European Review for Medical and Pharmacological Sciences 2020; 24: 9139-9146

MiR-140a contributes to the pro-atherosclerotic phenotype of macrophages by downregulating interleukin-10

H.-X. YANG[1], H.-B. JIANG[2], L. LUO[1]

[1]YongZhou Vocational Technical College, Yongzhou City, Hunan Province, China
[2]Xintian County Hospital of Traditional Chinese Medicine, Yongzhou City, Hunan Province, China

Abstract. – OBJECTIVE: The dysfunction of immune cells plays important roles in promoting the progression of atherosclerosis (AS). This study aims to investigate the role of miR-140a in modulating the function of AS-associated macrophages.

PATIENTS AND METHODS: The expression of miR-140a in human monocytes was evaluated by quantitative PCR. For *in vitro* studies, macrophages were transfected with miR140a mimic or miR140a inhibitor, and then, stimulated with oxidized low-density lipoprotein (ox-LDL). The production of cytokines was evaluated by quantitative PCR and enzyme-linked immunosorbent assay (ELISA). Flow cytometry was used to determine the phosphorylation of Signal Transducer and Activator of Transcription 3 (STAT3).

RESULTS: MiR-140a expression was upregulated in monocytes from AS patients. MiR140a overexpression enhanced the pro-inflammatory ability of ox-LDL-stimulated human macrophages. In addition, miR140a was found to target interleukin-10 (IL-10) in macrophages, thus reducing IL-10-mediated anti-inflammatory responses.

CONCLUSIONS: MiR-140a serves as a pro-atherosclerotic microRNA by modulating the phenotypic switch of AS-associated macrophages.

Key Words:
MiR-140a, Atherosclerosis, Macrophages, Interleukin-10.

Introduction

The incidence and mortality of cardio- and cerebrovascular diseases (CCVDs) have been rising continuously in recent years, becoming a leading threat to human health worldwide[1]. Atherosclerosis (AS) is widely considered to be the critical pathological hallmark of CCVDs. AS is characterized by the aggregation of lipoproteins, immune cells, and extracellular matrix in arterial intima[2]. Although AS is initially thought to be a consequence of the dysregulated lipid mechanism, it is increasingly recognized that chronic inflammatory plays indispensable roles in the pathological processes of AS[3]. Accumulated lipoproteins in arterial intima resulted in the recruitment of monocytes and the subsequent activation of innate and adaptive immune responses, leading to plaque formation. The sustained inflammatory responses contribute to the thinning of the fibrous cap and the eventual occurrence of plaque rupture[2,4].

Macrophages, a vital component of the host innate immunity, are involved in all stages of AS. Macrophages play dual roles in AS progression[2]. Generally, classically-activated macrophages (M1 macrophages) contribute to the development of plaque rupture by secreting various pro-inflammatory cytokines (such as TNF-α, IL-6, IL-18, IL-12, IL-15) and lipid mediators[2,5]. On the other hand, alternatively-activated macrophages (M2 macrophages) often inhibit the formation of plaque by clearing dead cells and enhancing tissue repair[6]. Environmental factors[7] and cell energy metabolism status[8] have been implicated in regulating the functions of AS-associated macrophages. However, the molecular elements modulating macrophage phenotypic switch in the development of AS remain further identification.

Some studies[9,10] have implicated that non-coding RNAs play important roles in affecting AS pathology. MicroRNAs (MiRNAs) are highly conserved small non-coding RNAs (18-24 nucleotides) which are known as gene expression regulators at the post-transcriptional level. Over the past decade, miRNAs have been found to serve as versatile regulators in the pathogenic immune responses in AS development, such as modulating the production of inflammatory cy-

Corresponding Author: Hengbo Jiang, MD; e-mail: HengboJ@yeah.net

tokines, or affecting lipid metabolism[9,11-14]. Of note, miR-33 disrupted efferocytosis, lysosomal synthesis, and apoptotic cell clearance functions of macrophages by modulating cell autophagy[13]; miR-210 improved fibrous cap stability by downregulating the expression of adenomatous polyposis coli (APC) and promoting smooth muscle cell survival[11]; miR-30c inhibited the secretion and accumulation of apoB proteins, thus reducing plaque formation in AS[15]. Thus, further identification of miRNAs that regulate AS progression is of potential significance for the diagnosis or treatment of this disease[16].

In the present study, we investigated the role of miR-140a in modulating the pro-inflammatory capability of AS-associated macrophages. The results showed that miR140a plays a pro-atherosclerotic role by downregulating the expression of IL-10, an anti-inflammatory cytokine.

Patients and Methods

Human Samples

Human peripheral blood samples were collected from AS patients and healthy volunteers using sodium-heparin containing collection tubes in Xintian County Hospital of Traditional Chinese Medicine. This study was approved by Ethics Committee of Xintian County Hospital of Traditional Chinese Medicine and the informed consent was obtained from all subjects.

Isolation of Monocytes

Monocytes were isolated from human peripheral blood samples in accordance with the instructions of Monocyte Isolation Kit (Haoyang Biological Manufacture, Tianjin, China). Briefly, the density gradient medium was prepared in a tube and whole blood was added. After centrifuging for 30 min (500 g), monocytes in the second layer were drawn into a new tube and washed twice. Thereafter, monocytes were collected for miR-140a quantification.

Cell Culture

Human acute monocytic leukemia cell line, THP-1, was purchased from Cell Bank of Chinese Academy of Sciences (Shanghai, China) and maintained in RPMI-1640 media supplemented with 10% fetal bovine serum. THP-1 cells were induced by phorbol 12-myristate 13-acetate (PMA, 100 nM) to differentiate into macrophages[17] and then treated with different concentrations of oxidized low-density lipoprotein (oxLDL, 20, 50, 100 μg/mL) for 0, 6, 12 h, respectively. oxLDL-treated cells were collected for miR-140a quantification.

Cell Transfection

miR-140 mimic, inhibitor and corresponding negative controls (RiboBio, Guangzhou, China) were transfected into macrophages induced from THP-1 cells using riboFECT™ reagent, respectively. Forty-two hours later, transfected cells were stimulated with oxLDL (50 μg/mL) for 6 h and subsequently these cells and culture supernatants were collected for quantitative PCR, enzyme-linked immunosorbent assay (ELISA), and flow cytometry, respectively.

RNA Isolation and Quantitative PCR

Cell RNA was extracted using TRIzol reagent (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions and then reversely transcribed into cDNA using PrimeScript™ RT reagent Kit (TaKaRa, Otsu, Shiga, Japan). Quantitative PCR was conducted on a 7500 fast Real-time PCR instrument (Applied Biosystems, Foster City, CA, USA) using Ultra SYBR mixture (Cwbiotech, Beijing, China). We used 18s mRNA expression as the integral control and gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method and normalized to respective negative control. The primer sequences are given below: TNF-α: forward, 5'-CCTCTCTCTAATCAGCCCTCTG-3'; reverse, 5'-GAGGACCTGGGAGTAGATGAG-3' IL-6: forward, 5'-ACTCACCTCTTCAGAACGAATTG-3'; reverse, 5'-CCATCTTTGGAAGGTTCAGGTTG-3' CCL-2: forward, 5'-CAGCCAGATGCAATCAATGCC-3'; reverse, 5'-TGGAATCCTGAACCCACTTCT-3' IL-10: forward, 5'-GACTTTAAGGGTTACCTGGGTTG-3'; reverse, 5'-TCACATGCGCCTTGATGTCTG-3' SOCS3: forward, 5'-CCTGCGCCTCAAGACCTTC-3'; reverse, 5'-GTCACTGCGCTCCAGTAGAA-3'.

MiRNA Quantification

miRcute Isolation Kit (Tiangen Biotech, Beijing, China) was used for miRNA extraction and miScript II RT Kit (QIAGEN, Hilden, Germany) was employed for reverse-transcription. Thereafter, quantitative PCR was conducted on the 7500 fast Real Time-PCR instrument using miScript SYBR Green PCR Kit (QIAGEN, Hilden, Germany) and U6 RNA was used for normalization.

Neutralization of IL-10

PMA-induced THP-1 cells transfected with miR140a inhibitor or control were treated with an anti-IL-10 neutralizing antibody (600 ng/mL, Abcam, Cambridge, United Kingdom) or IgG (Beyotime, Shanghai, China) for 2 h, followed by oxLDL stimulation for 6 h. Subsequently, cell culture supernatants were collected for ELISA.

ELISA

The concentrations of TNF-α, IL-6, CCL-2, IL-10 in collected cell culture medium supernatants were examined by ELISA kits (Boster, Wuhan, China) according to the manufacturer's instructions, respectively. For each inflammatory factor, three biological replicates were done.

Flow Cytometry

Transfected cells that were stimulated with oxLDL were digested by trypsin and fixed at 37°C for 10 min. Then, the cells were permeabilized, washed, and incubated in 100 μL PBS containing 1 μL PE anti-p-STAT3 (Tyr 705) antibody (Biolegend, San Diego, CA, USA) in the dark for 30 min. After a subsequent wash and resuspension with PBS, they were analyzed on a flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA).

Statistical Analysis

Results were expressed as mean ± SEM. All statistical analysis was performed on SPSS 20.0 (IBM Corp., Armonk, NY, USA). Two-tailed Student *t*-test was used to compare statistical differences and $p<0.05$ was considered to be significant.

Results

MiR140a Expression is Upregulated in Monocytes from AS Patients and oxLDL-Stimulated Macrophages

To investigate the possible expressional changes of miR140a in the setting of AS, we isolated peripheral monocytes (precursors of macrophages) from AS patients and healthy controls. By performing quantitative PCR, we found that miR140a expression was significantly upregulated in monocytes from AS patients, when compared with those from healthy donors (Figure 1A). We further evaluated the expression of miR140a in macrophages stimulated with oxidized low-density lipoprotein (oxLDL), which is the main pathological mediator for the formation of atherosclerotic plaque[18]. We chose THP-1 monocytes and differentiated them into macrophages with PMA. In the presence of phorbol 12-myristate-13-acetate (PMA), THP-1 cells gradually acquire macrophage phenotypes, including adherence capacity and high responsiveness to many external stimuli. As shown in Figure 1B, oxLDL stimulation significantly increased miR140a expression in THP-1 macrophages in a time-dependent and dose-dependent manner. These results suggest that miR140a might regulate macrophage functions in AS.

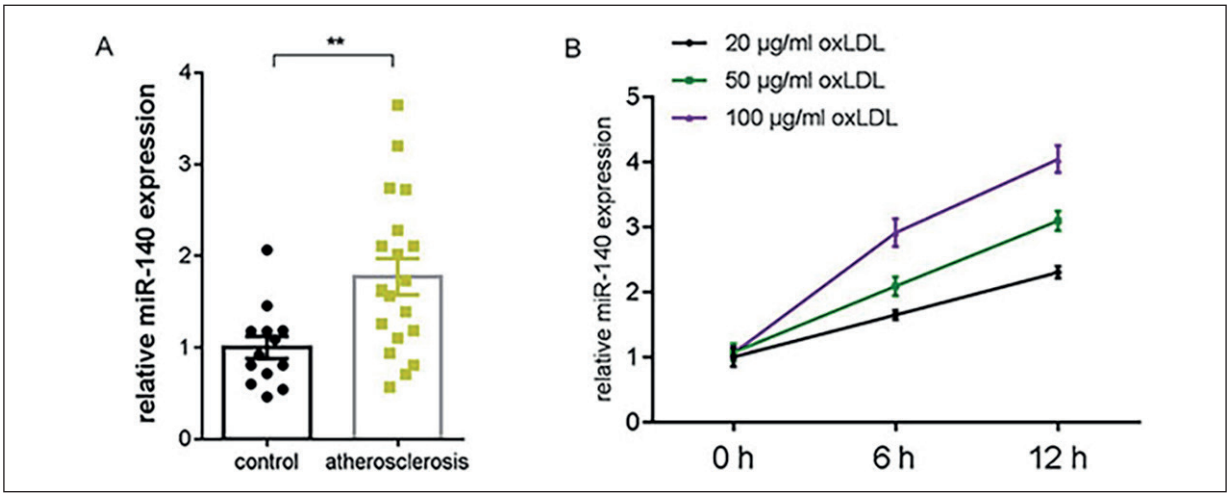

Figure 1. Increased expression of miR140a in AS-associated monocytes and macrophages. **A,** miR140a expression level in peripheral monocytes of atherosclerosis patients and healthy donors was examined by quantitative PCR. **B,** THP1 macrophages were treated with different concentrations (20, 50, 100 μg/mL) of oxidized low density lipoprotein (oxLDL), miR140a expression level was examined by quantitative PCR. Data are expressed as Mean ± SEM. **, $p < 0.01$.

MiR140a Enhances the Pro-Inflammatory Activity of Macrophages

We next investigated the possible role of miR140a in pro-inflammatory responses of macrophages. To this end, we transfected THP-1 macrophages with miR140a mimic or miR140a inhibitor. As shown in Figure 2A-B, miR140a overexpression markedly increased the mRNA expression of tumor necrosis factor alpha (TNF-α), C-C Motif Chemokine Ligand (CCL2), and IL-6, while miR140a inhibition led to the opposite effect. Consistently, the concentration of TNF-α, CCL2, and IL-6 in macrophage culture supernatant was also increased by miR140a overexpression. However, the levels of these pro-inflammatory cytokines were decreased by miR140a inhibition. Therefore, miR140a enhances the pro-inflammatory capacity of macrophages.

MiR140a Targets IL-10 and Downregulates the Anti-Inflammatory IL-10/STAT3 Signaling

Next, we searched the potential miR140a targets in TargetScan website. The results showed that the 3'-untranslated region (UTR) of IL-10 gene contained the binding site of miR140a (Figure 3A). To verify this prediction, we transfected macrophages with miR-140a mimic or miR-140a inhibitor, and then, IL-10 level was examined at both mRNA and protein levels. MiR140a overexpression reduced the mRNA and protein levels of IL-10, which were increased by miR140a inhibition (Figure 3B-C). We then examined the effect of miR140a on the expression of Suppressor Of Cytokine Signaling 3 (SOCS3), a well-known IL-10 inducible target gene[19]. As expected, the expression of SOCS3 was decreased in miR140a-overexpressed macrophages. In contrast, SOCS3 expression was increased in miR140a-silenced macrophages (Figure 3D). In addition, we evaluated the phosphorylation of Signal Transducer and Activator of Transcription 3 (STAT3), which is the key transcription factor downstream of IL-10 signaling[19,20]. Flow cytometry results showed that ox-LDL treatment induced the phosphorylation of STAT3 in macrophages. However, the augmented STAT3 phosphorylation was largely ab-

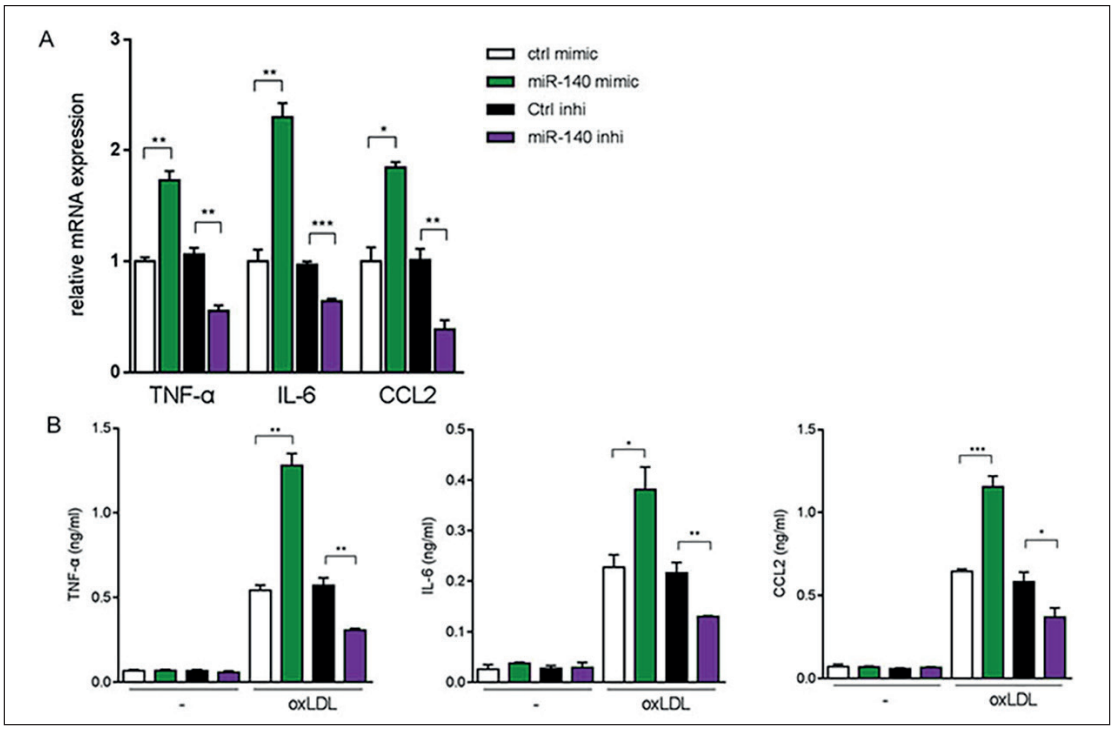

Figure 2. MiR140a promotes the production of pro-inflammatory cytokines in oxLDL-treated macrophages. **A,** THP-1 macrophages were transfected with miR140a mimic, miR140a inhibitor and corresponding controls followed by ox-LDL stimulation (50 μg/mL) for 6 h, mRNA levels of TNF-α, IL-6 and CCL2 were examined by quantitative PCR. **B,** The concentrations of TNF-α, IL-6 and CCL2 in supernatants of THP-1 macrophages were analyzed by ELISA. Data are expressed as Mean ± SEM. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$.

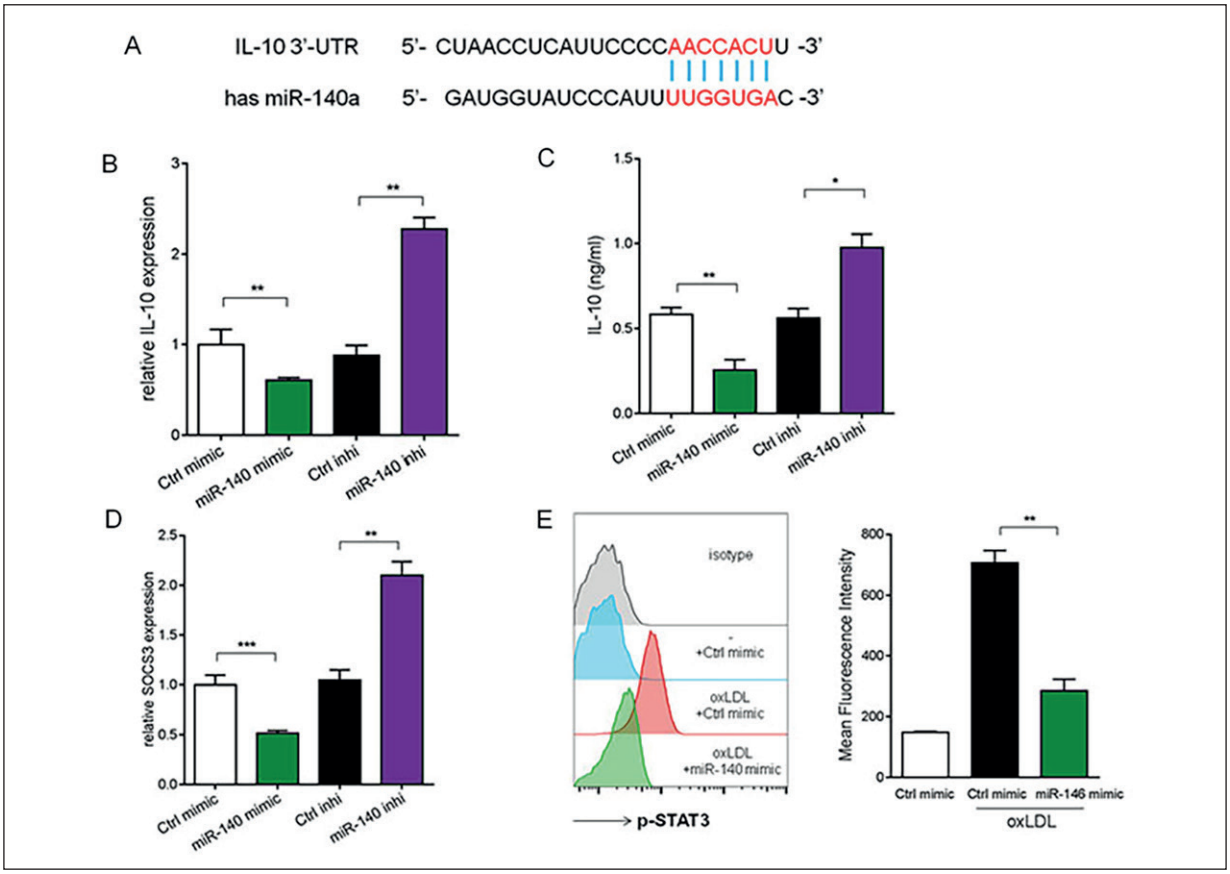

Figure 3. MiR140a targets IL-10 and downregulates IL-10 signaling. **A,** Sequence comparison between miR-140a and 3' UTR of IL-10 mRNA. **B-C,** THP-1 macrophages were transfected with miR140a mimic, miR140a inhibitor and corresponding controls followed by ox-LDL stimulation (50 μg/mL) for 6 h, mRNA **(B)** and proteins **(C)** levels of IL-10 were examined by quantitative PCR and ELISA, respectively. **D,** The SOCS3 mRNA level was examined by quantitative PCR. **E,** The level of p-STAT3 was detected by flow cytometry. Data are expressed as Mean ± SEM. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$.

rogated by miR140a overexpression (Figure 3E). Taken together, miR140a targets IL-10 and thus attenuates the anti-inflammatory IL-10/STAT3/SOCS3 cascade.

IL-10 Neutralization Reverses MiR140a Inhibition-Induced Downregulation of Pro-Inflammatory Cytokine Production in Macrophages

In order to further testify miR140a/IL-10 axis in macrophages, we neutralized IL-10 in miR140a inhibitor-transfected macrophages, and determined the production of pro-inflammatory cytokines. As shown in Figure 4, miR140a inhibition reduced the concentrations of TNF-α, CCL2, and IL-6 in the supernatant of ox-LDL-stimulated macrophages. However, this effect was largely reversed in the presence of IL-10-neutralizing antibody (Figure 4). Therefore, miR140a increases the pro-inflammatory activity of macrophages by impairing IL-10 production.

MiR140a Expression Is Negatively Correlated With IL-10 Level in AS Monocytes

To further validate the relationships between miR140a and IL-10, we analyzed the levels of miR140a and IL-10 in monocytes from AS patients. We found a negative correlation between the levels of miR140a and IL-10 in AS monocytes (Figure 5A). By contrast, a positive correlation between the levels of miR140a and TNF-α or IL-6 was found in AS monocytes (Figure 5B, C). Collectively, miR140a increases the pro-inflammatory ability in AS-associated macrophages.

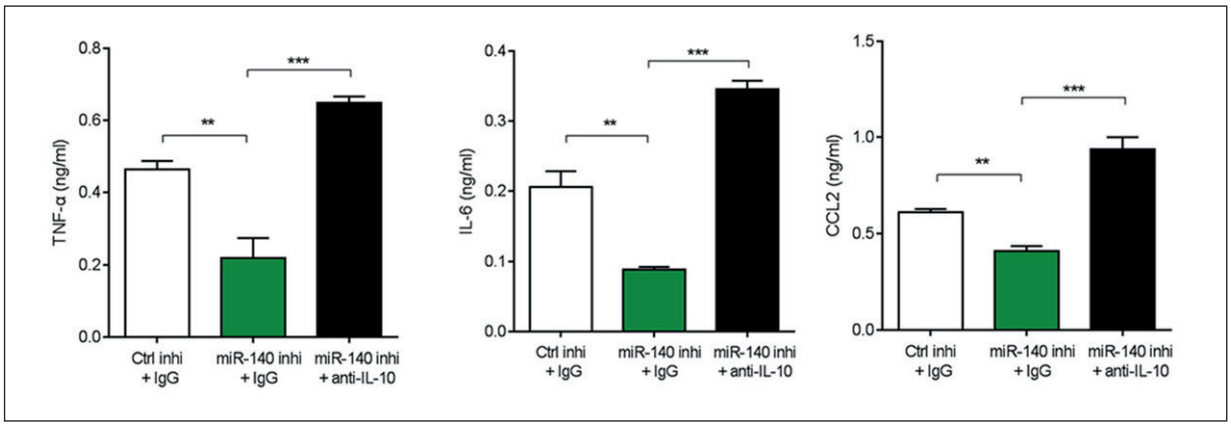

Figure 4. Neutralization of IL-10 rescues downregulated pro-inflammatory cytokine production induced by miR140a inhibition. THP-1 macrophages were transfected with miR140a mimic, miR140a inhibitor and corresponding controls followed by ox-LDL stimulation (50 μg/mL) for 6 h, in the presence of control IgG or anti-IL-10 (600 ng/mL), protein levels of TNF-α, IL-6 and CCL2 were examined by ELISA. Data are expressed as Mean ± SEM. ** means $p < 0.01$, ***means $p < 0.001$.

Discussion

The crucial roles of inflammatory immune cells and their products in AS progression have been increasingly recognized[3,4]. Among these pro-inflammatory immune cells, macrophages are the prominent players in the pathogenic processes of AS. It is thought that macrophages act as a double-edged sword *via* exerting both pro-inflammatory and anti-inflammatory capabilities[2]. Hence, a deeper understanding into the mechanism that modulates macrophage phenotypic switch is of great significance for AS diagnosis and treatment. In our study, we identified miR140a as a new AS-related microRNA that negatively regulates IL-10 expression, thus augmenting the pro-inflammatory phenotype of macrophages in AS.

As a well-known immunosuppressive cytokine, IL-10 is mainly produced by macrophages, and functions as an athero-protective factor at all stages of AS[21,22]. The effects of IL-10 on macrophages are multifaceted, including suppressing the production of pro-inflammatory cytokines or chemokines, inhibiting the apoptosis of lipid-laden macrophages, and maintaining lipid homeostasis through regulating cholesterol uptake and efflux[21]. In our case, we observed that the miR140a-induced downregulation of IL-10 was associated with the increased production of pro-inflammatory cytokines and chemok-

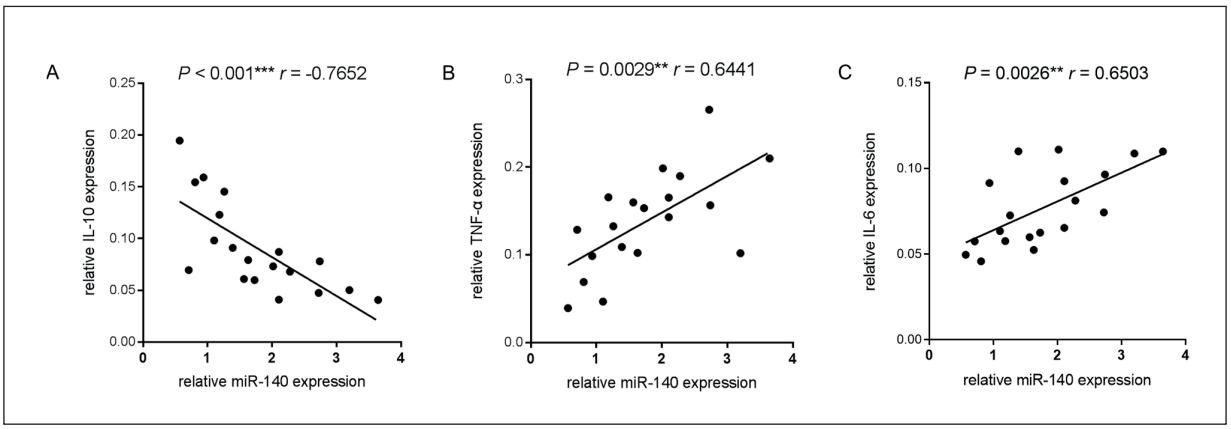

Figure 5. The correlation between the expression of miR140a and IL-10 or TNF-α in AS-associated monocytes. **A-C,** MRNA levels of IL-10, miR140a, TNF-α and IL-6 in blood monocytes from atherosclerosis patients were evaluated by quantitative PCR, their correlations were analyzed by Spearman's rank correlation test. **$p < 0.01$, ***$p < 0.001$.

ines (TNF-α, IL-6 and CCL2). This is in line with the anti-inflammatory role of IL-10. It is well-accepted that IL-10/STAT3/SOCS3 axis is the predominant signaling pathway responsible for the anti-inflammatory function of IL-10[20,23]. In our study, miR140a mimic impaired STAT3 phosphorylation and SOCS3 expression in ox-LDL stimulated macrophages, indicating the negative role of miR140a in regulating IL-10/STAT3/SOCS3 cascade.

Some studies[9,14] indicated the important roles of microRNA in regulating the functions of AS-associated macrophages. So, miR33 inhibition promotes M2 macrophage polarization by targeting AMP-Activated Protein Kinase (AMPK), thereby exerting an athero-protective function[14]. MiR140-3p expression was reported to be significantly downregulated in atherosclerosis obliterans, and high expression level of miR140-3p predicted a high risk of restenosis in patients with lower extremity arterial occlusive disease[24]. In addition, miR140 contributed to the hypertension and oxidative stress in AS through regulating the expression of Nuclear factor E2-Related Factor 2 (Nrf2) and Sirtuin 2 (Sirt2)[25]. Moreover, miR140a attenuated pulmonary arterial hypertension progression *via* targeting Smurf1[26]. These results indicate comprehensive roles of miR140a in AS and other vascular diseases. Here, we firstly reported the function of miR140 in regulating the pro-inflammatory capability of AS-related macrophages, by suppressing the production of anti-inflammatory cytokine IL-10. Moreover, the positive correlation between TNF-α and miR140a level in AS monocytes suggests that miR140a might serve as a diagnostic marker in AS patients. On the other hand, *in vivo* studies using mouse AS model are also needed in the future to further explore the physiological function of miR140a. In summary, our findings provide new insights into the role of miR140a in macrophage phenotypic switch in AS.

Conclusions

Briefly, miR140a downregulates IL-10 production in AS-associated macrophages. Therefore, miR140a functions as a pro-atherosclerotic microRNA by enhancing macrophage inflammatory capacity.

Funding

This study was supported by the Excellent Youth Project of Hunan Provincial Education Department (No. 17B274).

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Roth GA, Johnson C, Abajobir A, Abd-Allah F, Abera SF, Abyu G, Ahmed M, Aksut B, Alam T, Alam K, Alla F, Alvis-Guzman N, Amrock S, Ansari H, Arnlov J, Asayesh H, Atey TM, Avila-Burgos L, Awasthi A, Banerjee A, Barac A, Barnighausen T, Barregard L, Bedi N, Belay Ketema E, Bennett D, Berhe G, Bhutta Z, Bitew S, Carapetis J, Carrero JJ, Malta DC, Castaneda-Orjuela CA, Castillo-Rivas J, Catala-Lopez F, Choi JY, Christensen H, Cirillo M, Cooper L, Jr., Criqui M, Cundiff D, Damasceno A, Dandona L, Dandona R, Davletov K, Dharmaratne S, Dorairaj P, Dubey M, Ehrenkranz R, El Sayed Zaki M, Faraon EJA, Esteghamati A, Farid T, Farvid M, Feigin V, Ding EL, Fowkes G, Gebrehiwot T, Gillum R, Gold A, Gona P, Gupta R, Habtewold TD, Hafezi-Nejad N, Hailu T, Hailu GB, Hankey G, Hassen HY, Abate KH, Havmoeller R, Hay SI, Horino M, Hotez PJ, Jacobsen K, James S, Javanbakht M, Jeemon P, John D, Jonas J, Kalkonde Y, Karimkhani C, Kasaeian A, Khader Y, Khan A, Khang YH, Khera S, Khoja AT, Khubchandani J, Kim D, Kolte D, Kosen S, Krohn KJ, Kumar GA, Kwan GF, Lal DK, Larsson A, Linn S, Lopez A, Lotufo PA, El Razek HMA, Malekzadeh R, Mazidi M, Meier T, Meles KG, Mensah G, Meretoja A, Mezgebe H, Miller T, Mirrakhimov E, Mohammed S, Moran AE, Musa KI, Narula J, Neal B, Ngalesoni F, Nguyen G, Obermeyer CM, Owolabi M, Patton G, Pedro J, Qato D, Qorbani M, Rahimi K, Rai RK, Rawaf S, Ribeiro A, Safiri S, Salomon JA, Santos I, Santric Milicevic M, Sartorius B, Schutte A, Sepanlou S, Shaikh MA, Shin MJ, Shishehbor M, Shore H, Silva DAS, Sobngwi E, Stranges S, Swaminathan S, Tabares-Seisdedos R, Tadele Atnafu N, Tesfay F, Thakur JS, Thrift A, Topor-Madry R, Truelsen T, Tyrovolas S, Ukwaja KN, Uthman O, Vasankari T, Vlassov V, Vollset SE, Wakayo T, Watkins D, Weintraub R, Werdecker A, Westerman R, Wiysonge CS, Wolfe C, Workicho A, Xu G, Yano Y, Yip P, Yonemoto N, Younis M, Yu C, Vos T, Naghavi M, Murray C. Global, regional, and national burden of cardiovascular diseases for 10 causes, 1990 to 2015. J Am College Cardiol 2017; 70: 1-25.

2) Tabas I, Lichtman AH. Monocyte-macrophages and T cells in atherosclerosis. Immunity 2017; 47: 621-634.

3) Vaccarezza M, Balla C, Rizzo P. Atherosclerosis as an inflammatory disease: doubts? No more. IJC Heart Vascul 2018; 19: 1-2.

4) Viola J, Soehnlein O. Atherosclerosis--a matter of unresolved inflammation. Sem Immunol 2015; 27: 184-193.

5) Huang WC, Sala-Newby GB, Susana A, Johnson JL, Newby AC. Classical macrophage activation up-regulates several matrix metalloproteinas-

es through mitogen activated protein kinases and nuclear factor-kappaB. PLoS One 2012; 7: e42507.

6) Trogan E, Feig JE, Dogan S, Rothblat GH, Angeli V, Tacke F, Randolph GJ, Fisher EA. Gene expression changes in foam cells and the role of chemokine receptor CCR7 during atherosclerosis regression in ApoE-deficient mice. Proc Natl Acad Sci USA 2006; 103: 3781-3788.

7) Lavin Y, Winter D, Blecher-Gonen R, David E, Keren-Shaul H, Merad M, Jung S, Amit I. Tissue-resident macrophage enhancer landscapes are shaped by the local microenvironment. Cell 2014; 159: 1312-1326.

8) Van den Bossche J, O'Neill LA, Menon D. Macrophage immunometabolism: where are we (going)? Trends Immunol 2017; 38: 395-406.

9) Feinberg MW, Moore KJ. MicroRNA regulation of atherosclerosis. Circ Res 2016; 118: 703-720.

10) Li H, Zhu H, Ge J. Long noncoding RNA: recent updates in atherosclerosis. Int J Biol Sci 2016; 12: 898-910.

11) Eken SM, Jin H, Chernogubova E, Li Y, Simon N, Sun C, Korzunowicz G, Busch A, Backlund A, Osterholm C, Razuvaev A, Renne T, Eckstein HH, Pelisek J, Eriksson P, Gonzalez Diez M, Perisic Matic L, Schellinger IN, Raaz U, Leeper NJ, Hansson GK, Paulsson-Berne G, Hedin U, Maegdefessel L. MicroRNA-210 enhances fibrous cap stability in advanced atherosclerotic lesions. Circ Res 2017; 120: 633-644.

12) Nazari-Jahantigh M, Egea V, Schober A, Weber C. MicroRNA-specific regulatory mechanisms in atherosclerosis. J Mol Cell Cardiol 2015; 89: 35-41.

13) Ouimet M, Ediriweera H, Afonso MS, Ramkhelawon B, Singaravelu R, Liao X, Bandler RC, Rahman K, Fisher EA, Rayner KJ, Pezacki JP, Tabas I, Moore KJ. MicroRNA-33 regulates macrophage autophagy in atherosclerosis. Arterioscl Throm Vas 2017; 37: 1058-1067.

14) Ouimet M, Ediriweera HN, Gundra UM, Sheedy FJ, Ramkhelawon B, Hutchison SB, Rinehold K, van Solingen C, Fullerton MD, Cecchini K, Rayner KJ, Steinberg GR, Zamore PD, Fisher EA, Loke P, Moore KJ. MicroRNA-33-dependent regulation of macrophage metabolism directs immune cell polarization in atherosclerosis. J Clin Invest 2015; 125: 4334-4348.

15) Soh J, Iqbal J, Queiroz J, Fernandez-Hernando C, Hussain MM. MicroRNA-30c reduces hyperlipidemia and atherosclerosis in mice by decreasing lipid synthesis and lipoprotein secretion. Nat Med 2013; 19: 892-899.

16) Friedman RC, Farh KK, Burge CB, Bartel DP. Most mammalian mRNAs are conserved targets of microRNAs. Gene Res 2009; 19: 92-105.

17) Tsuchiya S, Kobayashi Y, Goto Y, Okumura H, Nakae S, Konno T, Tada K. Induction of maturation in cultured human monocytic leukemia cells by a phorbol diester. Cancer Res 1982; 42: 1530-1536.

18) Qin Z. The use of THP-1 cells as a model for mimicking the function and regulation of monocytes and macrophages in the vasculature. Atherosclerosis 2012; 221: 2-11.

19) Niemand C, Nimmesgern A, Haan S, Fischer P, Schaper F, Rossaint R, Heinrich PC, Muller-Newen G. Activation of STAT3 by IL-6 and IL-10 in primary human macrophages is differentially modulated by suppressor of cytokine signaling 3. J Immunol 2003; 170: 3263-3272.

20) Qasimi P, Ming-Lum A, Ghanipour A, Ong CJ, Cox ME, Ihle J, Cacalano N, Yoshimura A, Mui AL. Divergent mechanisms utilized by SOCS3 to mediate interleukin-10 inhibition of tumor necrosis factor alpha and nitric oxide production by macrophages. J Biol Chem 2006; 281: 6316-6324.

21) Han X, Boisvert WA. Interleukin-10 protects against atherosclerosis by modulating multiple atherogenic macrophage function. Thromb Haemost 2015; 113: 505-512.

22) Sabat R, Grütz G, Warszawska K, Kirsch S, Witte E, Wolk K, Geginat J. Biology of interleukin-10. Cytokine Growth F R 2010; 21: 331-344.

23) Murray PJ. Understanding and exploiting the endogenous interleukin-10/STAT3-mediated anti-inflammatory response. Curr Opin Pharmacol 2006; 6: 379-386.

24) Sun X, Liu A, Jiang Z, Cui J, Zhang W. Predictive value of plasma microRNA-140-3p for restenosis in patients with lower extremity arterial occlusive disease undergoing interventional procedures. Int J Clin Exp Med 2017; 10: 5578-5585.

25) Liu QQ, Ren K, Liu S-H, Li W-M, Huang C-J, Yang X-H. MicroRNA-140-5p aggravates hypertension and oxidative stress of atherosclerosis via targeting Nrf2 and Sirt2. Int J Mol Med 2019; 43: 839-849.

26) Rothman AMK, Arnold ND, Pickworth JA, Iremonger J, Ciuclan L, Allen R, Guth-Gundel S, Southwood M, Morrell Nicholas W, Francis Sheila E, Rowlands D, Lawrie A. Abstract 11498: MicroRNA 140 regulates disease phenotype in experimental pulmonary arterial hypertension and identifies Smurf1 as a novel therapeutic target. Circulation 2015; 132: Issue suppl 3.