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J Immunol published online 16 May 2014
http://www.jimmunol.org/content/early/2014/05/16/jimmunol.1400203

Supplementary Material
http://www.jimmunol.org/content/suppl/2014/05/16/jimmunol.1400203.DCSupplemental

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miR-27a Regulates Inflammatory Response of Macrophages by Targeting IL-10

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Although microRNAs were shown to participate in innate immune responses, it is not completely understood how they regulate negative immunomodulatory events. IL-10 is an important anti-inflammatory mediator that prevents excessive inflammation and associated immunological pathologies. Although the regulation of IL-10 expression has been well studied at both the transcriptional and translational levels, it is less clear how microRNAs control IL-10 expression during inflammation. In this study, we found that miR-27a is downregulated in macrophages following stimulation through TLR2 and TLR4, but not TLR3. Upregulation of miR-27a enhanced the expression of proinflammatory cytokines in TLR2/4-activated macrophages. Conversely, knockdown of miR-27a diminished cytokine expression. Mechanistically, we found that miR-27a negatively regulates IL-10 expression; upregulation of miR-27a decreases, whereas downregulation of miR-27a increases, IL-10 expression in activated macrophages. Likely due to the decreased expression of IL-10, upregulation of miR-27a diminished IL-10–dependent STAT3 phosphorylation in TLR4-activated macrophages. Consistent with IL-10 being a potential mediator for the role of miR-27a in the immune response, blocking IL-10 abolished the enhancing effect of miR-27a on TLR4-activated inflammation. In conclusion, our study identified miR-27a downregulation as a negative-regulatory mechanism that prevents overly exuberant TLR2- and TLR4-driven inflammatory responses. The Journal of Immunology, 2014, 193: 000–000.

Imnate immunity constitutes a first line of immune defense against host invasion by microbial pathogens. Immune cells, such as macrophages and neutrophils, respond to microorganisms through a series of unique receptors that detect pathogen associated molecular patterns (1–4). Among these, TLR4 and TLR2 recognize LPS and lipoproteins, which are important bacterial cell wall components (1–4). Engagement of TLRs with pathogen associated molecular patterns activates immune cells to produce proinflammatory mediators, such as cytokines and reactive oxygen species, which are essential to the eradication of microbial infection (1–5). However, overly exuberant inflammation can cause collateral tissue damage that leads to organ injury, such as acute lung injury. Multiple negative-regulatory mechanisms that are used by the immune system, such as induced expression of IL-10 and IκB-α, were shown to prevent excessive inflammatory response (6–9).

IL-10 is a potent anti-inflammatory cytokine that plays an important role in preventing inflammatory and autoimmune pathologies (7, 8, 10). This role of IL-10 is evidenced by spontaneously developing chronic enterocolitis in IL-10–knockout mice (11). IL-10 is expressed by various inflammatory cell types, including macrophages, neutrophils, dendritic cells, mast cells, NK cells, and T cell subsets (7, 8, 10). Diminished expression of IL-10 was shown to be associated with a variety of immunological disorders, such as cancer, rheumatoid arthritis, asthma, and inflammatory bowel disease (7, 8, 10).

MicroRNAs (miRNAs) are a class of small noncoding RNAs that target the 3′ untranslated region (UTR) of mRNAs, thereby inhibiting their expression (12–16). A number of miRNAs participate in innate immune responses (17–20). However, how miRNAs target negative-regulatory mechanisms in innate immunity is not completely understood (21). Given that IL-10 was found to be underexpressed in numerous inflammatory pathologies, we set out to identify the regulatory mechanisms of IL-10 expression by miRNAs. We were particularly interested in miRNAs whose expression is decreased in inflammatory activated macrophages and that potentially target IL-10. Inferably, aberrant expression of those miRNAs could lead to diminished IL-10, thereby contributing to the development of inflammatory diseases. In the process of characterizing miRNAs that had altered expression in LPS-treated macrophages, we found that miR-27a demonstrates such a role in the TLR2/4-induced innate immune response. Our data suggest that miR-27a downregulation serves as an important negative-feedback mechanism by which macrophages restrain an excessive inflammatory response.

Materials and Methods

Reagents

LPS from Escherichia coli 0111:B4 was from Sigma-Aldrich (St. Louis, MO). Ultrapure LPS from Salmonella minnesota R595, PAM3CSK4...
(PAM), and polyinosine-polycytidylic acid (poly I:C) were from InvivoGen (San Diego, CA). Isotype rat IgG and rat anti–IL-10–blocking Ab were from eBioscience (San Diego, CA). RAW 264.7 cells were from American Type Culture Collection (Manassas, VA).

Generation of mouse bone marrow–derived macrophages, mouse peritoneal macrophages, and human PBMC–derived macrophages

Mouse bone marrow–derived macrophages (BMDMs) were derived from bone marrow cells of C57BL/6 mice (National Cancer Institute-Frederick, Frederick, MD). Briefly, after lysis of RBCs, bone marrow cells were cultured in DMEM containing 10% FBS and 50 ng/ml murine M-CSF (R&D Systems, Minneapolis, MN) for 5 d. The cells were trypsinized and plated for treatment or transfection. Peritoneal macrophages were elicited from C57BL/6 mice by i.p. injection of 1 ml sterile 4% Brewer thioglycollate. Cells were harvested 4 d later by peritoneal lavage and plated. After 1 h at 37°C, nonadherent cells were removed by washing, and adherent macrophages were used for treatment or transfection. Human PBMCs were purchased from ZenBio (Research Triangle Park, NC). PBMCs were cultured in DMEM containing 10% FBS and 50 ng/ml murine M-CSF (R&D Systems) for 5 d. The cells were then trypsinized and plated for treatment or transfection. The animal protocol was approved by the University of Alabama at Birmingham Institutional Animal Care and Use Committee.

miRNA array

Total RNAs were purified from macrophages with a miRNeasy Mini Kit (Qiagen, Venlo, The Netherlands). The miRNA array was performed by Exiqon (Woburn, MA) using a miCURY LNA microRNA Array. The data were submitted to the Gene Expression Omnibus under accession number GSE55414 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE55414).

Quantitative real-time PCR

Probe Master Mix kit (Roche, Indianapolis, IN) was used for amplification of miRNAs. TaqMan probes for miR-27a and internal references,Probe Master Mix kit (Roche, Indianapolis, IN) was used for amplification of miRNAs. TaqMan probes for miR-27a and internal references,Probe Master Mix kit (Roche, Indianapolis, IN) was used for amplification of miRNAs. TaqMan probes for miR-27a and internal references,Probe Master Mix kit (Roche, Indianapolis, IN) was used for amplification of miRNAs. TaqMan probes for miR-27a and internal references,Probe Master Mix kit (Roche, Indianapolis, IN) was used for amplification of miRNAs. TaqMan probes for miR-27a and internal references, were determined by real-time PCR assay. Levels of miR-27a were determined by real-time PCR assay. Representative of two experiments are shown. *p < 0.05, ***p < 0.001 versus time “0.”

Levels of TNF-α, IL-6, and IL-10 in supernatants were quantified using DuoSet ELISA Development kits (R&D Systems), according to the manufacturer’s instructions.

Western blotting

Western blotting was performed as previously described (22). Anti–p-STAT3 and anti-STAT3 Abs were from Cell Signaling (Danvers, MA). Western blotting was performed as previously described (22). Anti–p-STAT3 and anti-STAT3 Abs were from Cell Signaling (Danvers, MA). Western blotting was performed as previously described (22). Anti–p-STAT3 and anti-STAT3 Abs were from Cell Signaling (Danvers, MA). Western blotting was performed as previously described (22). Anti–p-STAT3 and anti-STAT3 Abs were from Cell Signaling (Danvers, MA). Western blotting was performed as previously described (22). Anti–p-STAT3 and anti-STAT3 Abs were from Cell Signaling (Danvers, MA). Western blotting was performed as previously described (22). Anti–p-STAT3 and anti-STAT3 Abs were from Cell Signaling (Danvers, MA).
264.7 cells were transfected with 20 nM control mimics or miR-27a mimics. Six hours after transfection, cells were transfected again with 5 or 100 ng/pMir-Report constructs. After 48 h of transfection, luciferase activity in the cells was determined using a Luciferase Assay System (Promega, Madison, WI).

Transfection of miRNAs

Macrophages were transfected with 20 nM miRNA mimics or 20 nM miRNA inhibitors using HiPerFect transfection reagent (QIAGEN), according to the manufacturer’s instructions. Control mimics, miR-27a mimics, control inhibitors, and anti–miR-27a inhibitors were from Life Technologies.

Statistical analysis

One-way ANOVA, followed by the Bonferroni test, was performed for multiple-group comparisons. The Student t test was used for comparisons between two groups. The p values < 0.05 were considered statistically significant.

Results

miR-27a is downregulated in macrophages after TLR4 and TLR2 stimulation

To study the role of miRNAs in the innate immune response, we performed an miRNA array assay on RNAs of untreated and LPS-treated macrophages (GSE55414). We found that a number of miRNAs had altered expression in LPS-treated cells. Of those upregulated miRNAs, miR-147 and miR-125a-5p were found to inhibit inflammatory response through a negative-feedback mechanism (23, 24). Among those downregulated miRNAs, miR-27a was particularly interesting because it was predicted to potentially target IL-10. We first validated the expression of miR-27a by real-time PCR and found that miR-27a expression is indeed downregulated in LPS-treated mouse BMDMs (Fig. 1A). To determine whether miR-27a is subject to similar regulation by IL-10. We found no change in miR-27a expression in macrophages treated with IL-10 for 6 h (Fig. 1D). These data suggest that miR-27a downregulation at early time points after LPS treatment is unlikely to be an autocrine effect of IL-10. However, IL-10 treatment for 24 h decreased miR-27a levels in macrophages (Fig. 1D), sug-

FIGURE 2. Upregulation of miR-27a enhances inflammatory response of macrophages after TLR4 stimulation. (A) BMDMs were transfected with 20 nM control mimics or miR-27a mimics. Forty-eight hours after transfection, cells were treated with 100 ng/ml LPS for 6 h. The mRNA and protein levels of proinflammatory cytokines, IL-1β, IL-6, IL-12, and TNF-α, were determined by real-time PCR and ELISA assays. (B) Mouse peritoneal macrophages were transfected with 20 nM control mimics or miR-27a mimics. Forty-eight hours after transfection, cells were treated with 100 ng/ml LPS for 6 or 24 h. The protein levels of IL-6 and IL-12 were determined by ELISA assays. Experiments were done two or three times, and similar results were obtained. *p < 0.05, **p < 0.01, ***p < 0.001 versus “con miR” group.

FIGURE 3. Upregulation of miR-27a enhances inflammatory response to TLR2 stimulation. BMDMs were transfected with 20 nM control mimics or miR-27a mimics. Forty-eight hours after transfection, cells were treated with 1 μg/ml PAM for 6 h. The mRNA and protein levels of IL-6, IL-12, and TNF-α were determined by ELISA assays (A) and real-time PCR (B). Experiments were done two or three times, and similar results were obtained. *p < 0.05, **p < 0.01 versus “con miR” group.
gesting that IL-10 may still contribute to miR-27a downregulation at later time points after LPS treatment.

To determine whether activation of other TLRs also downregulates miR-27a, we treated macrophages with the synthetic TLR2 ligand, PAM, and the TLR3 ligand, poly I:C. Treatment with PAM, but not poly I:C, decreased the expression of miR-27a in macrophages (Fig. 1E, 1F).

**Upregulation of miR-27a enhances inflammatory response of macrophages after TLR4 stimulation**

In our initial experiments, we found that miR-27a is downregulated in LPS-treated macrophages. These data suggest that miR-27a may participate in inflammatory responses associated with TLR4 activation. To test this hypothesis, we increased the level of miR-27a in BMDMs by transfecting the cells with miR-27a mimics. We found that upregulation of miR-27a increased the LPS-induced expression of proinflammatory cytokines, including IL-1β, IL-6, IL-12, and TNF-α, compared with that found in BMDMs transfected with control mimics (Fig. 2A). Consistent with the findings in BMDMs, upregulation of miR-27a also enhanced LPS-induced expression of IL-6 and IL-12 in peritoneal macrophages (Fig. 2B). To rule out the possibility of nonspecific effects caused by impurity of LPS from *E. coli* 0111:B4, we performed the same experiments using ultrapure LPS from *S. minnesota* R595. As shown in Supplemental Fig. 1A and 1B, and consistent with the data found with LPS from *E. coli* 0111:B4, upregulation of miR-27a increased the ultrapure LPS-induced expression of proinflammatory cytokines, including IL-6, IL-12, and TNF-α, compared with that found in BMDMs transfected with control mimics. These data suggest that miR-27a is a positive regulator of the TLR4-induced inflammatory response.

**Upregulation of miR-27a enhances inflammatory response to TLR2 stimulation**

Because miR-27a is also downregulated in PAM-treated macrophages, we next investigated whether miR-27a participates in the
TLR2-induced inflammatory response. As shown in Fig. 3, up-regulation of miR-27a enhanced the expression of proinflammatory cytokines, including IL-6, IL-12, and TNF-α, compared with that found in PAM-treated macrophages infected with control mimics. Taken together, these data suggest that miR-27a regulates the immune response after both TLR4 and TLR2 engagement.

**Downregulation of miR-27a attenuates inflammatory response to TLR4 stimulation**

Because we found that upregulation of miR-27a increased the inflammatory response to TLR4 activation, we asked whether downregulation of miR-27a would act in an opposite manner. As shown in Fig. 4, downregulation of miR-27a by anti-miR-27a inhibitors diminished the LPS-induced expression of TNF-α and IL-6 in macrophages compared with that found in cells transfected with control inhibitors. These data provide further evidence that miR-27a is a positive regulator of the inflammatory response in macrophages.

**miR-27a negatively regulates the expression of IL-10**

We found that miR-27a is downregulated in TLR4- and TLR2-activated macrophages. Furthermore, miR-27a downregulation diminished the inflammatory response to TLR4 activation. These data suggest that miR-27a downregulation may be a negative-feedback mechanism in macrophages that prevents overly activated inflammatory responses. Because IL-10 is one of the most important anti-inflammatory mediators (7, 8), we asked whether miR-27a exerts its effect on the immune response through regulating IL-10. We showed that IL-10 expression is upregulated early in LPS-treated macrophages (Fig. 5A), in line with the decreased expression of miR-27a at the same time point (Fig. 1A, 1B). Of more importance, upregulation of IL-10 in LPS-treated BMDMs was diminished by miR-27a at both the protein and mRNA levels (Fig. 5B, 5C, Supplemental Fig. 1C). Additionally, miR-27a downregulated IL-10 expression in LPS-treated peritoneal macrophages (Fig. 5D). Similar to its effect in LPS-stimulated cells, miR-27a decreased IL-10 expression in PAM-treated macrophages (Fig. 5E). These data suggest that miR-27a is a negative regulator of IL-10. Consistent with the findings with miR-27a upregulation, downregulation of miR-27a enhanced the expression of IL-10 in LPS-treated macrophages (Fig. 5F).

**miR-27a targets IL-10**

Because we showed that miR-27a decreases IL-10 expression, we asked whether miR-27a does so by targeting IL-10 3’ UTR, a general mechanism by which miRNAs regulate target expression (12–16). Using TargetScan and miRWalk (31), computational programs predicting miRNA targets, we identified a site at the 3’ UTR of the IL-10 transcript that is predicted to be targeted by miR-27a (Fig. 6A), with a p value < 0.05 for the miRNA/target association. We cloned the 3’ UTR of the IL-10 mRNA into a luciferase reporter vector downstream of the luciferase gene. We also created mutations in the 3’ UTR of the IL-10 mRNA at the site targeted by miR-27a (Fig. 6A) and cloned the mutant 3’ UTR into the same luciferase reporter. We found that miR-27a decreased the activity of the luciferase reporter that contained the wild-type 3’ UTR of IL-10 mRNA (Fig. 6B). However, miR-27a had no effect on the activity of a luciferase reporter that contained the mutant 3’ UTR of IL-10 mRNA (Fig. 6C). To determine whether LPS-enhancing IL-10 expression is mediated, at least in part, by regulating the 3’ UTR of IL-10, we performed the luciferase reporter assay in a macrophage cell line (RAW 264.7). As shown in Fig. 6D, miR-27a decreased the activity of the luciferase reporter that contains the wild-type 3’ UTR of IL-10 mRNA in untreated RAW cells, consistent with those found in HEK-293 cells. More importantly, we found that LPS treatment enhanced the activity of the luciferase reporter (Fig. 6D), consistent with decreased expression of miR-27a in LPS-treated macrophages.

**FIGURE 6.** miR-27a targets IL-10. (A) Schematic illustration of the miR-27a targeting site at the 3’ UTR of the mouse IL-10 gene. The created point mutations are depicted in italic letters. (B) HEK-293 cells were transfected with 20 nM control mimics or specific miR-27a mimics. Six hours after transfection, cells were transfected again with 5 ng pMir-Report luciferase constructs that contained the wild-type IL-10 3’ UTR. Two days after transfection, the cells were lysed, and luciferase activity was measured. The absolute luciferase activity in the “con miR” group was regarded as 100%. **p < 0.01 versus “con miR” group. (C) HEK-293 cells were transfected with 20 nM control mimics or specific miR-27a mimics. Six hours after transfection, cells were transfected again with 5 ng pMir-Report luciferase constructs that contained the mutant IL-10 3’ UTR. Two days after transfection, luciferase activity in the cells was measured. Experiments were done twice, and similar results were obtained. (D) RAW 264.7 cells were transfected with 20 nM control mimics or specific miR-27a mimics. Six hours after transfection, cells were transfected again with 100 ng pMir-Report luciferase constructs that contained the wild-type IL-10 3’ UTR. Two days after transfection, the cells were treated or not with ultrapure LPS for 6 h. Cells were lysed, and luciferase activity was measured. The absolute luciferase activity in the untreated “con miR” group was regarded as 100%. *p < 0.05, **p < 0.01 versus untreated “con miR” group. #p < 0.05 versus LPS-treated “con miR” group. (E) Experiments were performed as in (A), except for the pMIR-Report luciferase construct that contained the human wild-type IL-10 3’ UTR. ***p < 0.001 versus “con miR” group. (F) BMDMs were transfected with 20 nM control mimics or miR-27a mimics. Forty-eight hours after transfection, protein levels of IL-10 in the supernatants were determined by ELISA. ***p < 0.001 versus “con miR” group.
Such enhanced activity of the luciferase reporter in LPS-treated RAW cells was again diminished by miR-27a mimics (Fig. 6D). miR-27a also decreased the activity of the luciferase reporter that contained the human wild-type 3' UTR of IL-10 mRNA (Fig. 6E), suggesting the conservation of IL-10 targeting by miR-27a across species. Of note, miR-27a also downregulated the basal expression of IL-10 in BMDMs (Fig. 6F). These data suggest that IL-10 is a direct target of miR-27a.

**miR-27a regulates IL-10–dependent signaling events in LPS-treated macrophages**

It was shown that LPS-induced phosphorylation of STAT3 is dependent on IL-10 (32). Because we found that miR-27a diminishes LPS-induced IL-10 expression, we asked whether miR-27a regulates STAT3 phosphorylation in LPS-treated macrophages. As shown in Fig. 7A, upregulation of miR-27a diminished LPS-induced STAT3 phosphorylation. We reasoned that downregulation of STAT3 phosphorylation by miR-27a in LPS-treated macrophages is likely due to decreased IL-10 expression in the same cells. We first confirmed that miR-27a decreased IL-10 expression at 1 h after LPS treatment (Fig. 7B). However, there remains the possibility that the diminished STAT3 phosphorylation in LPS-treated macrophages may be caused by miR-27a targeting IL-10–signaling events. To test this hypothesis, we examined whether miR-27a affects IL-10–induced downstream events. As shown in Fig. 7C, miR-27a had no effect on IL-10–induced STAT3 phosphorylation. Taken together, these data suggest that miR-27a selectively targets IL-10 expression, but not IL-10 downstream-signaling events, to limit the anti-inflammatory capacity of macrophages.

**Blocking IL-10 abolishes the regulation of inflammatory response by miR-27a in macrophages**

The findings above suggest that the mechanism by which miR-27a regulates immune responses in macrophages is through targeting IL-10. If this is true, blocking IL-10 should abolish the effects of miR-27a. To test this hypothesis, we treated miR-27a mimics–transfected macrophages with anti–IL-10–blocking Ab or isotype-control IgG. As shown in Fig. 8, upregulation of miR-27a enhanced LPS-induced expression of IL-12, IL-6, IL-1β, and TNF-α in isotope-control IgG-treated macrophages. Anti–IL-10 Ab enhanced LPS-induced expression of these proinflammatory cytokines, consistent with IL-10 acting as an anti-inflammatory molecule (Fig. 8). More importantly, the enhancement of LPS-induced expression of IL-12, IL-6, IL-1β, and TNF-α in macrophages with miR-27 overexpression was attenuated by anti–IL-10 Abs (Fig. 8). Taken together, these data suggest that regulation of IL-10 in BMDMs (Fig. 6F). These data suggest that IL-10 is a direct target of miR-27a.

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the inflammatory response by miR-27a is mediated, at least in part, by IL-10.

Discussion

Inflammatory responses mediated by TLR2 and TLR4 play a crucial role in combating bacterial infection (1–4). However, excessive inflammation is a major causal factor that leads to clinically important morbidities in infected patients (1–4). The immune system is equipped with a wide range of negative regulatory mechanisms that dampen inappropriately excessive inflammatory responses at multiple levels in the activation process (1–4). These include transcriptional factors and corepressors, protein-modification enzymes, and RNA-binding proteins.

miRNAs were shown to be major regulators in the innate immune response (17–20). However, only a limited number of miRNAs have been identified to function in negative-feedback loops that ameliorate inflammation. In this study, we found that miR-27a is downregulated in macrophages activated through TLR2 or TLR4. Downregulation of miR-27a diminishes activation of macrophages by TLR2/4 stimulation and coincides with increased IL-10 expression. Therefore, miR-27a downregulation appears to be a prerequisite to keep the inflammatory response under control during macrophage activation.

IL-10 is one of the most important anti-inflammatory mediators (7, 8). Dysregulation of IL-10 was shown to participate in inflammatory pathologies (7, 8). Therefore, appropriate regulation of IL-10 expression appears required to maintain immunological homeostasis (7, 8, 10). IL-10 expression is subject to both transcriptional and translational regulation (7, 8). For example, Sp1 and Sp3 play an indispensable role in controlling IL-10 expression at the transcriptional level. In addition, IL-10 expression is subject to translational regulation through adenylate-uridylate-rich elements in the 3′ UTR of IL-10 mRNA (7, 8). However, additional mechanisms that participate in the posttranscriptional control of IL-10 expression remain to be elucidated. In this study, we identified miR-27a as a negative regulator of IL-10 expression and highlight the complexity of the immunoregulatory network involved in modulation of IL-10 expression. It should be noted that IL-10 had returned to its basal level at the late time point of LPS treatment when miR-27a expression was still suppressed. These data suggest that miR-27a is not a predominant regulator of IL-10 expression at the late stage of the inflammatory response, and downregulation of miR-27a is insufficient for sustainably high IL-10 expression.

Binding of IL-10 to its receptor results in activation of the JAK1-STAT3 pathway (33). The transcriptional factor, STAT3, then enters the nucleus to diminish the inflammatory response through incompletely characterized mechanisms (7, 8). We found that miR-27a upregulation decreases STAT3 phosphorylation in LPS-treated macrophages and concluded that such a decrease in STAT3 phosphorylation is due to reduced IL-10 expression caused by miR-27a upregulation. This conclusion is supported by the finding that miR-27a upregulation does not affect STAT3 phosphorylation in macrophages treated with exogenous IL-10. These data also suggest that inhibition of IL-10 expression is directly responsible for the ability of miR-27a to enhance inflammatory responses.

Although miR-27a decreases IL-10 expression in LPS-treated macrophages, the increase in the expression of proinflammatory cytokines by upregulation of miR-27a is smaller than that induced by IL-10-blocking Ab. This can be explained by the finding that miR-27a only partially decreases, and does not completely block, IL-10 expression in LPS-treated macrophages. However, anti–IL-10 Abs, at the concentration used in these studies, are able to completely block secreted IL-10 in LPS-treated macrophages (data not shown).

Upregulation of miR-27a has been found in various types of cancers and is often associated with adverse outcome from malignancy (34–37). A chronic inflammatory microenvironment is a risk factor that contributes to cell transformation, proliferation, and resistance to programmed cell death (38, 39). Given that miR-27a is a positive regulator of inflammation, it is possible that it functions as an oncomir by promoting a deleteriously proinflammatory environment that drives pathological progression of cancer. Importantly, our study lends further evidence to the correlation between inflammatory diseases and cancer (38, 39).

In summary, our study identified miR-27a as a potential therapeutic target for the treatment of inflammatory diseases and cancer. Our data also provide solid rationale for future studies that investigate correlations between the expression of miR-27a and/or single-nucleotide polymorphisms of the miR-27a gene and clinicopathological features of inflammatory diseases.

Disclosures

The authors have no financial conflicts of interest.

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Supplementary Figure 1: Upregulation of miR-27a enhances inflammatory response of macrophages after TLR4 stimulation. (A-B) BMDMs were transfected with 20 nM control mimics or miR-27a mimics. 48h after transfection, cells were treated with 100 U/ml ultra-pure LPS for 6h. The mRNA or protein levels of pro-inflammatory cytokines, IL-6, IL-12 and TNF-a, were determined by real-time PCR and ELISA assays. (C) Experiments were performed as in "A". The mRNA levels of IL-10 were determined by real-time PCR. **p<0.01, ***p<0.001 compared to the "con miR" group. Experiments were done twice with similar results obtained.