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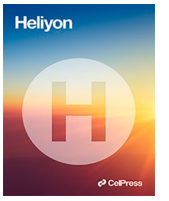

Research article

MiR-130a-3p inhibits endothelial inflammation by regulating the expression of MAPK8 in endothelial cells

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ABSTRACT

MicroRNA-130a-3p (miR-130a-3p) has been reported as closely related to atherosclerosis (AS). This study is to survey the effects of miR-130a-3p in endothelial cells (ECs) treated with oxidized low-density lipoprotein (ox-LDL) and explore underlying mechanisms. The proliferation and apoptosis of ox-LDL-treated HUVEC cells were determined by CCK-8, EdU, and flow cytometry assays. ELISA and Western blot analysis measured the expressions of cytokines and protein levels. Bioinformatics and dual-luciferase reporter assay were performed to predict and confirm that Mitogen-activated protein kinase 8 (MAPK8) was a direct target of miR-130a-3p, and MAPK8 was negatively associated with miR-130a-3p. As expected, miR-130a-3p was down-regulated in ox-LDL-treated HUVEC cells, and up-regulation of miR-130a-3p promoted proliferation and inhibited apoptosis of ox-LDL-treated HUVEC cells. Furthermore, miR-130a-3p mimics suppressed the expressions of TNF-α and IL-6 and decreased the protein levels of VCAM-1, ICAM-1 and E-selectin. MAPK8 was highly expressed in ox-LDL-treated HUVEC cells, and silence of MAPK8 promoted proliferation inhibited apoptosis, suppressed inflammatory responses, and decreased the levels of VCAM-1, ICAM-1, and E-selectin, over-expression of MAPK8 partially restored the functional effects of miR-130a-3p on proliferation, inflammatory responses, and the expressions of VCAM-1, ICAM-1 and E-selectin. This study indicates that miR-130a-3p may emerge as an effective target for treating AS.

1. Introduction

Cardiovascular disease is one of the most common diseases in China. It is also the main cause of disability and death [1]. The increasing morbidity and mortality of cardiovascular disease in China year by year seriously increases the medical burden of the people [2]. According to research statistics, the number of people who die of cardiovascular disease accounts for more than 40 % of the total death rate of residents in China every year, topping any other cause [3]. Atherosclerosis (AS) is the pathological basis of most cardiovascular diseases caused by endothelial cell (EC) dysfunction [4]. AS is the leading cause of acute coronary syndrome (ACS). ACS causes unstable angina pectoris (UAP) and acute myocardial infarction (AMI), which easily leads to sudden cardiac death (SCD). Therefore, the study of its pathogenesis is of great value to the prevention and treatment of AS and to improving patients' quality of life [5,6].

One of the characteristics of AS is plaque accumulation. It is a progressive, chronic inflammatory and immune disease. They are usually considered arterial wall diseases [7]. The pathological process of AS starts from endothelial dysfunction, lipid deposition,

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adhesion and aggregation of phagocytes and platelets, release of inflammatory factors, collagen decomposition, and gradual evolution into unstable plaques [8]. In this progress, vascular ECs and platelets play an essential role [9]. Endothelial dysfunction plays a vital role in the early stage of AS. It may be the starting point of plaque formation. It is characterized by the accumulation of monocytes and smooth muscle cells in the intima, the increase of vascular permeability, the easy infiltration of lipids into the vascular wall, the entry of monocytes and phagocytes, and the infiltration of inflammatory cells [10]. After exposure to subendothelial collagen tissues, platelet adhesion, aggregation, thrombosis, and inflammatory cell infiltration can be induced [11]. In addition, ECs mediate the regulation of vascular tension, the maintenance of blood flow, the regulation of inflammation and immune response, and neovascularization [12]. The integrity of vascular endothelium is the basis for maintaining blood vessels' normal regulatory function [11]. The destruction of vascular integrity leads to the accumulation of plasma, protein, and cells in the interstitial space, which leads to an inflammatory reaction, endothelial dysfunction, and vascular injury [13]. Therefore, maintaining normal endothelial function is of great significance in reducing the subsequent pathological changes and prevent the occurrence of AS.

Oxidized low-density lipoprotein (ox-LDL) deposition is also one of the characteristics of AS. Furthermore, Ox-LDL has been reported to increase in AS lesions and significantly improve the occurrence and development of AS [14]. Therefore, a study on HUVEC cells stimulated by ox-LDL could further clarify the pathogenesis of AS.

MicroRNA (miRNA) is a class of small non-coding RNA with a highly conserved evolutionary origin and about 18–24 bp in length [15]. They can bind to 3′- untranslated regions (UTR) to form silencing complexes, thus inhibiting the translation of messenger RNA (mRNA) or degrading mRNA [16]. In recent years, more and more studies have confirmed that miRNA is involved in the biological process of normal cells, such as cell growth, development, survival, differentiation, proliferation, and apoptosis [17]. Besides, miRNA exhibits a vital role in the pathological process of many diseases, including AS [18].

In the progress of AS, miRNA regulates the inflammatory response, cell proliferation, apoptosis, and lipid metabolism. For example, in the coronary heart disease rat model, miR-22 inhibits the inflammatory signaling pathway by targeting NLRP3 [19]. MiR-122 affects the formation of atherosclerotic plaque by regulating lipid metabolism [20]. At the same time, many studies have shown that miRNA participates in the pathological processes of AS by regulating the biological functions of ECs. Xu et al. find that miR-135 b-5p and miR-499a-3p promote the proliferation and migration of ECs by down-regulating the expression of MEF2C [21]. Li et al. show that miR-210 promotes the apoptosis of ECs via targeting PDK1 [22]. Kong et al. show that miR-130a-3p is downregulated in breast cancer [23]. MiR-130a-3p, as a member of the miR-130a family, has been reported to be close with AS [24]. However, the detailed effects and underlying mechanisms of miR-130a-3p on the functions of ECs in AS have not been clarified yet. Therefore, the present study was designed to explore whether miR-130a-3p exerted its functional role in ECs of AS and investigate the possible mechanisms.

2. Materials and methods

2.1. Bioinformatic analysis

Genes were searched for relevant genes for AS using Genecard datasets (https://www.genecards.org/). To screen out AS-related miRNA, AS related genes were subjected to enrichment analysis with miRTarBase database using g: Profiler (https://biit.cs.ut.ee/gprofiler/gost). PaGenBase in Metascape (https://metascape.org/) was used for enrichment analysis to screen HUVEC-related target genes.

2.2. Cell culture and transfection

ECs (HUVEC) were obtained from the American Type Culture Collection (ATCC, USA) and routinely cultured in Dulbecco's modified Eagle medium (DMEM, Thermo Fisher Scientific, Waltham, MA, USA), which was added 10 % fetal bovine serum (FBS, Gibco, NY, USA) in an incubator at 37 °C and 5 % CO_2. In addition, streptomycin (0.1 mg/mL) and penicillin (100 U/Ml) (Gibco, USA) were also added to the culture process.

MiR-130a-3p mimics, NC mimics, si-NC, si-MAPK8, pcDNA-NC, and pc-DNA-MAPK8 were provided by GeneScript (Nanjing, China), and lipofectamine 3000 (Invitrogen, USA) was used for HUVEC cell transfection. After 24 h transfection, the HUVEC cells were treated with 60 μg/mL ox-LDL for 24 h [25].

2.3. Cell proliferation analysis

CCK-8 (Sigma-Aldrich, USA) assay was carried out to detect the proliferation of HUVEC cells. Ox-LDL-treated HUVEC cells (1×10^3 cells per well) were seeded in the 96-well plates. Then 10 μL CCK-8 reagent was added and incubated for 45 min, and the absorbance was detected at 450 nm.

For the EdU assay, transfected ox-LDL-treated HUVEC cells were incubated with 10 μM EdU reagents for 2 h in the dark. Cells were then fixed in phosphate buffer saline (PBS, containing 4 % paraformaldehyde (PFA; Sigma-Aldrich), Thermo Fisher Scientific) for 15 min. Subsequently, 2 mg/mL glycine (Solarbio, Beijing, China) and 0.5 % Triton X-100 (Solarbio) were added to each well. After that, DAPI (Vector Laboratories) was added to each well. Then, cells were incubated in the dark for 30 min. Images were observed using a fluorescence microscope (Olympus IX 51 fluorescence microscope, Japan) [26].

2.4. Cell apoptosis analysis

For cell apoptosis, the transfected ox-LDL-treated HUVEC cells were harvested with trypsin, washed with PBS, and re-suspended in 500 μL binding buffer. Next, the cells were treated with 5 μL Annexin V-FITC and 10 μL PI for 15 min in the dark. Finally, a flow cytometer determined apoptotic cells (BD Biosciences, USA).

2.5. Enzyme-linked immunosorbent assay (ELISA)

According to the manufacturer's instructions, the concentrations of cytokines, including TNF-α and IL-6, collected from ox-LDL-treated HUVEC cells were examined by ELISA (Beyotime, Nanjing, China).

2.6. Target prediction and dual-luciferase reporter assay

MiRDB, RNA Society, LiENCORI, and TargetScan were performed to predict the targets of miR-130a-3p, and MAPK8 was identified as a potential target of miR-130a-3p. Then, 3′-UTR (Noncoding regions) encoding wild-type MAPK8 (MAPK8-WT) and mutant MAPK8 (MAPK8-MUT) was generated by inserting the amplified wild-type or mutant MAPK8 sequence into the psiCHECK™-2 luciferase plasmid (Promega, Madison, USA). Afterward, the miR-130a-3p mimic or miR-NC was co-transfected into HUVEC cells. Finally, the reporter mentioned the above vector using Lipofectamine 3000 (Genomeditech, China).

2.7. qPCR analysis

According to the Kit instructions, total RNA was isolated from HUVEC cells using TRIzol reagent (Leagene Biotechnology, China) and reversely transcribed to cDNA by HiScript III RT SuperMix (Vazyme, Nanjing, China) for qPCR. ChamQ SYBR Color qPCR Master Mix (Vazyme, Nanjing, China) was used to perform the qPCR analysis. We used the $2^{-\Delta\Delta Ct}$ method to determine the relative RNA expression and normalized RNA and target gene expression levels by U6 and GAPDH. The primer sequences used were as follows: miR-29 b-3p forward, 5′-TGCGGTAGCACCATTTGAA AT-3′ and reverse 5′-CCAGTGCAGGGTCCGAGGT-3'. miR-130a-3p forward, 5′-GATGCTCTCAG TGCAATGTTA-3′ and reverse, 5′-CTCTGTCTCTCGTCTTGTTGGTAT-3'. miR-199a-5p forward, 5′-GCCAAGCC-CAGTGTTCAGAC-3′ and reverse, 5′-GTGCAGGGTCCGAGGTATTC-3'. miR-9-5p forward, 5′-GAGGCAGACAGCCAGACA-3′ and reverse, 5′-CAAGGGTCCGAGGTGGGT-3'. miR-29a-3p forward, 5′-GCGGCGGTAGCACCATCTGAAAT-3′ and reverse, 5′-ATCCAGTGCAGG GTCCGAGG-3'. FGF2 forward, 5′-GTTGCTGTAGCCAAATTCGTTGT-3′ and reverse, 5′-CCAAA AGACGACGAGGGGTACAA-3'. MAPK8 forward, 5′-GGGCAGCCCTCTCCTTTA-3′ and reverse 5′-CATTGACAGACGACGATGATG-3'. VCL forward, 5′-G GAATTCAATG-GAGGGGATCAGTA TATACAC-3′ and reverse 5′-GGGGTACCAAGCTGGAGTGAAAATTGAAGACTCAGAC-3'. TJP1 forward, 5′-CGGGCCAGCAACAAAGTG-3′ and reverse, 5′-AGTGTAAGGACCCATCGGAG AA-3'. KLF4 forward, 5′-ATCTTTCTCCACGTTCGCGT-3′ and reverse, 5′-GGAAGTCGCTTCATG TGGGA-3'. RORA forward, 5′-TTCTTTCCCTACTGTTCGTTC A-3′ and reverse, 5′-CAGGTTTCC AGATGCGATTTAG-3'. U6 forward, 5′-GCTTCGGCAGCACATATACTAAAAT-3′ and reverse, 5′-CGCTTCACGAATTTGCGTGTCAT-3'. β-actin forward, 5′-AGAAGGCTGGGGCTCATTTG-3′, and reverse, 5′-AGGGGCCATCCACAGTCTTC-3'. Conditions for qRT-PCR were used: 95 °C for 10 min, 40 cycles of 95 °C for 15 s, and 60 °C for 1 min.

2.8. Western blotting assay

The cells were lysed using RIPA buffer (Beyotime Biotechnology, Shanghai, China). The lysate was added with a protease inhibitors cocktail (Sigma-Aldrich, USA) on the ice simultaneously. The vortex was used to mix the mixture for 20 min. Then the mixture was centrifuged at 12 000 g for 10 min. Then, the supernatant was collected and mixed with 5 × loading buffer (Elabscience, Wuhan, China) and denatured at 100 °C for 10 min. Then the samples were separated using SDS-PAGE for 2 h. Denatured and separated protein was transferred to PVDF membranes (Millipore, USA). Skim milk was used to block the PVDF membranes for 2 h at 37 °C. The primary antibodies including VCAM-1 (ab134074, 1: 1, 000), ICAM-1 (ab179707, 1: 1, 000), E-selectin (ab137732, 1: 1, 000), and β-actin (ab8226, 1: 1, 000) were added. Afterward, the membranes were washed using TBST and the secondary antibody for 2 h at 37 °C. At last, protein blots were observed by an enhanced chemiluminescence kit (ECL, Millipore, Bedford, MA, USA) and quantified using ImageJ software (NIH, version 4.3).

2.9. Statistical analysis

The data were displayed using the means ± standard deviation (SD), and all statistical analyses were analyzed by GraphPad Prism 6.0. Differences among groups were treated using Unpaired Student's *t*-test and One-way ANOVA unless otherwise indicated. The values of $P < 0.05$ were reputed as significant. Each experiment was repeated in triplicate.

3. Results

3.1. miR-130a-3p is down-regulated in ox-LDL-treated HUVEC cells

To explore the possible role of miRNA in AS. First, we used genecards (https://www.genecards.org/) to query the genes related to

A

ID	Source	Term ID	Term Name	padj (query_1)
1	MIRNA	MIRNA:hsa-miR-29b-3p	hsa-miR-29b-3p	2.234×10^{-3}
2	MIRNA	MIRNA:hsa-miR-130a-3p	hsa-miR-130a-3p	2.842×10^{-3}
3	MIRNA	MIRNA:hsa-miR-199a-5p	hsa-miR-199a-5p	3.929×10^{-3}
4	MIRNA	MIRNA:hsa-miR-9-5p	hsa-miR-9-5p	4.934×10^{-3}
5	MIRNA	MIRNA:hsa-miR-29a-3p	hsa-miR-29a-3p	1.825×10^{-2}

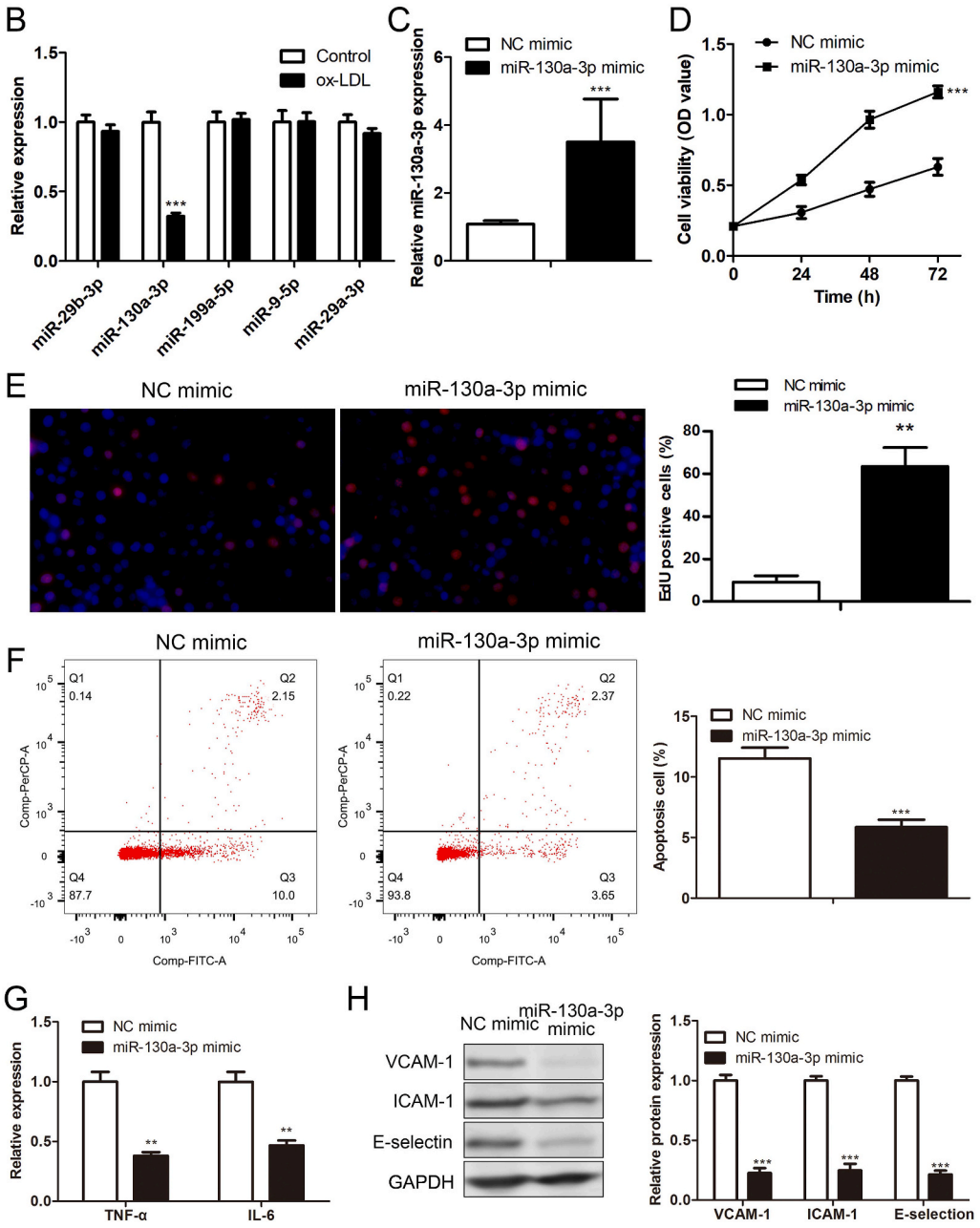

Fig. 1. Expression and effects of miR-130a-3p in ox LDL treated HUVEC cells. (A) Genecards and g: Profiler was performed to select appropriate miRNA related to AS. (B) The expressions of selected miRNA in ox-LDL-treated HUVEC cells were detected by the qRT-PCR assay. ***$P < 0.001$ *vs.* Control group. (C) The expression of ox-LDL-treated HUVEC cells transfected with miR-130a-3p mimic or NC mimic was assessed by qRT-PCR assay. (D) The proliferation of ox-LDL-treated HUVEC cells transfected with miR-130a-3p mimic or NC mimic was assessed by CCK-8 assay. (E) The ability of cell proliferation was examined by EdU assay. (F) The apoptosis of ox-LDL-treated HUVEC cells transfected with miR-130a-3p mimic or NC mimic was assessed by flow cytometer analysis. (G) The expressions of TNF-α and IL-6 in ox-LDL-treated HUVEC cells transfected with miR-130a-3p mimic

or NC mimic were assessed by ELISA assay. (H) The protein levels of VCAM-1, ICAM-1 and E-selectin in ox-LDL-treated HUVEC cells transfected with miR-130a-3p mimic or NC mimic were assessed by Western blot assay. **P < 0.01, ***P < 0.001 vs. NC mimic group. All data were presented as mean ± SD. n = 3.

AS. We then performed enrichment analysis with the g: profiler (https://biit.cs.ut.ee/gprofiler/gost) to screen the miRNA related to AS. As shown in Fig. 1A, a total of 5 miRNA was selected including miR-29 b-3p, miR-130a-3p, miR-199a-5p, and miR-29a-3p. The levels of miRNA in Ox-LDL-treated HUVEC cells were performed to evaluate by qRT-PCR. The data of Fig. 1B indicated that only miR-130a-3p was down-regulated in ox-LDL-treated HUVEC cells. These data suggested that miR-130a-3p might play an important role in AS.

3.2. Up-regulation of miR-130a-3p promotes proliferation and inhibits apoptosis of ox-LDL-treated HUVEC cells

To investigate the possible role of miR-130a-3p in AS, ox-LDL-treated HUVEC cells were transfected with miR-130a-3p mimics or

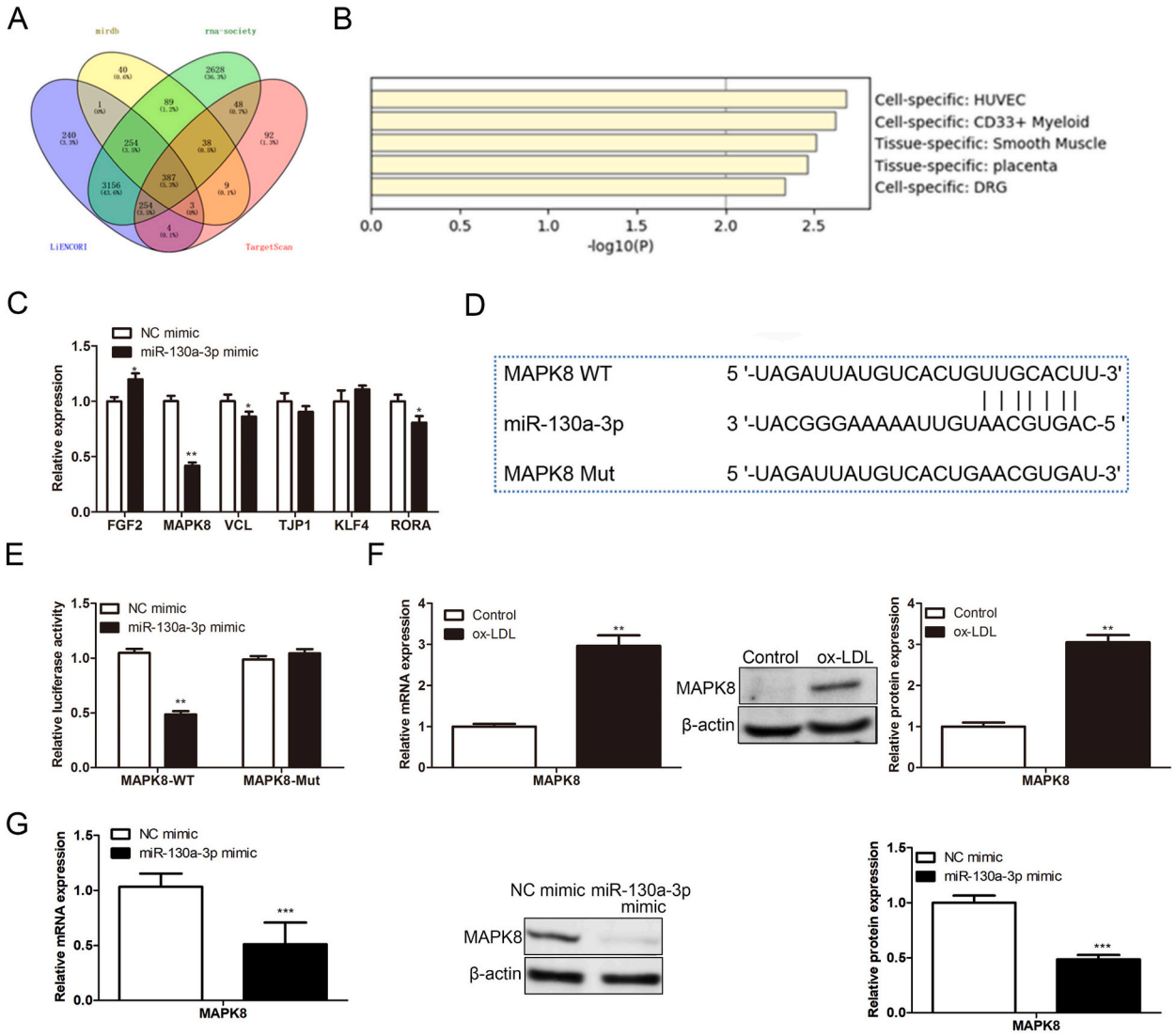

Fig. 2. MAPK8 is a direct target of miR-130a-3p. (A) miRDB, RNA Society, LiENCORI and TargetScan were performed to predict the targets. (B) PaGenBase was used to select the appropriate targets. (C) The expressions of selected targets in HUVEC cells transfected with miR-130a-3p mimic or NC mimic were assessed by qRT-PCR assay. (D) The binding sites between miR-130a-3p and MAPK8. (E) Dual-luciferase-reporter analysis was employed to validate the coactions between miR-130a-3p and MAPK8. (F) The mRNA and protein levels of MAPK8 in ox-LDL-treated HUVEC cells were detected by western blotting assay. (G) The protein levels of MAPK8 in ox-LDL-treated HUVEC cells transfected with miR-130a-3p mimic or NC mimic were assessed by Western blot assay. *P < 0.05, **P < 0.01 *vs.* NC mimic or Control group. All data were presented as mean ± SD. n = 3.

NC mimics. MiR-130a-3p was significantly upregulated in ox-LDL-treated HUVEC cells after transfection with miR-130a-3p mimics (Fig. 1C). CCK-8 assay was adopted to evaluate the role of miR-130a-3p in the proliferation of ox-LDL-treated HUVEC cells. Fig. 1D showed that compared with the NC mimics group, over-expression of miR-130a-3p promoted the proliferation of ox-LDL-treated HUVEC cells. This result has been verified in the EdU experiment assay (Fig. 1E). Then flow cytometer was performed to determine the effects of miR-130a-3p on the apoptosis of ox-LDL-treated HUVEC cells. The data of Fig. 1F displayed that relative to the NC mimics group miR-130a-3p mimics inhibited apoptosis of ox-LDL-treated HUVEC cells. These data suggested that up-regulation of miR-130a-3p promoted proliferation and inhibited apoptosis of ox-LDL-treated HUVEC cells.

3.3. Up-regulation of miR-130a-3p inhibits inflammatory responses of ox-LDL-treated HUVEC cells

In addition, an ELISA assay was carried out to assess the impact of miR-130a-3p on inflammatory responses of ox-LDL-treated HUVEC cells. As displayed in Fig. 1G, compared with the NC mimics group, up-regulation of miR-130a-3p significantly inhibited the expressions of TNF-α and IL-6. Besides, western blotting analysis was used to illustrate the role of miR-130a-3p in the expressions of VCAM-1, ICAM-1, and E-selectin. The data of Fig. 1H indicated that relative to the NC mimics group, miR-130a-3p mimics decreased the protein levels of VCAM-1, ICAM-1, and E-selectin. These data suggested that up-regulation of miR-130a-3p inhibited inflammatory responses of ox-LDL-treated HUVEC cells.

3.4. MAPK8 is a direct target of miR-130a-3p

To study the possible targets of miR-130a-3p involved in the occurrence of AS, miRDB, RNA Society, LiENCORI, and TargetScan were performed to predict the targets (Fig. 2A). We screened 6 highly correlated target genes, including FGF2, MAOK8, VCL, TJP1, KLF4, and RORA, using PaGenBase in Metascape (https://metascape.org/) [27] and analyzed them at the cell and tissue levels (Fig. 2B). Then qRT-PCR was performed to evaluate the expressions of selected targets in HUVEC cells transfected NC mimic and miR-130a-3p mimics. Fig. 2C showed that only MAPK8 was negatively associated with miR-130a-3p. Moreover, dual-luciferase reporter analysis was used to validate the interaction between miR-130a-3p and MAPK8. The upshots revealed that miR-130a-3p decreased the luciferase activity of the 3′-UTR of MAPK8 in HUVEC cells but did not affect the 3′-UTR of MAPK8 (Fig. 2D and E). Furthermore, a Western blot assay was used to assess the MAPK8 expression in ox-LDL-treated HUVEC cells. Fig. 2F showed that MAPK8 was up-regulated in ox-LDL-treated HUVEC cells. After transfection with miR-130a-3p mimics, the mRNA and protein levels of MAPK8 were significantly decreased in ox-LDL-treated HUVEC cells compared with the NC mimics group (Fig. 2G). These data

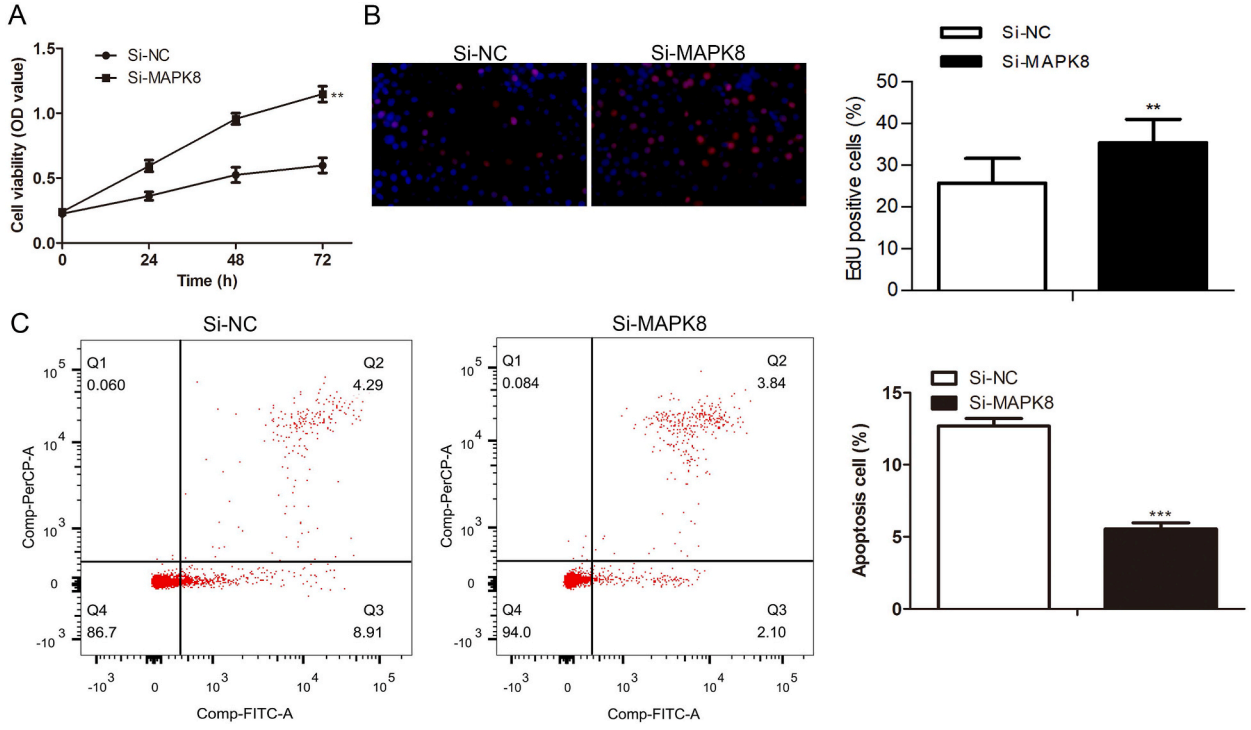

Fig. 3. Silence of MAPK8 promotes proliferation and inhibits apoptosis of ox-LDL-treated HUVEC cells. (A) The proliferation of ox-LDL-treated HUVEC cells transfected with si-MAPK8 or si-NC was assessed by CCK-8 assay. (B) The ability of cell proliferation was examined by EdU assay. (C) The apoptosis of ox-LDL-treated HUVEC cells transfected with si-MAPK8 or si-NC was assessed by flow cytometer analysis. $^{**}P < 0.01$ *vs.* si-NC group. All data were presented as mean ± SD. n = 3.

suggested that MAPK8 was a direct target of miR-130a-3p and was negatively associated with miR-130a-3p in AS.

3.5. Silence of MAPK8 promotes proliferation and inhibits apoptosis of ox-LDL-treated HUVEC cells

To explore the role of MAPK8 in ox-LDL-treated HUVEC cells, firstly, a CCK-8 assay was used to evaluate the role of MAPK8 in the proliferation of ox-LDL-treated HUVEC cells. The data of Fig. 3A showed that compared with the si-NC group, down-regulation of MAPK8 notably promoted the proliferation of ox-LDL-treated HUVEC cells. This result has been verified in the EdU assay (Fig. 3B). Then flow cytometer was performed to determine the effects of MAPK8 on the apoptosis of ox-LDL-treated HUVEC cells. The data of Fig. 3C displayed that relative to the si-NC group, the silence of MAPK8 inhibited apoptosis of ox-LDL-treated HUVEC cells. These data suggested that down-regulation of MAPK8 promoted proliferation and inhibited apoptosis of ox-LDL-treated HUVEC cells.

3.6. Silence of MAPK8 inhibits inflammatory responses of ox-LDL-treated HUVEC cells

In addition, an ELISA assay was carried out to assess the impact of MAPK8 on inflammatory responses of ox-LDL-treated HUVEC cells. As displayed in Fig. 4A, compared with the si-NC group, down-regulation of MAPK8 significantly inhibited the expressions of TNF-α and IL-6. Besides, western blotting analysis was also used to illustrate the role of MAPK8 in the expressions of VCAM-1, ICAM-1, and E-selectin. The data of Fig. 4B indicated that relative to the si-NC group, si-MAPK8 decreased VCAM-1, ICAM-1, and E-selectin protein levels. These data suggested that down-regulation of MAPK8 inhibited inflammatory responses of ox-LDL-treated HUVEC cells.

3.7. miR-130a-3p exhibits its role in AS via targeting MAPK8

To further investigate the effects of miR-130a-3p on AS, pcDNA-MAPK8 was transfected into ox-LDL-treated HUVEC cells transfected with miR-130 b mimics. Then CCK-8 assay was performed to evaluate the role of miR-130a-3p/MAPK8 in the proliferation of ox-LDL-treated HUVEC cells. The data of Fig. 5A showed that over-expression of MAPK8 partially restored the promoting effects of miR-130a-3p mimics on the proliferation of ox-LDL-treated HUVEC cells. Besides, an ELISA assay was used to determine the effects of miR-130a-3p/MAPK8 on inflammatory responses of ox-LDL-treated HUVEC cells. The data of Fig. 5B indicated that over-expression of MAPK8 partially restored the inhibitory effects of miR-130a-3p mimics on expressions of TNF-α and IL-6 of ox-LDL-treated HUVEC

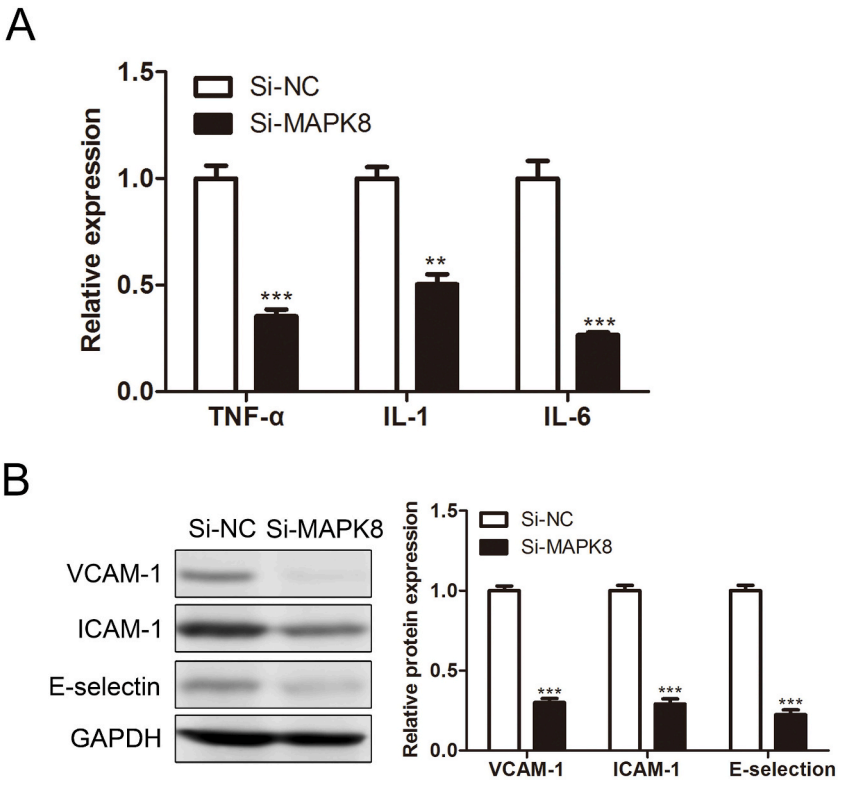

Fig. 4. Silence of MAPK8 inhibits inflammatory responses of ox-LDL-treated HUVEC cells. (A) The expressions of TNF-α and IL-6 in ox-LDL-treated HUVEC cells transfected with si-MAPK8 or si-NC were assessed by ELISA assay. (B) The protein levels of VCAM-1, ICAM-1 and E-selectin in ox-LDL-treated HUVEC cells transfected with si-MAPK8 or si-NC were assessed by Western blot assay. $^{**}P < 0.01$, $^{***}P < 0.001$ *vs.* si-NC group. All data were presented as mean ± SD. n = 3.

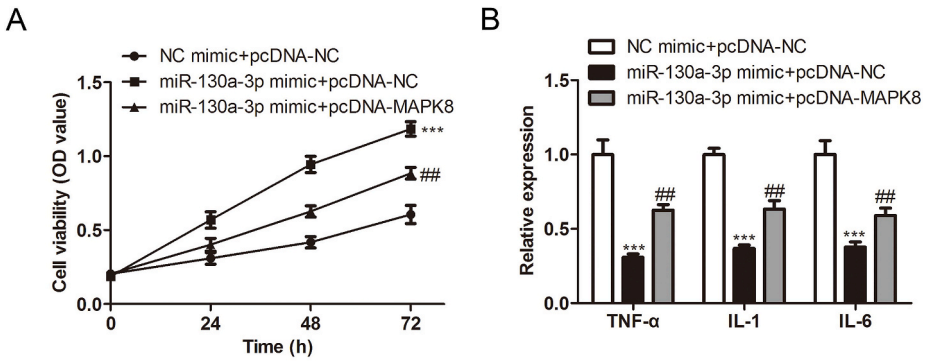

Fig. 5. MiR-130a-3p exhibits its role in AS via targeting MAPK8. (A) The proliferation of ox-LDL-treated HUVEC cells transfected with pcDNA-MAPK8 was assessed by CCK-8 assay. (B) The expressions of TNF-α and IL-6 in ox-LDL-treated HUVEC cells transfected with pcDNA-MAPK8 were assessed by ELISA assay. $^{**}P < 0.01$, $^{***}P < 0.001$ *vs.* NC mimic + pcDNA-NC group, $^{\#\#}P < 0.01$ *vs.* miR-130a-3p mimic + pcDNA-NC group. All data were presented as mean ± SD. n = 3.

cells. These data suggested that up-regulation of miR-130a-3p inhibited proliferation and inflammatory responses of ox-LDL-treated HUVEC cells via targeting MAPK8.

4. Discussion

As the leading cause of cardiovascular diseases such as peripheral vascular disease, myocardial infarction, ischemic stroke, and heart failure, AS has brought a major medical and socio-economic problem with high mortality and disability [28]. Studies have shown that damage to vascular endothelial integrity leading to dysfunction is the initial factor for the occurrence and development of AS [29]. Furthermore, endothelial integrity is mainly related to the proliferation, migration, and apoptosis of ECs [12]. Therefore, elucidating the mechanisms of regulating the biological function of ECs can provide a theoretical basis for clarifying the pathogenesis of AS. At present, the in-depth study of miRNA, provides a new direction for further understanding the development process of AS. Previous studies have shown that miR-130a-3p is involved in the pathogenesis of various diseases, including Crohn's disease, renal fibrosis, cancer [30]. Furthermore, it has been reported that miR-130a-3p is closely related to AS [24]. However, the biological role and potential mechanisms of miR-130a-3p in AS have not been fully studied.

This study showed that miR-130a-3p expression was down-regulated in ox-LDL-treated HUVECs. Based on these, we further explored the role of miR-130a-3p in AS. As expected, over-expression of miR-130a-3p notably promoted proliferation and inhibited inflammatory responses and apoptosis of ox-LDL-treated HUVECs.

Meanwhile, there is evidence that miRNA regulates the expression of nearly 30 % of protein-coding genes in the human genome [31]. Furthermore, studies have shown that mir-130a-3p can protect against Alzheimer's disease by targeting DAPK1 [32]. Mir-130a-3p can inhibit colorectal cancer growth by targeting WNT1. To further study the mechanisms of miR-130a-3p regulating EC function, bioinformatics software was used to predict the potential targets of miR-130a-3p. The predicted results showed a miR-130a-3p binding site in the 3′-UTR region of MAPK8. Furthermore, the results of dual-luciferase reporter assay, qRT-PCR, and Western blotting confirmed that MAPK8 was a direct target of miR-130a-3p and was negatively associated with miR-130a-3p.

Moreover, the knockdown of MAPK8 promoted proliferation and suppressed inflammatory responses and apoptosis of ox-LDL-treated HUVECs. Furthermore, over-expression of MAPK8 partially restored the functional effects of miR-130a-3p mimics on proliferation and inflammatory responses of ox-LDL-treated HUVECs. Thus, miR-130a-3p exhibited its functional role in AS by regulating the expression of MAPK8.

Mitogen-activated protein kinases (MAPKs) are a family of serine-threonine protein kinases that play essential regulatory roles in cells [33]. MAPK8, as a member of the MAPKs family, phosphorylates and activates the expression of a series of downstream genes. Exhibiting a critical regulatory role in cell proliferation, cell differentiation, cell survival, cell death, inflammation, and other pathological progress [34]. At present, the role of MPAK8 in AS has been widely discussed. In AS, MAPK8 is highly expressed, and its inhibition suppresses the proliferation and metastasis of ECs [35,36]. Moreover, many studies have shown that MAPK8 expression is regulated by cytokines in the pathological progress of AS [37,38]. Our data confirmed that miR-130a-3p negatively regulated MAPK8.

However, our experiments still have certain limitations. In our study, clinical samples were not used to explore the interaction between miR-130a-3p and MAPK8, which makes the experiment lack of evidence for human treatment; Moreover, it lacks in vivo experimental verification, which makes the conclusion lack of in vivo reliability. Further clinical samples and animal experimental studies are needed to verify the feasibility of miR-130a-3p in the treatment of AS.

5. Conclusion

MiR-130a-3p was lowly expressed in ox-LDL-treated HUVEC cells. The over-expression of miR-130a-3p notably promoted proliferation and inhibited inflammatory responses and apoptosis of ox-LDL-treated HUVEC cells accomplished by targeting MAPK8. This

experiment was the first time to explain the role of miR-130a-3p in AS. Regulation of miR-130a-3p and its downstream targets provided new treatment and diagnosis methods for AS.

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Ethics approval and consent to participate

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Availability of data and material

Data will be made available on request.

CRediT authorship contribution statement

Mingming Gu: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Kun Liu:** Visualization, Methodology, Investigation, Formal analysis, Data curation. **Hui Xiong:** Visualization, Methodology, Investigation, Formal analysis, Data curation. **Qingsheng You:** Writing – review & editing, Visualization, Validation, Supervision, Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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