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IL-8 negatively regulates ABCA1 expression and cholesterol efflux via upregulating miR-183 in THP-1 macrophage-derived foam cells

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ABSTRACT

Objective: Previous studies suggest that IL-8 has an important role in the regulation of cholesterol efflux, but whether miRNAs are involved in this process is still unknown. The purpose of this study is to explore whether IL-8 promotes cholesterol accumulation by enhancing miR-183 expression in macrophages and its underlying mechanism.

Methods and results: Treatment of THP-1 macrophage-derived foam cells with IL-8 decreased ABCA1 expression and cholesterol efflux. Using bioinformatics analyses and dual-luciferase reporter assays, we found that miR-183 was highly conserved during evolution and directly inhibited ABCA1 protein and mRNA expression by targeting ABCA1 3′UTR. MiR-183 directly regulated endogenous ABCA1 expression levels. Furthermore, IL-8 enhanced the expression of miR-183 and decrease ABCA1 expression. Cholesterol transport assays confirmed that IL-8 dramatically inhibited apolipoprotein AI-mediated ABCA1-dependent cholesterol efflux by increasing miR-183 expression. In contrast, treatment with anti-IL-8 antibody reversed these effects.

Conclusion: IL-8 enhances the expression of miR-183, which then inhibits ABCA1 expression and cholesterol efflux. Our studies suggest that the IL-8-miR-183-ABCA1 axis may play an intermediary role in the development of atherosclerosis.

1. Introduction

Atherosclerosis is the common pathological basis of cardiovascular disease, a leading cause of morbidity and mortality worldwide [1,2]. Macrophages, the major cells in the atherosclerotic lesion, undergo foam cell formation, which plays an important role in lipid accumulation and cholesterol homeostasis [3]. The imbalance in lipid metabolism and the accumulation of cholesterol-laden macrophages in the artery wall plays a decisive role in the development of atherosclerosis [4–6].

ATP-binding cassette transporter A1 (ABCA1), an important membrane protein, is highly expressed in macrophages and plays a key role in the control of cellular cholesterol homeostasis [7–9]. Also, several lines of evidence have demonstrated that increased expression of ABCA1 promotes cholesterol efflux to lipid-free apolipoprotein A1 for the generation of high density lipoprotein (HDL), serving as the first step in reverse cholesterol transport (RCT) that inhibits the formation of foam cells and development of atherosclerosis [10–12]. Accordingly, a proper understanding of the relationship between ABCA1 and its regulators is of great importance.

Pro-inflammatory cytokine IL-8 has emerged as a key factor in cholesterol homeostasis associated with atherosclerosis. Wang et al. [13] demonstrated that IL-8 is induced in human monocyte-derived macrophage cells treated with acetylated-low density lipoprotein (acLDL) and the regulation of its production is correlated with cholesterol loading. More recently, Chen et al. [14] found that IL-8 plays a

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negative role in cholesterol efflux in THP-1 cells. Several lines of evidence suggest that inflammatory cytokines IL-6 and TNF-α activate the transcription of miRNAs, which inhibit the expression of ABCA1 and cholesterol efflux in human THP-1 macrophages [15,16].

miRNAs are endogenous small (22-nt) non-coding RNAs that can regulate gene expression at the post-transcriptional level by binding to complementary sequences in the 3′UTR of mRNAs and targeting them for translational inhibition or mRNA cleavage [17–19]. MiR-183 is involved in intracellular lipid homeostasis. Recent studies have demonstrated that miR-183 is highly expressed in adipose tissue [20–22]. Furthermore, Chen et al. [23] demonstrated that miR-183 positively enhances adipocyte differentiation, lipid accumulation and adipogenesis. Moreover, sterol regulatory element-binding protein 2 (SREBP-2) induces the expression of a cluster of miR-96, miR-182 and miR-183 and then controls cholesterol homeostasis by affecting nuclear SREBP accumulation [24]. In addition, miR-183 was found to promote the proliferation and inhibit the apoptosis in colon cancer by targeting ABCA1 [25]. Our preliminary studies revealed that miR-183 directly binds to 3′UTR of ABCA1 and decreases its mRNA and protein levels in THP-1 macrophages. Subsequently, we found that IL-8 significantly enhances the expression of miR-183 in human THP-1 macrophages. Thus, we hypothesized that IL-8 promotes the expression of miR-183, and then suppresses ABCA1 expression and cholesterol efflux in THP-1 macrophages.

To test this hypothesis, we investigated the mechanism underlying IL-8 suppression of ABCA1 expression and cholesterol efflux and the roles of miR-183 in THP-1 macrophages.

2. Materials and methods

2.1. Cell culture and reagents

HEK 293T and THP-1 cells were obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). HEK 293T cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS). The THP-1 human monocytic cells were cultured in RPMI-1640 growth medium supplemented with 10% FBS, penicillin (100 U/ml) and streptomycin (100 mg/ml) at 37 °C with 5% CO2. THP-1 cells were differentiated into macrophages by incubation with 100 ng/ml phorbol-12-myristate acetate (PMA, Sigma, America) for 24 h. To induce foam cell formation, macrophages were incubated in RPMI-1640 containing 0.2% (w/v) bovine serum albumin (BSA) and 50 μg/ml acetylated-low density lipoprotein (acLDL) or oxidized-low density lipoprotein (oxLDL) for 48 h in a serum-free medium.

2.2. DNA constructs and 3′UTR luciferase reporter assays

cDNA fragments corresponding to the entire 3′UTR of ABCA1 were amplified by reverse transcription-polymerase chain reaction (RT-qPCR) from total RNA extracted from THP-1 cells with XhoI and NotI linkers. The PCR products were directionally cloned downstream of the Renilla luciferase open reading frame (ORF) in the psiCHECK™-2 vector (Promega) that also contains a constitutively expressed firefly luciferase gene, which is used to normalize transfections. Site-directed mutagenesis introduced in the seed region of predicted miR-183 binding sites within the 3′UTR of ABCA1 was generated using Multisite-QuickChange (Stratagene, America) according to the manufacturer's protocol. All constructs were confirmed by commercial sequencing. HEK293T cells were seeded at 1×10^6 cells/well into 12-well plates (Costar, America) and co-transfected with 1 μg of the indicated 3′UTR luciferase reporter vectors and miR-183 mimic utilizing RiboFECT™CP reagent (Ribobio, China) for 24 h. Luciferase activity was measured using the Dual-Glo Luciferase Assay System (Promega). Renilla Luciferase activity was normalized to the corresponding firefly luciferase activity and plotted as a percentage of the control (Con-miR). All experiments were independently performed in triplicate wells of a 12-well plate and repeated three times.

2.3. MiR-183 and anti- miR-183 transfection

HEK 293T and THP-1 macrophage-derived foam cells were transfected with miR-183 mimic or inhibitor (anti-miR-183) (Ribobio, China) at the concentrations indicated in the corresponding figure legends for 24 h using RiboFECT™CP reagent (Ribobio, China) in accordance with the manufacturer's protocol. MiR-183 mimic is small, chemically modified double-stranded RNAs that mimic endogenous miR-183, whereas miR-183 inhibitor is chemically modified, single stranded nucleic acids designed to specifically bind to and suppress endogenous miR-183 molecules. A total volume of 1 ml per well contained 100 μl transfection buffer, 10 μl RiboFECT™CP reagent, different volumes of miR-183 mimic/inhibitor depending on the concentrations used, and RPMI 1640 containing 0.2% (w/v) BSA. All control samples were treated with an equal concentration of a nontargeting negative control sequence (scrambled miR-183) (Ribobio, China).

2.4. Quantitative real-time polymerase chain reaction (RT-qPCR)

For mRNA determination, total RNA was extracted from THP-1-derived foam cells using TRIzol reagent (Invitrogen) according to the manufacturer's instructions. Relative quantitative real-time PCR was carried out using Roche Light Cycler Run 5.32 Real-Time PCR System with SYBR Green detection chemistry. Melting curve analyses of all real-time PCR products were performed and shown to produce a single DNA duplex. Quantitative measurements were analyzed using the ΔΔCt method. In these experiments, β-actin was used as the internal control. Data are expressed as means ± SD of three repeats.

2.5. Western blot analysis

Cells were lysed for protein extraction using RIPA buffer containing proteinase inhibitor cocktails (Sigma). Proteins (5 μl lysates) were separated by SDS-PAGE (6% gels), and then transferred to polyvinylidene fluoride (PVDF) membranes. Membranes were blocked with 5% fat-free dry milk and then incubated with rabbit antibodies against ABCA1 (Abcam, USA) or β-actin (Boster, China), followed by incubation with horseradish peroxidase-conjugated goat anti-rabbit secondary antibody for 2 h at room temperature. Finally, the western blot bands were detected using a chemiluminescence method and quantified with the Tanon-5500 Chemiluminescent Imaging System (Tanon, Shanghai, China). Data are expressed as means ± SD of three repeats.

2.6. Cellular cholesterol efflux assays

Cholesterol efflux experiments were performed as previously described [26]. In brief, THP-1 macrophage-derived foam cells (1×10^6 cells) were seeded into 12-well plates. On day 3, cells were labeled with 5 μCi/ml of [3H]-cholesterol (PerkinElmer, Waltham, MA) in media containing 0.2% bovine serum albumin (BSA) for 24 h. Cells were then washed with fresh media and treated as indicated in the figures. The cells were washed again with PBS and incubated in the presence of apoA-I (10 μg/mL) for 24 h. Medium with cell-associated [^{3}H] cholesterol was then measured by liquid scintillation counting. Percent efflux was calculated by the following equation: [total media counts/(total cellular counts + total media counts)] × 100%. Data are expressed as means ± SD of three repeats.

2.7. Statistical analysis

Data are expressed as the means ± SD. Results were analyzed by one-way analysis of variance and Student's *t* test using SPSS 13.0 software. Difference was considered statistically significant when p values were < 0.05.

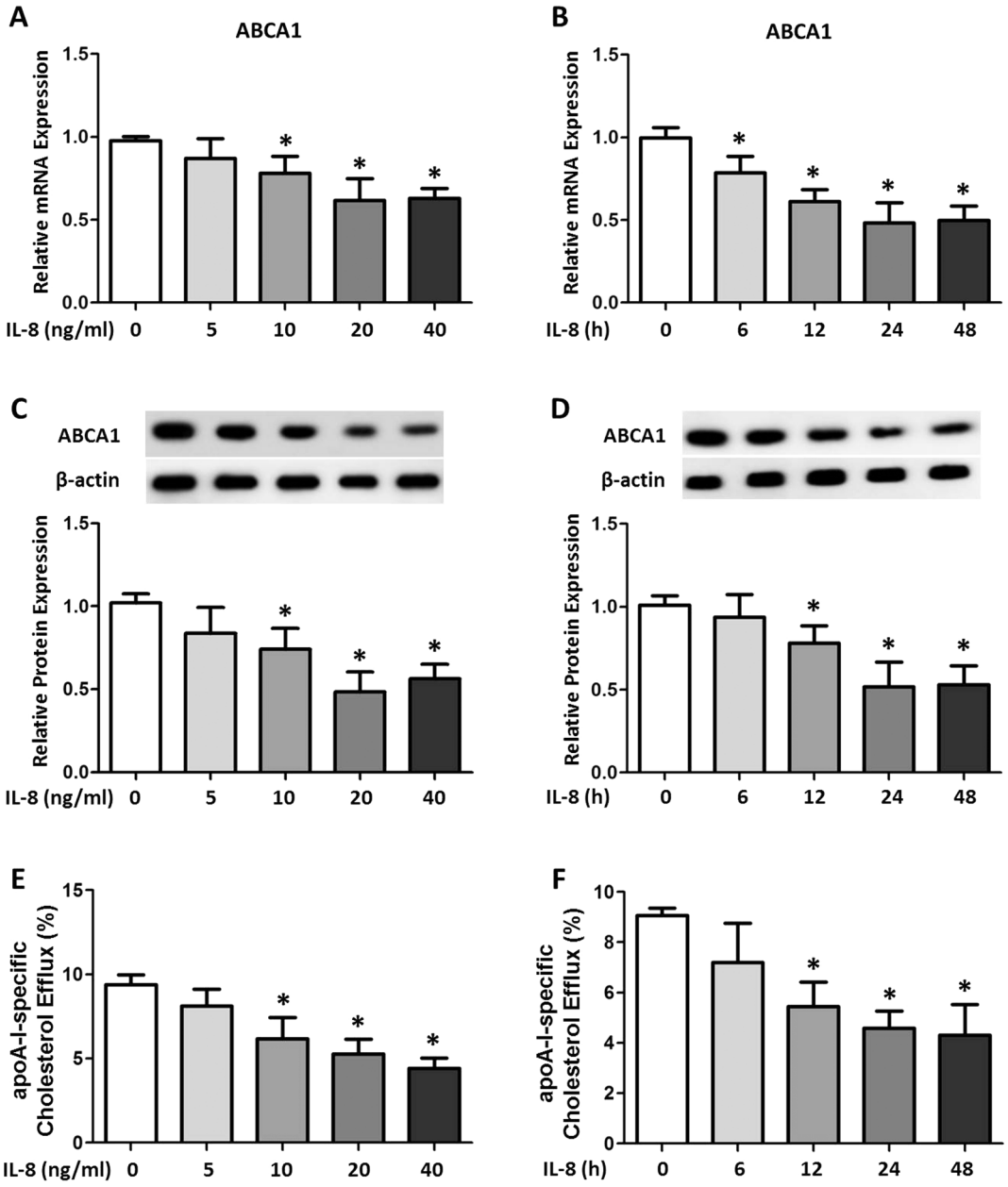

Fig. 1. IL-8 suppresses the expression of ABCA1 and decreases cholesterol efflux. A, B, C, D, E and F, THP-1 macrophage-derived foam cells were treated with IL-8 at different concentrations (0, 5, 10, 20, 40 ng/ml) for 24 h or with 20 ng/ml IL-8 at the indicated periods (0, 6, 12, 24, 48 h). A and B, Total RNA was extracted and quantitative real-time PCR was performed to determine the ABCA1 mRNA expression. C and D, Western blot assays using antibodies against ABCA1 and β-actin were conducted. E and F, ApoA1-specific cholesterol efflux was determined by liquid scintillation counting assays as described in Methods. All results are expressed as mean ± S.D. from three independent experiments with each performed in triplicate. $^{*}P < 0.05$ vs. control group.

3. Results

3.1. IL-8 represses the expression of ABCA1 and reduces cholesterol efflux.

We assessed the effects of IL-8 on ABCA1 expression and cholesterol efflux in cultured THP-1 macrophage-derived foam cells. Treatment with acLDL stimulated the expression of IL-8 receptor B (IL-8RB, CXCR2) in THP-1 macrophage (Fig. S1). Then the cells were treated with IL-8 at different concentrations (0, 5, 10, 20, 40 ng/ml) for 24 h or with 20 ng/ml IL-8 for different periods of time (0, 6, 12, 24, 48 h). The protein and mRNA levels of ABCA1 in foam cells were determined by western blot and RT-qPCR analyses, respectively. Compared with the control, treatment with IL-8 showed a significant reduction in ABCA1 mRNA (Fig. 1A and B) and protein levels (Fig. 1C and D) in concentration- and time-dependent manners. We then determined whether IL-8 regulates cholesterol efflux. THP-1 cells were treated with IL-8, followed by cholesterol efflux assay. Our results showed that IL-8 markedly reduced apoA1-specific efflux of cholesterol (Fig. 1E and F). Taken together, these data suggested that IL-8 inhibits ABCA1 expression and decreases cholesterol efflux.

3.2. MiR-183 is highly conserved in mammals and putatively binds to ABCA1 3′UTR.

Several target gene prediction algorithms predict that the mRNA transcripts of the cellular cholesterol transporter ABCA1 in most animal species contained the putative binding sites of miR-183 in its 3′UTR (Fig. 2A). The conservative binding site is highly conserved among

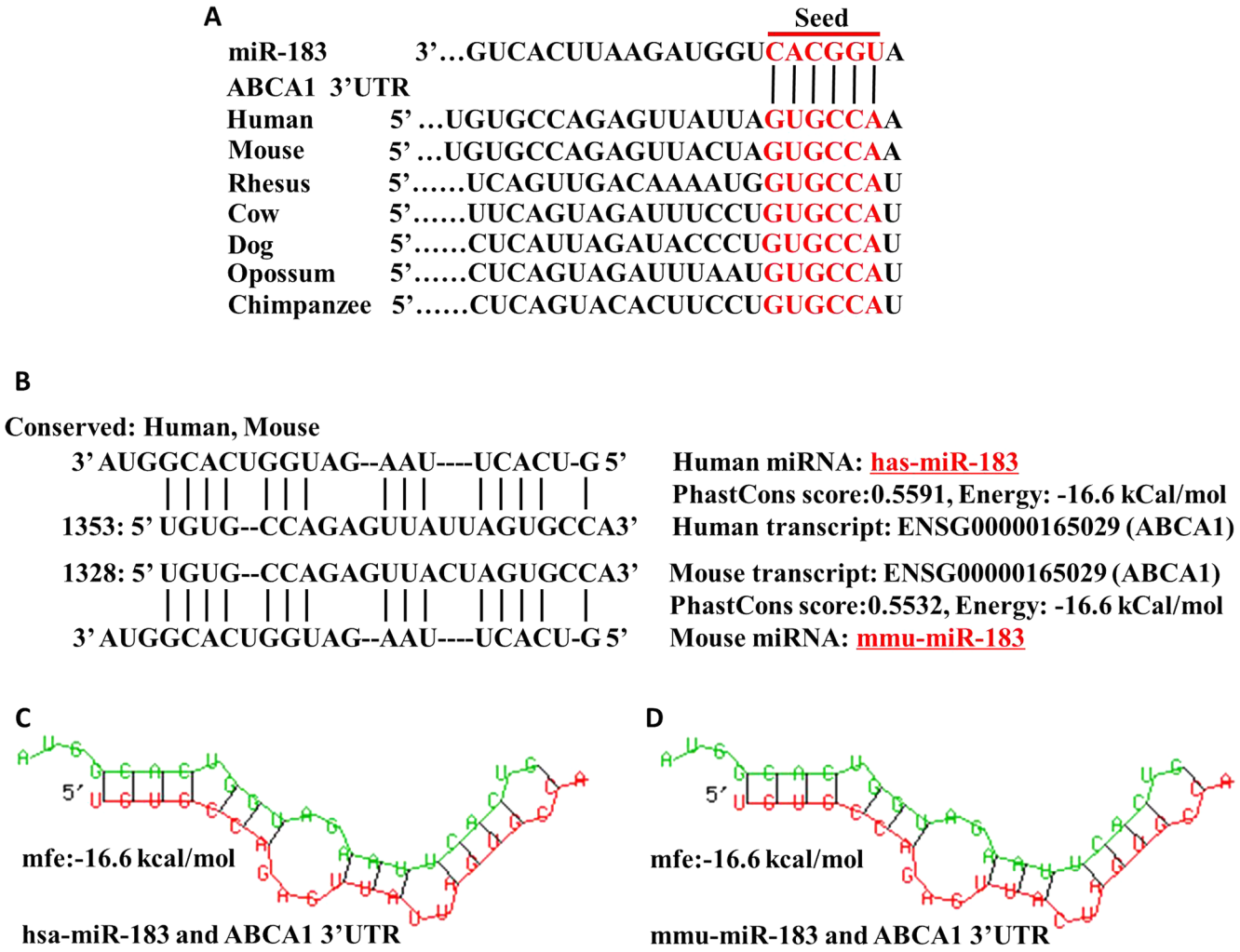

Fig. 2. Predicted annealing of miR-183 to ABCA1 3′UTR. A, miR-183 has the same bases and is highly conserved in different species (miRDB). B, Sequence alignment of the miR-183 mature sequence binding to the 3′UTR of ABCA1 in different species. C and D, The free energy scores (in RNAhybrid) for miR-183-ABCA1 hybridization in human (left panel) and mouse (right panel).

species, suggesting that the role of miR-183 is important in miR-183-mediated ABCA1 suppression (Fig. 2A and B). Importantly, RNAhybrid data showed a free energy score at 16.6 kcal/mol in human and at 16.6 kcal/mol in mice (Fig. 2C and D). These results suggest that miR-183 most likely binds directly to the 3′UTR of ABCA1 and then post-transcriptionally downregulates its expression under physiological and/or pathological conditions.

3.3. MiR-183 directly targets ABCA1 3′UTR and represses the expression of ABCA1

To determine whether ABCA1 is a direct target of miR-183, we performed luciferase reporter assay in HEK 293 T cells. Cotransfection of miR-183 mimic with the luciferase reporter gene associated with the wild-type (WT) segment of the ABCA1 3′UTR dramatically repressed luciferase activity, but did not affect the luciferase activity of the mutant (MUT) ABCA1 3′UTR (Fig. 3A). These findings suggest that the conservative miR-183 binding site plays an important role in post-transcriptional repression of ABCA1 by miR-183 and miR-183 likely targets ABCA1 3′UTR via this binding site.

We next examined the effect of miR-183 mimic on ABCA1 expression in cultured THP-1 macrophage-derived foam cells. The cells were transfected with different concentrations of miR-183 mimic (0, 10, 20, 40, 80 nM) for 24 h or with 40 nM miR-183 mimic for different periods of time (0, 6, 12, 24, 48 h). The mRNA and protein levels of ABCA1 in foam cells were determined by qPCR and Western blot, respectively. When compared with cells transfected with scrambled miR-183, foam cells transfected with miR-183 mimic showed a significant reduction in ABCA1 mRNA (Fig. 3B and C) and protein levels (Fig. 3D and E) in concentration- and time-dependent manners. We then examined the effects of TO901317, a liver X receptor (LXR) agonist which induces ABCA1 expression. We observed that miR-183 mimic abrogated TO901317-induced upregulation of ABCA1 expression (Fig. 3F and G). These data demonstrated that miR-183 regulates ABCA1 expression in THP-1 macrophages probably by targeting ABCA1 3′UTR to promote mRNA degradation or translational repression.

3.4. IL-8 suppresses ABCA1 expression and cholesterol efflux through upregulating the expression of miR-183

We then explored whether IL-8 suppressed ABCA1 expression and cholesterol efflux via regulating the expression of miR-183. THP-1 macrophage-derived foam cells were treated with IL-8 at different concentrations (0, 5, 10, 20, 40 ng/ml) for 24 h or with 20 ng/ml IL-8 at the indicated periods (0, 6, 12, 24, 48 h). The mRNA levels of miR-183 in foam cells were determined by RT-qPCR analyses. Treatment with IL-8 showed a significant increase in ABCA1 mRNA levels (Fig. 4A and B) in concentration- and time-dependent manners. Next, THP-1 macrophages were transfected with scrambled miR-183 or miR-183 inhibitor after incubation with IL-8. We found that treatment with IL-8 and

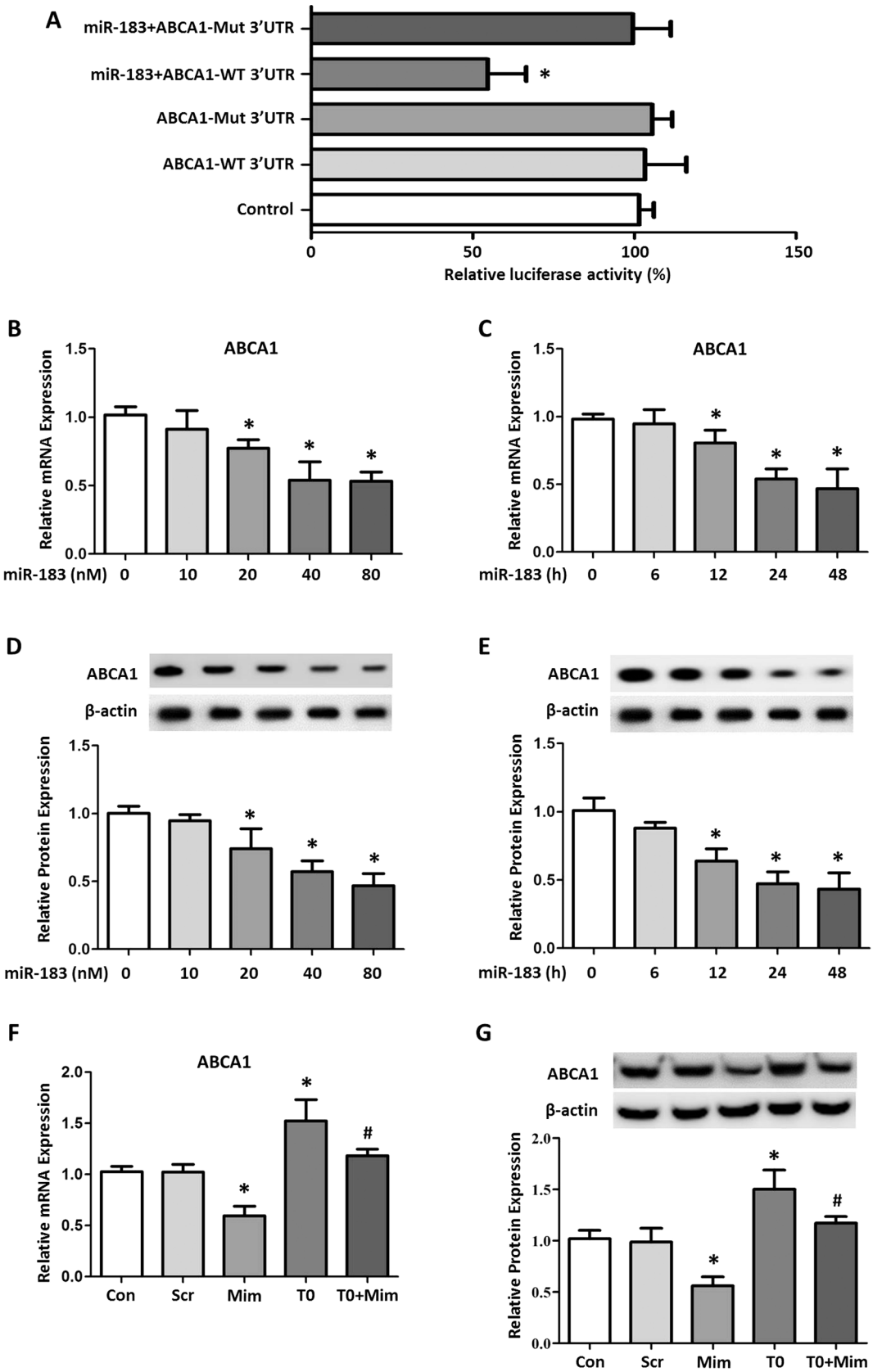

Fig. 3. MiR-183 directly targets ABCA1 3′UTR. A, Luciferase activity assay of the HEK 293 cells cotransfected with a luciferase reporter plasmid containing wild-type (WT) or mutant (Mut) ABCA1 3′UTR and miR-183 mimic for 24 h. B, C, D and E, THP-1 macrophage-derived foam cells were treated with miR-183 mimic at different concentrations (0, 10, 20, 40, 80 nM) for 24 h or with miR-183 mimic (40 nM) for different periods (0, 6, 12, 24, 48 h) to examine the effects of miR-183 mimic on ABCA1 expression. The mRNA and protein levels of ABCA1 in foam cells were determined by RT-qPCR and western blot analyses, respectively. F and G, ABCA1 protein and mRNA levels were determined by western blot and RT-qPCR assays in THP-1 macrophage-derived foam cells treated with 10 mM TO901317, 40 nM miR-183 mimic or both, respectively. All results are expressed as mean ± S.D. from three independent experiments (each performed in triplicate). $^{*}P < 0.05$ vs. control group or scrambled miR-183 group, $^{\#}P < 0.05$ vs. TO901317 group. Con, control; Scr, scrambled miR-183; Mim, miR-183 mimic; TO, TO901317, a liver X receptor agonist.

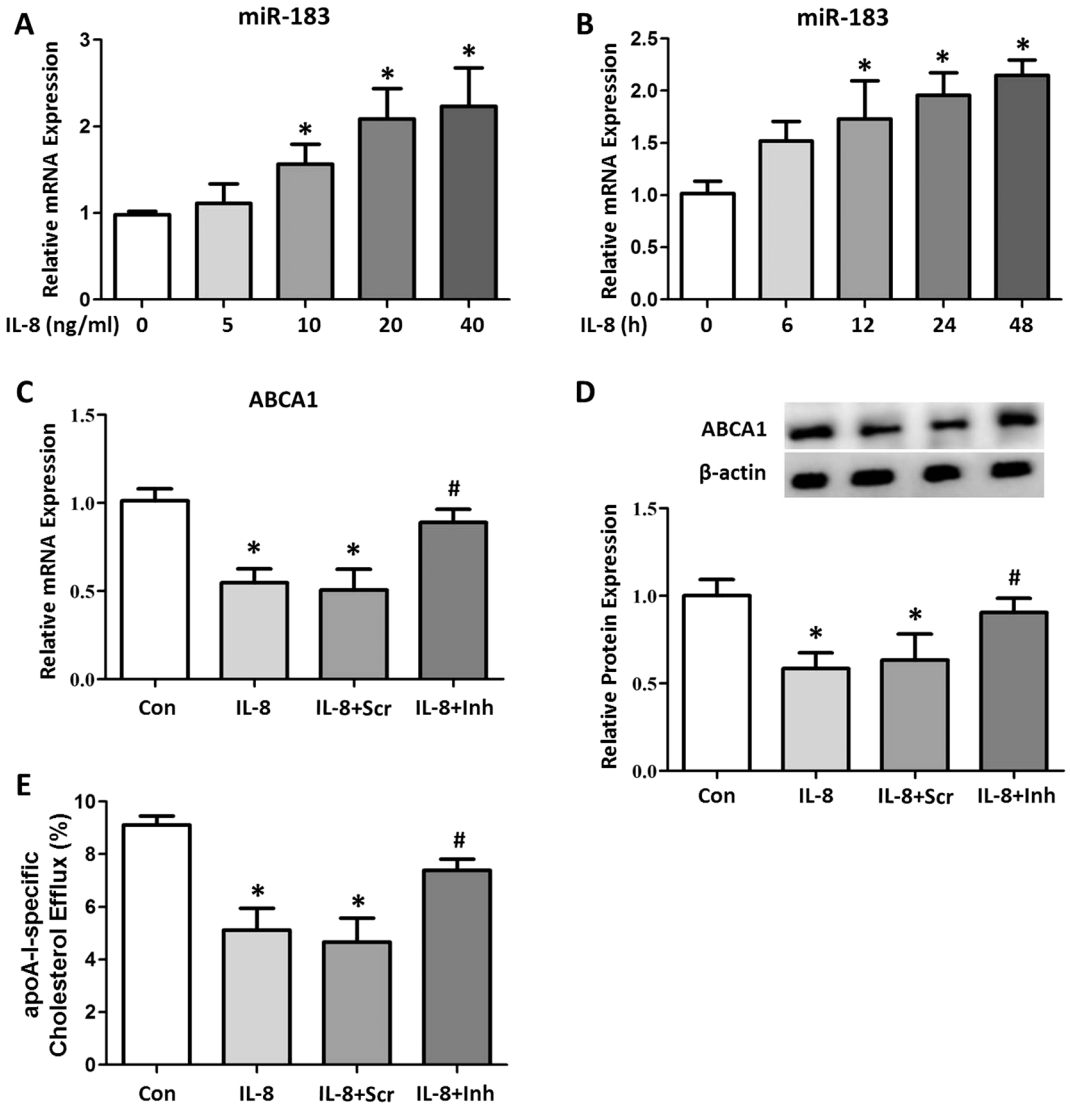

Fig. 4. IL-8 suppresses ABCA1 expression and cholesterol efflux through upregulating the expression of miR-183. A and B, THP-1 macrophage-derived foam cells were treated with IL-8 at different concentrations (0, 5, 10, 20, 40 ng/ml) for 24 h or with 20 ng/ml IL-8 at the indicated periods (0, 6, 12, 24, 48 h). The mRNA levels of miR-183 in foam cells were determined by RT-qPCR analyses. C and D, The mRNA and protein levels of ABCA1 were determined by RT-qPCR and western blot assays in THP-1 macrophage-derived foam cells treated with IL-8, alone or in combination with 40 nM scrambled miR-183 or 40 nM miR-183 inhibitor, respectively. E, ApoA1-specific cholesterol efflux was determined after THP-1 macrophage-derived foam cells treated with IL-8, alone or in combination with 40 nM scrambled miR-183 or 40 nM miR-183 inhibitor, respectively. All results are expressed as mean ± S.D. from three independent experiments (each performed in triplicate). $^{*}P < 0.05$ vs. control group, $^{\#}P < 0.05$ vs. control group or IL-8 group. Con, control; Scr, scrambled miR-183; Inh, miR-183 inhibitor.

scrambled miR-183 significantly decreased the mRNA and protein levels of ABCA1 (Fig. 4C and D) and cholesterol efflux (Fig. 4E), whereas treatment with IL-8 and miR-183 inhibitor abrogated IL-8-induced inhibition of ABCA1 expression (Fig. 4C and D) and cholesterol efflux (Fig. 4E). The similar results were obtained in ox-LDL-induced foam cells (Fig. S2).

To further determined the effects of anti-IL-8 antibody on ABCA1 expression, miR-183 expression and cholesterol efflux in THP-1 macrophage-derived foam cells, cells were treated with anti-IL-8 antibody or isotype control antibody for 24 h. As expected, anti-IL-8 antibody treatment markedly increased ABCA1 mRNA and protein levels (Fig. 5A and B) and cholesterol efflux (Fig. 5D), whereas significantly decreased miR-183 levels (Fig. 5C). Together, these results showed that miR-183 plays an important role in IL-8-induced suppression of ABCA1 expression and cholesterol efflux.

4. Discussion

Recent extensive studies have demonstrated that accumulation of cholesterol-laden macrophages in the arterial wall is the hallmark of atherosclerosis [3,4,27]. In macrophages, dysregulation of cholesterol efflux is a key factor contributing to the excessive accumulation of cholesterol and then transformation into foam cells and death [27,28]. In the current study, we investigated the effects of IL-8 on macrophage cholesterol accumulation and the underlying molecular mechanisms. First, we found that IL-8 suppresses ABCA1 expression and cholesterol efflux to ApoA-1. Next, we found that miR-183 directly targets the ABCA1 3′UTR and negatively regulates ABCA1 expression in the THP-1-derived macrophages. More specifically, the negative regulation of ABCA1 by IL-8 was mediated partly by miR-183. Lastly, IL-8 inhibition enhanced ABCA1 expression and cholesterol efflux while decreased

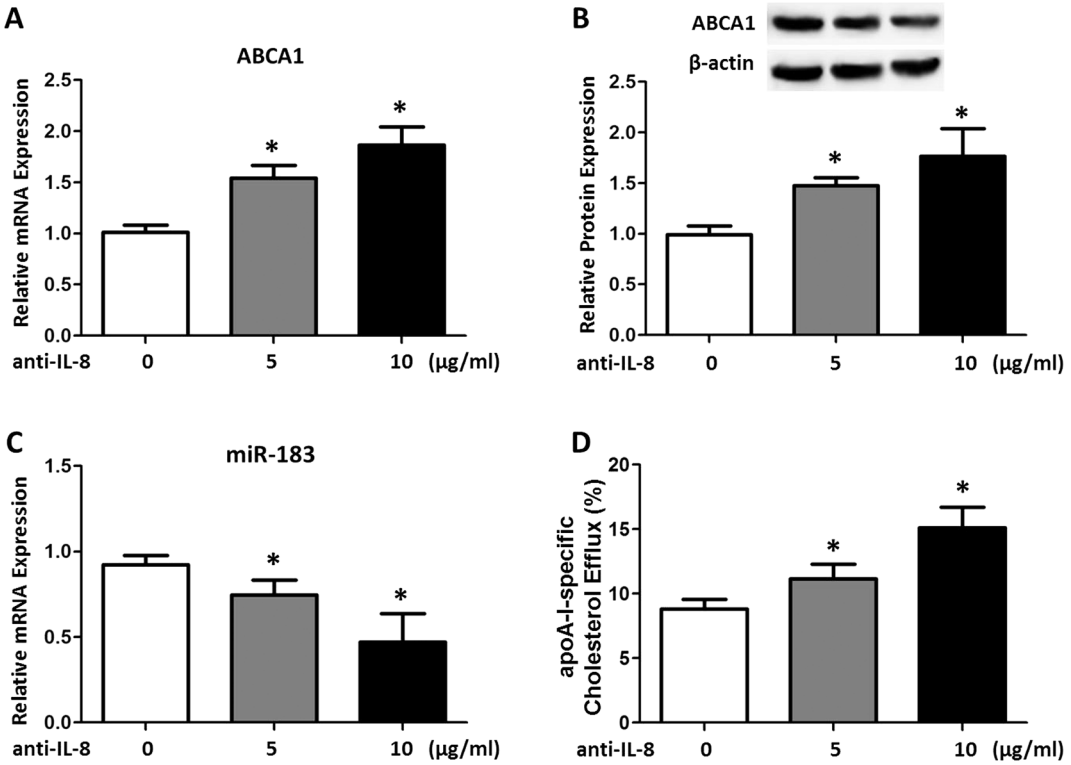

Fig. 5. Anti-IL-8 antibody treatment inhibits the expression of miR-183 and increases ABCA1 expression and cholesterol efflux. A, B, C and D, THP-1 macrophage-derived foam cells were treated with anti-IL-8 antibody (5, 10 μg/ml) or isotype control antibody (10 μg/ml) for 24 h. A, Total RNA was extracted and quantitative real-time PCR was performed to determine the ABCA1 mRNA expression. B, Western blot assays using antibodies against ABCA1 and β-actin were conducted. C, The mRNA levels of miR-183 in foam cells were determined by RT-qPCR analyses. D, ApoA1-specific cholesterol efflux was determined after THP-1 macrophage-derived foam cells treated with anti-IL-8 antibody. All results are expressed as mean ± S.D. from three independent experiments with each performed in triplicate. $^{*}P < 0.05$ vs. isotype control antibody group.

miR-183 expression. Our results have provided convincing evidence that IL-8 inhibits ABCA1 expression and cholesterol efflux to ApoA-1 through promoting miR-183 expression, suggesting that IL-8-miR-183-ABCA1 axis may play an important role in the development of atherosclerosis.

There is growing evidence suggesting that IL-8 is involved in the pathogenesis of atherosclerosis. Plasma IL-8 levels are increased in patients with type 2 diabetes and associated with worse inflammatory and cardiometabolic profile [29]. IL-8 is an independent predictor of long-term all-cause mortality in patients with acute coronary syndrome [30]. It is a proinflammatory cytokine that is produced by major cell types involved in atherosclerosis, including endothelial cells, macrophages and vascular smooth muscle cells. Although virtually all nucleated cells can produce IL-8, the principal sources of IL-8 are typically monocytes and macrophages [31]. Macrophages have been identified as the main source of IL-8 in atherosclerotic plaques [32]. We found that IL-8 significantly inhabits ABCA1 expression at both mRNA and protein levels in THP-1-derived macrophages. This finding is consistent with the results from Chen's laboratory [14], in which IL-8 inhibition markedly enhances cholesterol efflux and ABCA1 expression. These results may partly explain the roles of IL-8 in occurrence and development of atherosclerosis.

Our study also revealed that miR-183 directly targets ABCA1 3′UTR and decreases its mRNA and protein levels in THP-1 macrophage-derived foam cells, suggesting the roles for miR-183 in regulating intracellular lipid homeostasis by targeting ABCA1 and the cholesterol efflux to ApoA-1. In addition to the regulatory role of miR-183 in ABCA1 expression in THP-1 macrophages, miR-183 was previously reported to regulate several other proteins involved in the regulation of cholesterol homeostasis. For instance, miR-183 increases osteoclastogenesis in bone marrow-derived macrophages by repressing heme oxygenase-1 (HO-1) [33]. Various studies have shown that HO-1 expression in macrophages inhibits the development of atherosclerosis [34–36]. Recent studies showed that overexpression of HO-1 significantly suppress total cholesterol and increases cholesterol efflux in macrophages [37,38]. Moreover, another study by Li et al.[39] showed that HO-1 expression is required for the induction of ABCA1 in the regulation of cholesterol homeostasis. These data suggest that HO-1 may be another miR-183 target gene that may be also involved in the development of atherosclerosis via regulating cholesterol homeostasis. In addition, miR-183 was found to inhibit the canonical Wnt/β-catenin signaling pathway and promote 3T3-L1 adipogenesis via targeting low-density lipoprotein receptor-related protein 6 (LRP6) [23]. Based on these studies, further research is necessary to investigate whether HO-1 and LRP6 is involved in the regulation of lipid homeostasis by miR-183.

Aside from miR-183, several other miRNAs have been identified as negative regulators of ABCA1 expression in THP-1 macrophage. For example, the conservative miR-19b binding site in ABCA1 3′UTR plays important roles in posttranscriptional repression of ABCA1 by miR-19b [10]. MiR-33a suppresses ABCA1 expression in THP-1 macrophages and decreases circulating HDL-C levels [40]. MiR-20a/b decreases ABCA1 expression, which, in turn, decreases cholesterol efflux and increases cholesterol content in THP-1 [41]. In addition, miR-27a/b affects the efflux, influx, esterification and hydrolysis of cellular cholesterol by regulating the expression of ABCA1, apoA1, lipoprotein lipase (LPL), CD36 and ACAT1 [42]. As all the foregoing miRNAs have been shown to downregulate ABCA1 expression in THP-1 macrophages, miRNA-based therapeutics targeting atherosclerosis should endeavor to simultaneously target multiple miRNAs in order maximize therapeutic efficacy.

The precise mechanisms by which IL-8 suppresses ABCA1 expression remain unclear. Suppression of miR-183 could not completely

reverse the ABCA1 expression suppressed by IL-8. One possible explanation is that other molecular components may participate in this process, such as the liver X receptorα (LXRα), which is an important nuclear receptor that regulates the expression of ABCA1. Chen et al. [14] have previously revealed that IL-8 inhibition enhances the expression of peroxisome proliferator activated γ (PPARγ), LXRα, and ABCA1, suggesting that IL-8 may inhibit cholesterol efflux through suppressing the PPARγ/LXRα/ABCA1 signaling pathway in THP-1 macrophages. In the current study, we detected the effect of miR-183 in the suppression of ABCA1 induced by IL-8. It should be noted that the differential mechanisms underlying the effects of IL-8 on ABCA1 expression and cellular cholesterol efflux will need further investigation.

Although this study clearly demonstrates that miR-183 negatively regulates ABCA1 expression and cholesterol efflux through directly targeting the ABCA1 3′ UTR, there have been several limitations to be considered. Firstly, we did not conduct animal model experiments to assess the in vivo effects of miR-183 on plasma HDL-c and LDL-c levels and arterial plaque size. Secondly, although we revealed that IL-8 treatment promotes miR-183 expression and suppresses ABCA1 expression in THP-1 macrophages, we did not examine the signal transduction mechanism(s) underlying this phenomenon. Thirdly, we did not examine the effects of miR-183 on the expression of other proteins that may also regulate ABCA1 expression. The future studies should be designed to address these limitations.

In summary, the present study demonstrates that the proinflammatory cytokine IL-8 can downregulate ABCA1 cholesterol transporter with an important consequence for cholesterol trafficking in vitro. MiR-183 involved in this process post-transcriptionally regulates the ABCA1. Our findings suggest that the IL-8-miR-183-ABCA1 axis may provide a way to develop the therapeutic and prevention approaches to atherosclerosis.

5. Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgements

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.cyto.2018.04.028.

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