✤ *Author's Choice*

microRNA-181a represses ox-LDL-stimulated inflammatory response in dendritic cell by targeting c-*Fos*

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Abstract Oxidized LDL (ox-LDL) activates dendritic cells (DCs), thereby initiating inflammation responses in atherosclerosis, yet the modulatory mechanisms remain unclear. MicroRNAs (miRNAs) are important regulators for DC functions. This study evaluated the regulation by miRNAs of the ox-LDL-induced DC immune response. In CD11c$^+$ DCs from ApoE-deficient mice with hyperlipidemia, microRNA miR-181a was significantly up-regulated. In cultured bone marrow-derived DCs (BMDCs), ox-LDL promoted DC maturation and up-regulated miR-181a expression. Abundance of miR-181a attenuated ox-LDL-induced CD83 and CD40 expression, inhibited the secretion of interleukin (IL)-6 and TNF-α, and up-regulated IL-10, an important anti-inflammatory cytokine that was inhibited by ox-LDL. Inhibition of the endogenous miR-181a reversed the effects on CD83 and CD40 as well as the effects on IL-6 and TNF-α. The putative target genes of miR-181a were evaluated by gene ontology assessment, and the *c-Fos*-mediated inflammation pathway was identified. miR-181a targeted the 3′ untranslated region of c-*Fos* mRNA by luciferase experiments. Thus, abundance of miR-181a reduced *c-Fos* protein, whereas inhibition of miR-181a increased *c-Fos* protein in BMDCs. We therefore suggest that miR-181a attenuates ox-LDL-stimulated immune inflammation responses by targeting *c-Fos* in DCs.—Wu, C., Y. Gong, J. Yuan, W. Zhang, G. Zhao, H. Li, A. Sun, KaiHu, Y. Zou, and J. Ge. **MicroRNA-181a represses ox-LDL-stimulated inflammation response in dendritic cell by targeting c-Fos. *J. Lipid Res.* 2012.** 53: **2355–2363.**

Supplementary key words dendritic cells • hyperlipidemia • atherosclerosis

Atherosclerosis, the foremost cause of coronary heart disease, is a multifactorial and multistep disease. Chronic inflammation triggered by an autoimmune response toward the accumulated oxidized LDL (ox-LDL) is involved in atherosclerotic process, from initiation to progression and, eventually, plaque rupture. Dendritic cells (DCs) are the only "professional" and most powerful antigen-presenting cells in the inflammatory response. In atherosclerotic lesions, DCs accumulate in the subendothelium and undergo maturation stimulated by ox-LDL (1, 2), advanced glycosylation end products (3), or other antigen components. The maturation of DCs is characterized by increased secretion of cytokines; up-regulation of surface molecules such as CD80, CD86, and CD40; and enhanced efficiency in stimulating the differentiation of effector CD4$^+$ T cells from naive T-cell precursors (4).

Recently, negative immune regulatory functions of DCs in atherosclerosis have been reported. For example, apolipoprotein B-100-loaded DCs present a tolerogenic phenotype and attenuate atherosclerosis in hypercholesterolemic mice (5). In another study, oral administration of an active form of vitamin D3 (calcitriol) decreased atherosclerosis in $ApoE^{-/-}$ mice (6). Our group has recently reported that salvianolic acid B effectively suppressed ox-LDL-induced DC maturation through peroxisome proliferator-activated receptor γ activation (7). Nevertheless, until now, the process of differentiation of immature DCs to mature DCs (proinflammation) or tolerogenic DCs (regulatory DCs, negatively regulating inflammation) has remained unclear.

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Abbreviations: ox-LDL, oxidized low-density lipoprotein; DC, dendritic cell; miRNAs, microRNA; BMDC, bone marrow-derived dendritic cell; 3′-UTR, 3′ untranslated region; IL, interleukin; TNF, tumor necrosis factor; TGF, transforming growth factor; GO, gene ontology; AP-1, activating protein 1; LPS, lipopolysaccharide; Treg, regulatory T cell

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Recent studies have shown that microRNAs (miRNAs) regulate the immune functions of DCs and are involved in the pathogenesis of atherosclerosis (8). miRNAs represent a class of naturally occurring, small, noncoding RNAs. Single-stranded miRNAs bind to the 3′ untranslated region (3′-UTR) of target mRNAs and consequently decrease protein translation, increase degradation of the target message RNA, or both. In atherosclerosis, miRNAs miR-21 and miR-10a regulate neointimal lesion formation (9, 10), miR-146 and miR-155 regulate the inflammatory response in ox-LDL-stimulated macrophages (11), and miR-26a and miR-100 regulate the neovascularization and smooth muscle cell functions (12). For DC immune functions, regulation by miRNAs includes the capturing of the antigen via Toll-like receptors, the processing and presenting of antigen to T cells (such as by miR-148 and miR-152), etc. (13, 14). However, whether miRNAs have a regulatory role in DCs that affects the development of atherosclerosis has not been addressed.

The purpose of this study was to investigate the regulation by miRNAs of the DC-mediated immune inflammatory response stimulated by ox-LDL in the process of atherosclerosis.

METHODS

Animals and CD11c$^+$ DCs

ApoE-deficient mice (*ApoE*$^{-/-}$) were purchased from the Jackson Laboratory (Bar Harbor, Maine). Female *ApoE*$^{-/-}$ mice at 8 weeks of age were fed a control diet (ND) or a Western high-fat diet (HD; 21% fat, 0.15% cholesterol) for 2 weeks. *ApoE*$^{-/-}$ mice were anesthetized by inhalation of isoflurane (5% for induction, 1% for maintenance) for blood collection and euthanized by CO_2 inhalation. The spleens were crushed, and the purified CD11c$^+$ DCs were positively selected with anti-CD11c$^+$ microbeads (Miltenyi Biotec, Bergish Gladbach, Germany). C57BL/6 mice for culture of bone marrow-derived dendritic cells (BMDCs) were also anesthetized by inhalation of isoflurane (5% for induction, 1% for maintenance) and euthanized by CO_2 inhalation. The adequacy of anesthesia was determined by the loss of a pedal withdrawal reflex and any other reaction from the animal in response to pinching the toe, tail, or ear of the animal. The respiration rate of the animal under anesthesia was closely monitored, and an increased respiration rate was used as a sign that anesthesia was too light. All procedures and protocols were approved by the Institutional Review Board of the Zhongshan Hospital, Fudan University and Shanghai Institutes for Biological Sciences-CAS (A5894-01) and were conducted in conformity with the Public Health Service Policy on Humane Care and Use of Laboratory Animals.

miRNA microarray analysis

Real-time PCR for microRNAs was performed using the miRCURY LNA Universal RT microRNA PCR system and microRNA Ready-to-Use PCR Panel-I (mouse) (Exiqon, Vedbaek, Denmark) according to the manufacturer's instructions. Details are provided in the online supplement, miRNA microarray analysis.

Quantitative RT-PCR

Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA). The expression levels of miRNAs were determined by using specific primers and probes according to the manufacturer's protocol (Applied Biosystems, Foster City, CA), and U6 was used as an internal control. For quantitative RT-PCR analyses, we used a SYBR RT-PCR kit (Takara, Dalian, China). The primers for the genes for c-Fos, interleukin (IL)-1, IL-6, IL-12b, tumor necrosis factor (TNF)-α, interferon (IFN)-γ, and GAPDH are listed in supplementary Table I. The relative expression levels of the genes were normalized to that of GAPDH by using the $2^{-\Delta\Delta Ct}$ cycle threshold method.

Cell culture

BMDCs were from C57BL/6 mice. Bone marrow progenitors were cultured in medium containing 10 ng/ml granulocyte-macrophage colony-stimulating factor and 1 ng/ml IL-4. Nonadherent cells were gently washed out at 24 h. The remaining loosely adherent clusters were cultured, and the medium was changed every 2 days. On day 7, the cells were collected for ox-LDL treatment (48 h, 50 μg/ml) or transfection. The ox-LDL was purchased from Peking Union-Biology Co., Ltd. (Beijing, China). For detailed preparation methods, LDL from fresh human plasma was isolated, which has tested negative for hepatitis C, HIV-1, and HIV-II antibodies as well as hepatitis surface antigens after series ultracentrifugartion (density, 1.03–1.50). LDL was then oxidized using 10 μM Cu2So4 (oxidant) in PBS. Oxidation is terminated by adding excess EDTA. Each is analyzed on agarose gel electrophoresis for migration LDL.

Cell transfection

Cells were transfected with miR-181a precursor or inhibitor at 30 or 100 nM, respectively (Applied Biosystems). BMDCs (5×10^5) were seeded in each well of 6-well plates. The cells were mixed with 100 μl of OptiMEM (Gibco, Grand Island, NY), transferred to a 4-mm cuvette (Molecular Bioproducts, San Diego, CA), and pulsed in a GenePulser Xcell (400 V, 150 μF, 100 Ω) (Bio-Rad, Hercules, CA) (15). The cells were transferred to RPMI-1640 medium, and 1 h later and an equal amount of RPMI-1640 plus 20% FBS was added. A scrambled oligonucleotide (GenePharm, Shanghai, China) was used as a control. The cell viability has been examined by propidium iodide staining of nonviable cells.

Flow cytometry

Adherent cells were harvested and counterstained immunephenotypically using anti-CD83, anti-CD80, anti-CD86, anti-CD40, and anti-IAd (BD Pharmingen, San Diego, CA).

Western blotting

The protein levels of c-*Fos* and GAPDH were determined by Western blot analysis using specific antibodies (Cell Signaling Technology, Beverly, MA).

ELISA assay

The culture supernatant was analyzed for TNF-α, IL-6, and IL-10 with ELISA kits (R&D Systems, Minneapolis, MN).

Plasmids

The 3′-UTRs of c-*Fos* mRNA harboring the predicted miR-181a binding sequences were PCR amplified from mouse genomic DNA and cloned into *Hin*dIII and *Spe*I of the pMir-Report luciferase vector (Ambion, Austin, TX) to generate the c-*Fos*-3′-UTR reporter construct. Mutagenesis of predicted targets with a deletion of 7 bp from the site of perfect complementarity was performed using a site-directed Mutagenesis Kit (Takara). The primers are listed in supplementary Table I.

Luciferase assay

Two hundred nanograms of plasmid DNA (wt-*Luc*-c-*Fos* or mu-*Luc*-c-*Fos*) plus miR-181a precursor, miR-181a inhibitor, or a

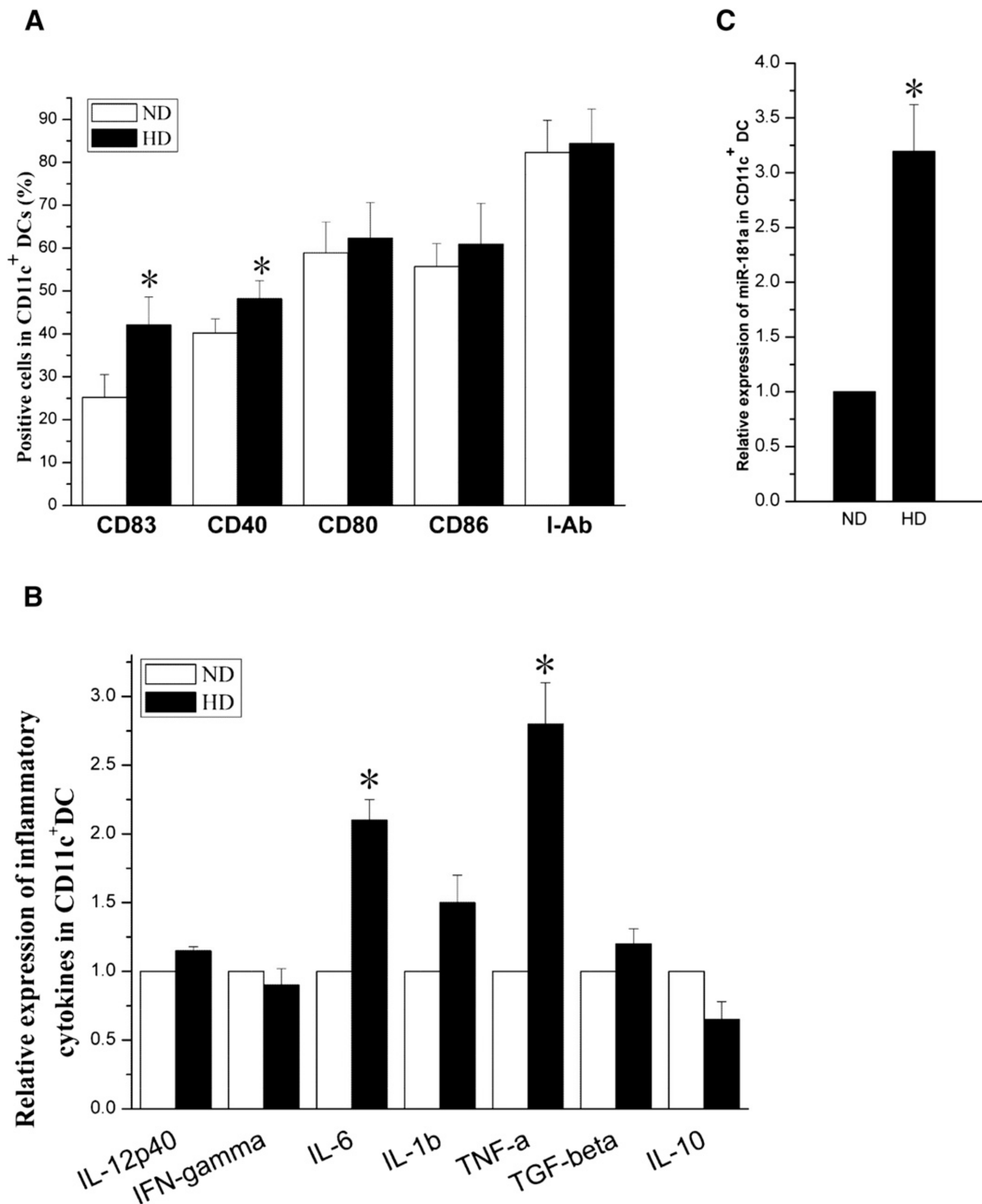


Fig. 1. Increased inflammation and miR-181a expression in CD11c$^+$ DCs from *ApoE*$^{-/-}$ mice with hyperlipidemia. A: Flow cytometric analysis of the immune phenotypes of the spleen CD11c$^+$ DCs. CD83 and CD40 were up-regulated in HD mice. The percentage of positive cells is presented. *$P < 0.05$. B: Expression of inflammatory cytokines by real-time RT-PCR in spleen CD11c$^+$ DCs. Results are shown as relative mRNA expression. *$P < 0.05$. C: Verification of miR-181a expression in CD11c$^+$ DCs by real-time PCR. *$P < 0.05$.

unrelated miRNA, miR-518b or scrambled oligonucleotide were transfected. The pSV-β-galactosidase plasmid was cotransfected with the luciferase reporter vectors to normalize the transfection efficiency. Forty-eight hours after transfection, luciferase activity was measured using the luciferase assay system (Ambion). Five replicates were performed.

In silico analysis

We identified the genes putatively targeted by miR-181a using TargetScan (http://www.targetscan.org) (16) and miRanda (http://www.microrna.org) (17). The KEGG pathways and gene ontology (GO) hierarchial categories analyses were carried out using the Capitalbio server (http://bioinfo.capitalbio.com/mas3/) (18). The Genes Regulatory Network was constructed using Cytoscape software (19).

Statistical analysis

Data are expressed as mean ± standard error of differences. One-way ANOVA followed by Dunnett's multiple comparison test or Mann-Whitney U test was used to calculate *P* values. $P < 0.05$ was considered statistically significant.

RESULTS

CD11c$^+$ DCs from *ApoE*$^{-/-}$ mice with hyperlipidemia had increased inflammatory response and up-regulated miR-181a expression

ApoE$^{-/-}$ mice (8 weeks of age) were fed a ND or HD. Consumption of HD for 2 weeks significantly increased

the circulating total cholesterol and LDL cholesterol in the mice (supplementary Table II). We observed significantly enhanced expression of CD83 and CD40 and a marginal increase of CD80 and CD86 in CD11c$^+$ DCs from the spleens of the HD mice (**Fig. 1A**). In addition, the expression of the TNF-α and IL-6 was significantly increased in CD11c$^+$ DCs (Fig. 1B). These data indicated activation of the immune inflammatory response by DCs in *ApoE*$^{-/-}$ mice with high lipidemia.

The miRNA expression profile of the CD11c$^+$ DCs was screened using miRNA PCR array. miR-181a was significantly increased approximately 3-fold in HD mice (Fig. 1C). Several other miRNAs were also significantly altered in CD11c$^+$ DCs of HD mice (data not shown). Because previous studies have showed that miR-181a regulates the T-cell immune inflammatory response (20, 21), we then asked whether miR-181a regulates ox-LDL-stimulated inflammatory response in DCs.

miR-181a inhibited the ox-LDL-induced DC immune inflammatory response

In cultured BMDCs, ox-LDL treatment produced an activated phenotype consisting of elongated cell morphology (supplementary Fig. I); up-regulated DC maturation markers such as CD83, CD40, CD80, and CD86 (**Fig. 2A**); and increased inflammatory cytokines, including TNF-a, IL-12p40, IL-1, IL-6, and IFN-γ (Fig. 2B). In this model, ox-LDL increased the expression of miR-181a approximately 4-fold (supplementary Fig. II).

The transfection efficiency of miRNAs into BMDCs was high (supplementary Fig. III). MiR-181a overabundance down-regulated CD83 and CD40 and suppressed the

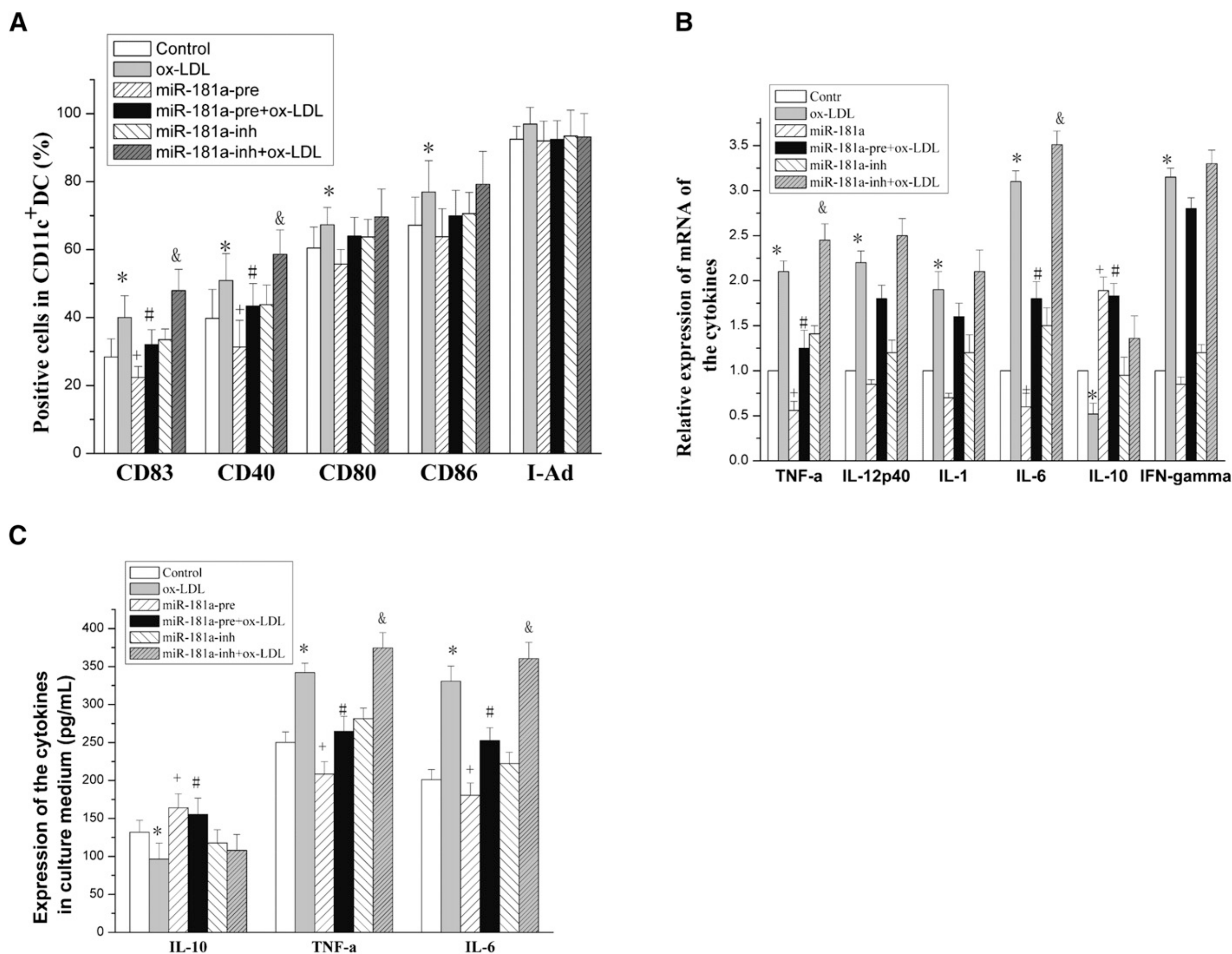
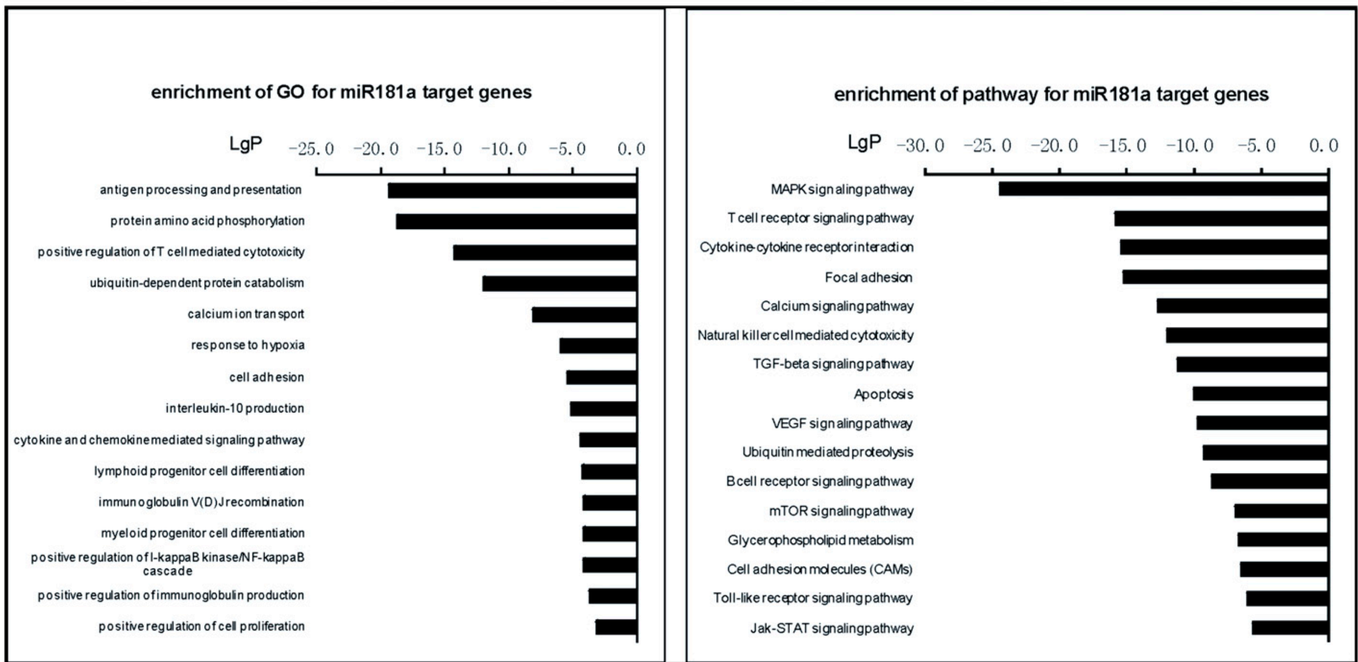


Fig. 2. miR-181a inhibited the ox-LDL-induced immune inflammatory response in BMDCs. A: Expression of cell-surface CD80, CD83, CD86, CD40, and I-Ad as determined by flow cytometry of BMDCs (n = 5). *ox-LDL versus control; $P < 0.05$. $^+$miR-181a-pre versus control; $P < 0.05$. $^\#$ox-LDL plus miR-181a-pre versus ox-LDL; $P < 0.05$. $^\&$ox-LDL plus miR-181a-inh versus ox-LDL; $P < 0.05$. B: Expression of the cytokines in BMDCs analyzed by real-time RT-PCR (n = 5). *ox-LDL versus control; $P < 0.05$. $^+$miR-181a-pre versus control; $P < 0.05$. $^\#$ox-LDL plus miR-181a-pre versus ox-LDL; $P < 0.05$. $^\&$ox-LDL plus miR-181a-inh versus ox-LDL; $P < 0.05$. C: Expression of cytokines in BMDCs analyzed by ELISA (n = 5). *ox-LDL versus control; $P < 0.05$. $^+$miR-181a-pre versus control; $P < 0.05$. $^\#$ox-LDL plus miR-181a-pre versus ox-LDL; $P < 0.05$. $^\&$ox-LDL plus miR-181a-inh versus ox-LDL; $P < 0.05$.

A



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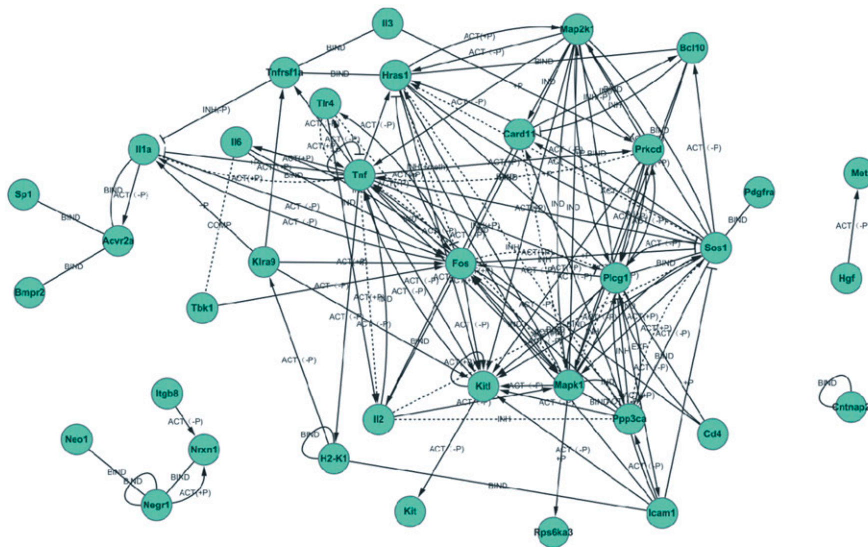

Fig. 3. In silico analysis of target mRNAs for miR-181a. A: Significant GO (left panel) and pathway (right panel) genes for miR-181a. The vertical axis is the GO category or the pathway name, and the horizontal axis is the enrichment of GO or pathway. B: The gene interaction networks of the genes involved in inflammation and putatively targeted by miR-181a. Edges describe the connectivity (indegree or outdegree) and the interaction effects of the gene with other genes. ACT (+P), activation phosphorylation; ACT (−P), activation dephosphorylation indirect effect; BIND, binding/association; COMP, compound; EXP, expression; IND, inhibition; INH, inhibition dephosphorylation. The gene *Fos* is distinctive with higher in-degree and out-degree values in the network.

ox-LDL-induced up-regulation of CD83 and CD40 (Fig. 2A). We also observed moderately inhibitory effects on CD86 and CD80 by miR-181a overbundance (Fig. 2A). Furthermore, inhibiting endogenous miR-181a by transfection of miR-181a inhibitor further increased ox-LDL-induced CD83 and CD40 expression (Fig. 2A).

For the cytokines, miR-181a overabundance inhibited TNF-α and IL-6 expression and attenuated the ox-LDL-induced TNF-α and IL-6 expression (Fig. 2B). In addition, IL-10, an important anti-inflammatory cytokine that was decreased by ox-LDL, was significantly increased by miR-181a (Fig. 2B). To support these data, inhibition of the endogenous miR-181a showed the reversed effects of miR-181a to TNF-α and IL-6. The altered cytokine levels were further verified by ELISA, which showed a consistent regulation by ox-LDL or miR-181a of cytokine expression (Fig. 2C).

In silico analysis of the target mRNAs by miR-181a

Based on miR-181a's putative targets, we undertook GO and pathway analysis of those genes. Among the most

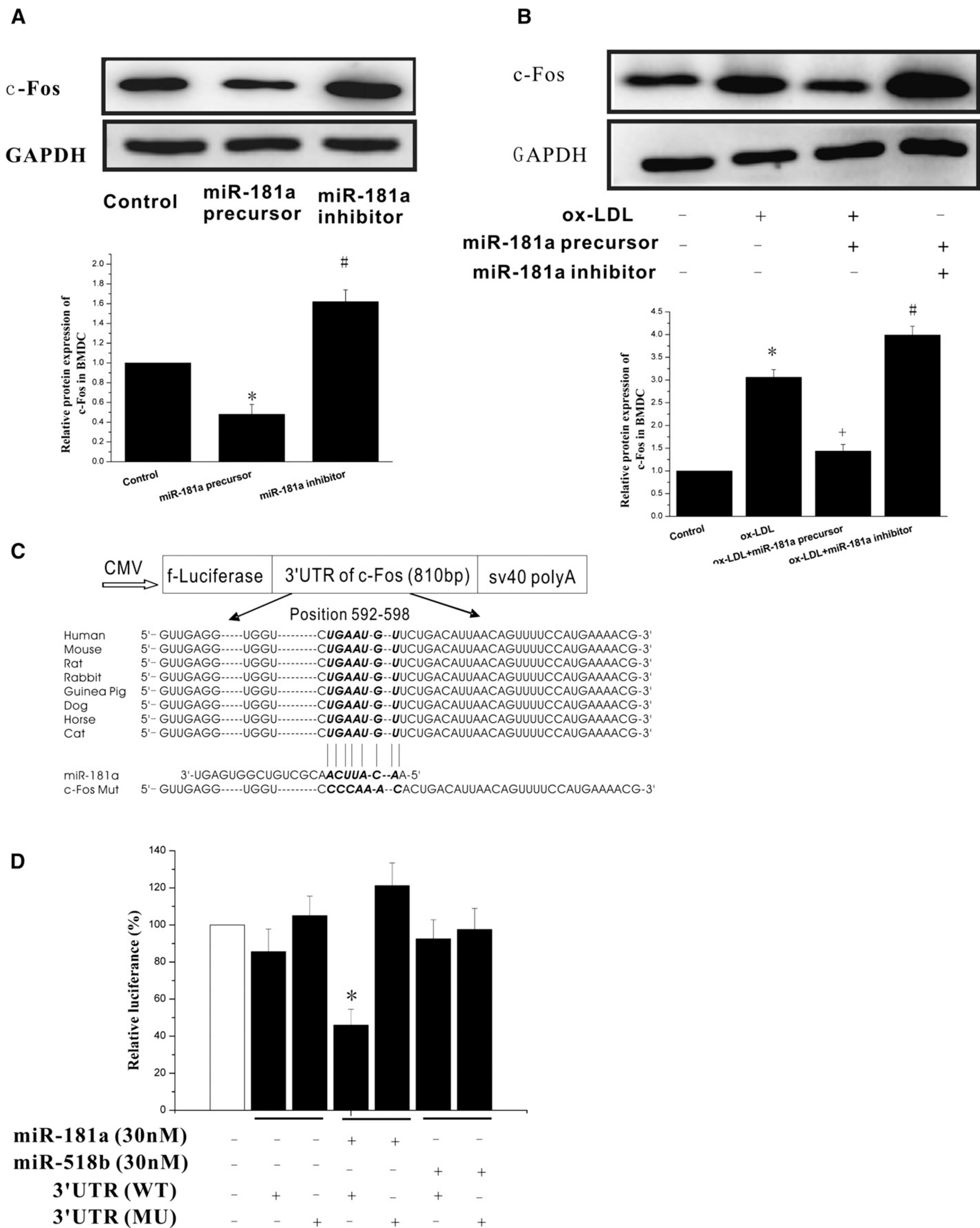

Fig. 4. c-*Fos* is a target gene for miR-181a. A: Representative immunoblot analyses of c-*Fos* transfected with miR-181a precursor or inhibitor (n = 5). *miR-181a-pre versus control; $P < 0.05$. #miR-181a-inh versus control; $P < 0.05$. B: Representative immunoblot analyses of c-*Fos* from BMDCs treated with ox-LDL and transfected with miR-181a precursor or inhibitor (n = 5). +ox-LDL versus control; $P < 0.05$. *miR-181a-pre+ox-LDL versus ox-LDL; $P < 0.05$. #miR-181a-inh+ox-LDL versus ox-LDL; $P < 0.05$. C: Luciferase reporter constructs containing the

highly enriched GO results for miR-181a were antigen processing and presentation of exogenous peptide antigen, positive regulation of T-cell-mediated cytotoxicity,IL-10 production, positive regulation of the I-κB kinase/NF-κB cascade, and IL-12 production (**Fig. 3A**, left panel). Among the most significant pathways were T-cell receptor signaling, cytokine-cytokine receptor interactions, natural killer cell-mediated cytotoxicity, the TGF-β signaling pathway, and the Toll-like receptor signaling pathway (Fig. 3A, right panel).

We then constructed a gene interaction network based on the genes in the immune inflammatory response targeted by miR-181a. The details of the gene names, the number of interactive genes, and the attributed pathways are listed in supplementary Table III. In the network, the most elementary characteristic of a node is its degree (connectivity; i.e., the number of the links the node has to other nodes) (20). The TNF-α node had the highest degree of 24 (in-degree of 11 and out-degree of 13; Fig. 3B and supplementary Table III). c-*Fos* was also worthy of attention, with an in-degree of 12 and an out-degree of 9. Because previous studies have suggested that c-*Fos* has an important role in DC-related immune functions (22, 23), we then asked whether it could be a crucial target gene for miR-181a in ox-LDL-stimulated DCs.

miR-181a targets c-*Fos* in ox-LDL-stimulated BMDCs

To ascertain whether miR-181a regulates c-*Fos*, we harvested BMDCs treated with ox-LDL and transfected them with miR-181a precursor or miR-181a inhibitor. miR-181a overabundance significantly inhibited c-*Fos* expression, whereas inhibition of endogenous miR-181a up-regulated c-Fos protein expression, as shown by Western blot (**Fig. 4A**). Ox-LDL enhanced the expression of c-Fos, but this effect was attenuated significantly by miR-181a overabundance (Fig. 4B). Inhibition of endogenous miR-181a significantly reversed the effects of miR-181a to c-Fos (Fig. 4B). We also examined the c-Fos expression in splenocytes $CD11c^{+}$DC and observed significantly up-regulated expression of c-Fos in HD mice compared with the ND mice (supplementary Fig. IV).

We then used the computational approaches to identify the miRNA target sequences based on complementarity to the miRNA seed region, favorable sequence context, stability of the miRNA-mRNA duplex, and conservation across multiple species (Fig. 4C). A construct containing the 3′-UTR of c-*Fos* mRNA or the sequence with the mutant seed region was cotransfected along with miR-181a or a negative control miRNA into HEK 293 cells. Cotransfection of the nonmutated construct with miR-181a precursors resulted in a significant reduction in luciferase activity (Fig. 4D). This effect was specific because no change was seen in luciferase reporter activity with transfection of the mutated construct (Fig. 4D).

DISCUSSION

The major findings of this study are that miR-181a was up-regulated under conditions of hyperlipidemia. Increased miR-181a attenuated the ox-LDL-induced DC immune inflammatory response through down-regulating DC maturation surface markers, regulating the inflammatory cytokines. In addition, miR-181a targets a network of inflammation-related genes, and miR-181a represses the expression of *c-Fos*, a crucial inflammatory transcription factor. Thus, miR-181a negatively regulates the ox-LDL-induced immune inflammatory response in DCs.

Previous studies have showed that dyslipidemia accelerates the immune inflammatory response in atherosclerosis (24, 25). In this study, we consistently observed an increased inflammatory response in spleen DCs from mice having an elevated lipid profile. In addition, miR-181a was abnormally up-regulated in spleen $CD11c^{+}$ DCs. MiR-181a had been reported to be an intrinsic modulator of T-cell sensitivity and selection (19) and to control the strength of T-cell antigen receptor signaling during T-cell maturation (20). The level of miR-181a has been correlated with the expression of genes involved in the immune and inflammatory responses, such as IRF7 and TLR4 in patients with acute myeloid leukemia (26). We thus evaluated the potential regulation of miR-181a to the ox-LDL-stimulated DC immune inflammatory response.

From functional experiments, we found that miR-181a inhibited the expression of CD83 and CD40 in cultured BMDCs and ox-LDL-stimulated BMDCs and that inhibition of the endogenous miR-181a reversed the effects to these two molecules. In addition, miR-181a produced a modest decrease in the expression of the costimulatory molecules (CD80/CD86). All CD83, CD40, and CD80/CD86 are characteristic surface markers for fully matured DCs. Evidence can be found for these molecules as regulatory targets in immune homeostasis for DC-T-cell interactions (27). CD83 is preferentially expressed on mature DCs, and blockage of CD83 inhibits T-cell inflammatory responses (28). CD40 is a member of the TNF receptor family. By binding to its ligand (CD40L), which is transiently expressed on T cells under inflammatory conditions, the CD40/CD40L complex promotes the development of the T-cell immune response (29). Thus, our results indicates that miR-181a can attenuate DC-T-cell interactions by down-regulating DC surface maturation molecules.

The activation and polarization of the Th1-cell inflammatory response is a complex process involving the

wild-type or mutated 3′-UTR of mouse c-*Fos* mRNA were transfected into HEK 293 cells. The sequence in italic and gray background indicates the predicted binding site for miR-181a. The sequence of miR-181a is shown aligned with its predicted target site in the 3′-UTR of c-*Fos* mRNA from the indicated species. The sequence of the mutated (Mu) 3′-UTR of mouse c-*Fos* mRNA is also shown. D: Luciferase reporter constructs containing the wild-type or mutated 3′-UTR of mice c-*Fos* mRNA was transfected into HEK 293 cells. Luciferase activity was normalized to the activity obtained with a β-galactosidase vector and is expressed as relative luciferase activity (n = 5). * miR-181a plus 3-UTR (WT) versus control; $P < 0.05$.

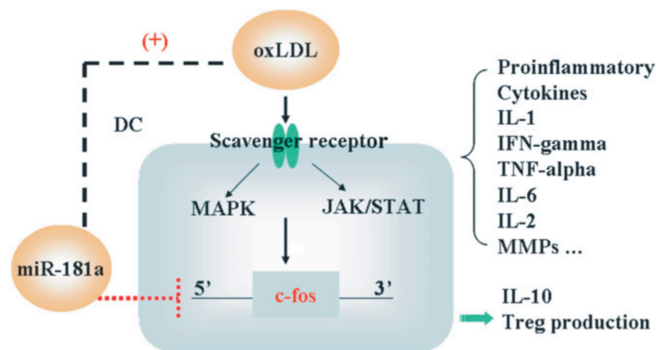

Fig. 5. Ox-LDL stimulates DCs to up-regulate the proinflammatory genes and to increase miR-181a expression. Meanwhile, miR-181a feedback blocks the ox-LDL induced inflammatory response by inhibiting the proinflammatory transcription factor of c-Fos. Thus, miR-181a promotes the production of Treg cells mediated potentially through increased IL-10 expression.

up-regulation of DCs surface markers and a corresponding increase in Th1 proinflammatory cytokines. This attenuation of miR-181a to DC-T interaction was further supported by the regulation of miR-181a to the inflammatory cytokine expression. In our study, ox-LDL increased the expression of the proinflammatory cytokines IL-6 and TNF-α but decreased the anti-inflammatory cytokine IL-10 (30, 31). In contrast, miR-181a down-regulated IL-6 and TNF-α and inhibited the ox-LDL-induced increase in IL-6 and TNF-α in BMDCs. These effects were reversed when inhibiting the endogenous miR-181a.

In our study, miR-181a significantly increased IL-10 expression. Previous reports showed that down-regulating DC surface markers and Th1 inflammatory cytokines but increasing IL-10 drive the differentiation of naive T cells to Treg cells (32). Treg cells have been reported to induce and maintain T-cell tolerance and to play a protective role in atherosclerosis (33, 34). Our data indicated that miR-181a might promote Treg cell production to help maintain immune atherosclerosis. This point is worthy of further investigation.

The regulation of miR-181a to DCs was also supported in GO and pathway analysis because most of the genes targeted by miR-181a are involved in the immune and inflammatory responses. In addition, c-*Fos* was especially distinctive in the gene interaction network based on the identified genes. c-*Fos* protein functions as one subunit of a complex of dimeric transcription factors collectively referred to as activating protein 1 (AP-1). Up-regulation of c-*Fos* is involved in LPS-induced up-regulation of MHC-II and IL-12p19 expression in DCs (35). Increased AP-1 was also involved in the process of minimally oxidized LDL-activating macrophages (36). In atherosclerosis, c-*Fos* was localized to plaques and was up-regulated in circulating monocytes from patients with coronary heart disease (37). Based on these data, we evaluated the potential functions of c-Fos in ox-LDL-stimulated BMDCs regulated by miR-181a.

Our results revealed that miR-181a repressed the expression of *c-Fos* and that the inhibition of endogenous miR-181a reversed this effect. In addition, the results of the in silico analysis and the luciferance assay suggested that miR-181a targeted the 3′-UTR of c-*Fos* mRNA to produce its post-transcriptional inhibitory function. Therefore, we suggested that the inhibition of *c-Fos* by miR-181a is a potentially novel mechanism contributing to immune homeostasis in the hypercholesterol-associated inflammatory response in atherosclerosis. Nevertheless, previous studies have also reported that *c-Fos* dampens the production of IFN-β and IL-12 in DCs stimulated by LPS (38) and that miR-155 inhibited the DC immune inflammatory response through up-regulating c-Fos expression. One potential explanation might be the differences in AP-1 complexes because of the different stimulation of LPS or ox-LDL (39).

In summary, we suggest that miR-181a feedback attenuates the ox-LDL-stimulated immune inflammatory response by targeting c-*Fos* in DCs (**Fig. 5**). Thus, miR-181a may be a new therapeutic target for ox-LDL-stimulated immune inflammation in atherosclerosis. Further elucidation of the antiatherogenic effects of miR-181a inhibition to DC immune response in atherosclerosis in vivo will lead to the in-depth understanding of the significance miRNA-regulated immunoinflammatory response in atherosclerosis.

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