactivation of MAPKs. A previous study by Kuwano et al. (41) has demonstrated that RNA-binding proteins HuR and NF90 regulate the stabilization of MKP-1 mRNA. In this study, we demonstrate for the first time, to our knowledge, that MKP-1 is a direct target of miR-101. miR-101 showed no significant effect on the level of MKP-1 mRNA but may attenuate the translation of MKP-1. Moreover, stimulation of the LPS-activated PI3K/Akt pathway to induce the expression of miR-101 and expression of miR-101 repressed the expression of MKP-1 to prolong the activation of MAPKs. Together, our data suggest an essential role for miR-101 in regulating innate immune responses to LPS stimulation. Because the expression of miR-101 also is enhanced by PGN and poly-IC, repression of MKP-1 by miR-101 may be a general mechanism by which the production of proinflammatory cytokines are regulated by different microbial stimuli.

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line RAW264.7, suggesting PI3K as a positive regulator of MAPKs. In our experiments, we also have found that inhibition of PI3K by LY294002 or specific inhibition of the expression of Akt by siRNA decreases the activation of MAPKs. In addition, we have found that inhibition of PI3K by LY294002 or RNAi of Akt enhances the expression of MKP-1, which is consistent with the findings of Leelawat et al. (56) that inhibition of PI3K leads to increased expression of MKP-1 in human hilar cholangiocarcinoma cells KKKU-100. Moreover, we found that treatment of cells with LY294002 markedly attenuated the expression of miR-101 induced by LPS. These results suggest that PI3K also may function as a positive regulator of innate immune responses, and the discrepancy may be due to the use of different cell types or different experimental conditions.

A more recent study shows that PI3K/Akt could positively regulate the expression of let-7e and miR-181c but negatively control the expression of miR-125b and miR-155 in LPS-activated macrophages (57). These miRNAs negatively regulate the TLR4 signaling pathway through targeting of TLR4, TNFSF11 (RANKL), SOCS1, and TNF-α. In the current study, we have found that PI3K/Akt regulates the expression of miR-101, thus upregulating MKP-1. Inhibition of PI3K/Akt by PI3K inhibitors suppressed the induction of miR-101 by LPS, leading to the enhanced production of MKP and subsequent inhibition of p38 and JNK. Thus, our study provides a mechanism by which PI3K/Akt negatively regulates the expression of MKP-1 through induction of miR-101.

It has been shown previously that TNF-α production by MKP-1–deficient macrophages was greater than that by wild-type macrophages at earlier time points. However, 12–24 h after LPS stimulation, the differences disappeared (16). If the effect of miR-101 is solely through MKP-1, then one would expect that regulation of TNF-α also is an early observation. However, we have found that introduction of miR-101 mimicking agent had no such effect on TNF-α secretion in the first 24 h, whereas TNF-α production was substantially higher in cells transfected with a miR-101 mimicking agent at 72 h. It has been shown that miR-101 can target different genes, such as EZH2/ENX-1, cyclooxygenase-2 (COX-2/PTGS2), MYCN, Mcl-1, and mTOR, or some other unknown genes (44–47). Therefore, the miR-101 also may regulate the production of TNF-α through targeting the expression of other genes.

GRs are the most effective drugs for chronic inflammatory diseases such as asthma (39), and their anti-inflammatory effect is primarily due to the inhibition of numerous proinflammatory mediators, including cytokines, chemotactic mediators, adhesion molecules, and other inflammatory proteins (39). In many cell types, GRs profoundly induce MKP-1 expression, thus attenuating the activation of MAPKs. To address the mechanism by which GRs induce MKP-1, a very recent study has indicated that GRs directly interact with PI3K to impede the phosphorylation of AKT (58).

Consistently, our data also indicate that treatment of cells with DEX attenuates the activation of PI3K/Akt by LPS. Furthermore, DEX inhibits the induction of miR-101 by LPS, whereas treatment of cells with miR-101 mimic together with DEX inhibits the induction of MKP-1 by LPS. These results suggest that part of the anti-inflammatory effect of DEX may be attributed to inhibition of the LPS-induced activation of PI3K/Akt, thus reducing the expression of miR-101, which enhances the MKP-1 expression level to deactivate the MAPKs. However, we also have observed that treatment of cells with DEX alone also increases the expression of MKP-1 but has no effect on the expression of miR-101, suggesting that DEX also can upregulate MKP-1 through a miR-101–independent pathway.

In summary, our results not only demonstrate that miR-101 directly targets mkp-1 to regulate the activation of MKP kinases and subsequent production of cytokines in response to LPS stimulation but also further reveal the complexity of signal transduction pathways involved in innate immune responses to microbial infection. Moreover, as a critical regulator of the innate immune system, miR-101 may serve as a potential pharmacological target for selective therapeutic intervention in microbial infection.

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Disclosures

The authors have no financial conflicts of interest.

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