

Original Article

The regulatory mechanism of miR-98-5p targeting ADAM15 on atherosclerotic plaque formation and vascular remodeling in mice with acute coronary syndrome through p38MAPK pathway

Rui Xue¹, Jiao Lei², Xueting Qiao¹, Yongping Liu³, Youlu Shen¹, Ming Ren¹, Ping Liu²

Departments of ¹Cardiology, ²Pharmacy, ³Traditional Chinese Medicine, QingHai University Affiliated Hospital, Xining, Qinghai Province, China

Received June 21, 2020; Accepted July 19, 2020; Epub October 15, 2020; Published October 30, 2020

Abstract: Objective: To investigate the regulatory mechanism of miR-98-5p targeting a disintegrin and metalloproteinase 15 (ADAM15) on the atherosclerotic plaque formation and vascular remodeling in mice with acute coronary syndrome (ACS) through the p38 mitogen-activated protein kinase (p38MAPK) pathway. Methods: The targeting relationship between miR-98-5p and ADAM15 was verified by dual-luciferase reporter assay. ACS mouse models were constructed. The mice were divided into the following groups: normal group, model group, miR-98-5p-NC group, miR-98-5p agomir group, ADAM15-NC group, si-ADAM15 group, miR-98-5p antagomir group and miR-98-5p antagomir + si-ADAM15 group. The serum inflammatory factors and vascular endothelial growth factor (VEGF) of the mice in each group were detected. The plaque stability in the mice with ACS was measured by intravascular ultrasound. Histopathological changes were examined by hematoxylin and eosin (H&E) staining. The expressions of miR-98-5p, ADAM15, p38MAPK, Bax, Fas and p53 in aortic vascular tissue were detected by quantitative real-time polymerase chain reaction (qRT-PCR) and Western blot. Results: ADAM15 was the target gene of miR-98-5p. Compared with those in the normal group, the expression of serum interleukin (IL)-6, tumor necrosis factor- α (TNF- α) and VEGF increased in the model group, while the expression of IL-10 decreased (all $P < 0.05$); the luminal area (LA), external elastic membrane area (EEMA), plaque area (PA) and plaque burden (PB) increased significantly (all $P < 0.05$); the expression of miR-98-5p decreased in aortic tissues, and the expression of messenger ribonucleic acid (mRNA) and protein levels of ADAM15, p38MAPK, Bax, Fas and p53 all increased significantly (all $P < 0.05$). The miR-132 mimics and si-PTEN groups had opposite results when comparing with those in the model group. The above indicators in the miR-98-5p agomir group and si-ADAM15 group significantly improved compared with those in the model group (all $P < 0.05$); however, the severity of above indicators in the miR-98-5p antagomir group increased when comparing with that in the model group (all $P < 0.05$). Conclusion: miR-98-5p can inhibit ADAM15 and the activation of p38MAPK signaling pathway, weaken the inflammatory response and plaque formation, and inhibit vascular remodeling. Thus, it plays a protective role in mice with ACS.

Keywords: miR-98-5p, ADAM15, p38MAPK, ACS, vulnerable plaque, vascular remodeling

Introduction

ACS is an acute myocardial ischemia syndrome caused by the rupture of coronary atherosclerosis plaques with superimposed thrombosis, causing sudden cardiac death, acute myocardial infarction, etc., and as such it is very harmful [1-3]. Current studies have found that inflammation is closely related to the occurrence of ACS [4].

miRNAs are a class of single-stranded non-coding RNA molecules that are involved in cell

proliferation, differentiation and apoptosis, as well as the negative regulation of gene transcription [5]. Studies have found that miRNA participates in the angiogenesis and the occurrence of cardiovascular diseases, suggesting that it may be a potential marker for the diagnosis and prognosis of cardiovascular diseases [6, 7]. Chen's study found that miR-98 participates in the occurrence and development of atherosclerosis (AS) and it postponed the progression of AS by inhibiting lectin-like oxidized-low-density-lipoprotein receptor-1

(LOX-1), suggesting that it may be a potential target for the treatment of AS [8].

As a type of membrane protein, ADAMs has the functions of hydrolyzing protein, cell adhesion, cell fusion and signal transduction, etc. Currently, more than 30 kinds of ADAMs have been found to participate in various physiological processes and the occurrence and development of diseases [9-11]. ADAM15 is the only disintegrin-metalloproteinase with the tripeptide sequence of arginine-glycine-aspartic acid in the ADAMs family, which is involved in the occurrence and development of inflammatory diseases [12]. Sun's study has found that ADAM15 is under-expressed in the blood vessels of normal mice, but it significantly increases at the injured vessel where AS occurs [13]. The p38MAPK pathway is a key pathway for signal transduction from extracellular to intracellular, and participates in multiple biological reactions such as transcription, inflammation, oxidative stress and apoptosis *in vivo* [14, 15]. Relevant studies have found that the ADAM15 disintegrin domain can inhibit the activity of p38 kinase, thereby regulating the proliferation of skin melanoma, revealing the possible relationship between ADAM15 and p38 [16]. In this study, bioinformatics tools were used to discover the existence of a targeted binding site in miR-98-5p and ADAM15, and a series of molecular biological methods were conducted to further explore the effect of the miR-98-5p/ADAM15 signaling pathway on the pathogenesis of ACS.

Materials and methods

Establishment of the ACS mouse model and grouping

The normal group consisted of 20, 6-week-old apolipoprotein E (ApoE) (-/-) mice (purchased from the Animal Experimental Center of Peking University) with no gender limitation and weighing (20.6±2.1) g. The experimental group consisted of 250 6-week-old ApoE (-/-) mice with no gender limitation and weighing (21.2±2.7) g. The mice in the experimental group were anesthetized by intraperitoneal injection of 60 mg/kg of pentobarbital sodium [17]. The mice were fixed on a horizontal on the operating table. The electrocardiogram electrodes were connected to the limbs and subcutane-

ous layer, and a ventilator was applied after endotracheal intubation. The chest cavity was opened up between the third and fourth intercostal space near the left side of sternum. The start of the anterior descending branch of the left coronary artery was found after cutting the pericardium open, one third of the middle coronary artery was ligated with 6-0 nylon suture (GL-885, Beijing Anbanghezong Supply Chain Management Co., Ltd., China) at about 3 mm from the aortic root, and the suture was performed after the thread was knotted. The electrocardiographic characteristic changes in myocardial infarction (arched ST-segment transient elevation in electrocardiogram (ECG) at the moment of ligation) were used as the basis of successful modeling. Follow-up experiments were conducted 3 hours after ligation. In this study, a total 223 mice were successfully modeled, with a success rate of 89.2%.

The mice with successful modeling in the experimental group were divided into 7 groups: 30 mice in the model group were injected with normal saline; 30 mice in the miR-98-5p-NC group with negative control of miR-98-5p; 30 mice in the miR-98-5p agomir group with miR-98-5p mimic; 30 mice in the miR-98-5p antagomir group with miR-98-5p inhibitor; 30 mice in the ADAM15-NC group with negative control of ADAM15; 30 mice in the si-ADAM15 group with si-ADAM15; 12 mice in the miR-98-5p antagomir + si-ADAM15 group with miR-98-5p inhibitor and si-ADAM15. The si-ADAM15 and negative control of ADAM15 were injected intraperitoneally at a dosage of 10 µL each time; the remaining reagents were injected via the tail vein at a dosage of 80 mg/kg each time. The mice were injected once a day and fasted overnight after continuous injection for 10 days. The mice were then executed by cervical dislocation under anesthesia, and the tissues were taken for the detection of various indicators. This study was approved by the Animal Research Ethics Committee of QingHai University Affiliated Hospital. Sequences of miR-98-5p agomir, miR-98-5p antagomir, si-ADAM15 and negative control plasmids were designed and synthesized by Shanghai Sangon Company (China). The remaining mice were euthanized under anesthesia.

miR-98-5p targets ADAM15 through p38MAPK

Verifying the targeting relationship between miR-98-5p and ADAM15 by dual-luciferase reporter assay

The target gene prediction of miR-98-5p was performed using Targetscan to verify if ADAM15 was a direct target gene of miR-98-5p, then the targeting relationship between miR-98-5p and ADAM15 was verified by dual-luciferase reporter assay. The predicted miR-98-5p binding site and the mutational binding site of ADAM15-3'UTR were cloned into the upstream of pmirGLO by double digests, named ADAM15-3'UTR-WT and ADAM15-3'UTR-MUT. The miR-98-5p mimic and NC were co-transfected into 293T cells with ADAM15-3'UTR-WT and ADAM15-3'UTR-MUT, respectively. The transfection procedure was performed according to the instructions of Lipofectamine™ 2000 (11668-019, Invitrogen, USA).

Cell cultures: The cells were inoculated into a 24-well cell culture plate, and 20 µL of complete medium with fetal bovine serum (SH300-68.03, Shanghai Yingxin Experimental Equipment Co., Ltd., China) and antibiotics (QN0075-VSN, Beijing Baiaolaibo Technology Co., Ltd., China) was added into each well. After incubating in a cell incubator at 37°C and 5% of CO₂ for 48 hours, the culture medium was discarded. Then, 40 µL of cell lysate (HR0271-AEL, Beijing Baiaolaibo Technology Co., Ltd., China) was added into each well, and the cells were collected after lysis. The luciferase activity was detected according to the instructions of dual-luciferase reporter assay gene kit (D0010, Beijing Solarbio Technology Co., Ltd., China) and with the application of a chemiluminescence detector (SMART 300, Wuhan Mingde Biotechnology Co., Ltd., China).

Enzyme-linked immunosorbent assay (ELISA)

The serum of 6 mice was diluted 100 times and inoculated into a 96-well plate. Three minutes later, the IL-6, IL-10, TNF-α and VEGF in serum were detected according to the instructions of IL-6 ELISA kit (ab100712, Abcam, UK), IL-10 ELISA kit (ab108870, Abcam, UK), TNF-α kit (ab208348, Abcam, UK) and VEGF ELISA kit (ab10075, Abcam, UK). The optical density (OD) of each well was measured by an enzyme-linked instrument (12454, Shandong Biobase biological Co., Ltd., China) at a wavelength of 450 nm.

Intravascular ultrasound (IVUS)

A 4F puncture needle was used to puncture the left femoral artery of 6 mice, and the 5F sheath tube was inserted along the guide wire and with the assistance of the expansion tube. A catheter-based probe with 2.5F and 40 MHz of IVUS (KD-00003, Shandong Kangda Medical Devices Co., Ltd.) was inserted into the distal thoracic aorta, and the probe was withdrawn at a rate of 0.5 mm/s. The intravascular plaque morphology was observed, the images of the distal and proximal plaques were marked, and the LA, PA, EEMA and PB were measured.

H&E staining

The aortae of 6 mice were harvested after opening up the chest cavity. The aorta of mice in each group was sliced up at a thickness of 4-5 µm after conventional paraffin embedding. After dewaxing by dimethylbenzene (5819096, Chongqing Tianyun Haocheng Chemical Co., Ltd., China), the slices were dehydrated in ethanol for 30 seconds at a concentration of 100%, 95%, 80% and 75%, respectively, then stained in hematoxylin (H8070, Beijing Solarbio Technology Co., Ltd., China) for 3 minutes. The residual dyeing solution was rinsed with running water. The slices were stained by Eosin (G1100, Beijing Solarbio Technology Co., Ltd., China) for 3 minutes and then rinsed by running water. After staining, the slices were dehydrated in alcohol for 30 seconds at a concentration of 100% and 95%, respectively. The slices were sealed with neutral balsam (G8590, Beijing Solarbio Technology Co., Ltd., China) after they were made transparent with dimethylbenzene. The thickness of aortic wall, vascular tissue structure, cell morphology and cellular arrangement were observed under an optical microscope (CX23, Olympus, Japan).

qPT-PCR

A total of 100 mg/mL of Trizol (10296028, Thermo Fisher, USA) was added to the aortic vascular tissues of 6 mice. After mixing up, the tissue suspension was shaken vigorously for 30 seconds and then let stand for 3 minutes. The precipitate RNA was extracted after centrifugation and the supernatant was discarded. RNA was fully dissolved by diethylpyrocarbonate (1609-47-8, Shanghai Jizhi Biochemical Technology Co., Ltd., China). The quality and

Table 1. Primers of qRT-PCR

Target gene	Primers
miR-98-5p	F: TGAGGTAGTAGTTTGTGCTGTT R: GCGAGCCACAGAATTAATACGAC
ADAM15	F: GCTGGCAGTGTCTCCTACCAGAGGGG R: GGTGCACCCAGCTGCAGTTCAGCTCAGTCC
p38MAPK	F: GCTCAGTAACAGTCCGCCTAGA R: GAGCAAGTCCAAAATCCA
Bax	F: TCCACCAAGAAGCTGAGCGAG R: GTCCAGCCCATGATGGTTCT
Fas	F: ACCCAGAATACCAAGTGCAG R: ATGTCTTCAAGTCCACACGAC
p53	F: ACGGTGACAACGCTTCCCTGGATTGG R: CTGTCACTGGGGAACAAGAAGTGGAG
GAPDH	F: TGCACCACCAACTGATTAG R: GATGCAGGGATGATGTTT
U6	F: CTCGCTTCGGCAGCACA R: AACGCTTCACGAATTTGCGT

Note: qRT-PCR: quantitative real-time polymerase chain reaction; ADAM15: a disintegrin and metalloproteinase 15; p38MAPK: p38 mitogen-activated protein kinase; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

concentration of the extracted RNA were measured by ultraviolet/visible light spectrophotometer (190100520, Shanghai Aoxi Scientific Instrument Co., Ltd., China) at the absorbance of 260 nm and 280 nm, respectively. Then, cDNA was synthesized using a reverse transcription (RT) kit (BPI01030, Beijing Huada Protein R&D Center Co., Ltd., China). After inactivating the reverse transcriptase at a temperature of 80°C for 5 minutes, the subsequent PCR reaction was performed. The reaction conditions and system were carried out according to the instructions of qRT-PCR kit (YB131042-25, Shanghai Yubo Biotechnology Co., Ltd., China). The internal reference of miR-98-5p was the U6 gene. The internal reference of ADAM15, p38MAPK, Bax, Fas and p53 was glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The primers were shown in **Table 1**. $2^{-\Delta\Delta Ct}$ refers to the relative expression of each target gene. $\Delta Ct = Ct \text{ target gene} - Ct \text{ internal reference gene}$, $\Delta\Delta Ct = \Delta Ct \text{ experimental group} - \Delta Ct \text{ control group}$.

Western blot

Pre-cooled tissue lysis buffer was added into the aortic vascular tissues of 6 mice for dissociation for 60 minutes. The samples were cen-

trifuged at a speed of 2,000 r/min for 30 minutes, then the protein concentration was detected with the use of Bicinchoninic acid (BCA) kit (ab102536, Abcam, UK). The proteins were isolated by polyacrylamide gel electrophoresis (SDS-PAGE) (M00655, GenScript, China). The samples were transferred to a polyvinylidene fluoride (PVDF) membrane by wetting transfer method, then blocked with tris buffered saline tween (TBST) containing 5% skim milk for 1 hours. The primary antibodies of ADAM15 (1:1,000) (ab194610, Abcam, UK), p-p38MAPK (1:2,000) (Shanghai Lianmai Bioengineering Co., Ltd., Shanghai, China), Bax (1:2000) (ab32503, Abcam, UK), Fas (1:1,000) (ab15285, Abcam, UK), p53 (1:1,000) (ab131442, Abcam, UK) and GAPDH (1:2,500) (ab9485, Abcam, UK) were added into the samples in sequence and incubated together at 4°C overnight. The samples were then washed 3 times with TBST, for 10 minutes each time, incubated with secondary antibody goat anti-rabbit IgG (1:1000) (ab-200699, Abcam, UK) for 1 hour at room temperature, and again washed 3 times with TBST, for 10 minutes each time. ECL development was conducted. GAPDH was used as internal reference and the relative expression of proteins was analyzed by Image J software.

Statistical analysis

SPSS 21.0 (SPSS, Inc, Chicago, IL, USA) was used for statistical analysis. The measurement data were expressed as mean $\pm$ standard deviation ($\bar{x} \pm sd$). One-way analysis of variance was used for the pairwise comparison among groups combined with the Bonferroni post-hoc test. The count data were expressed as percentage, chi-square test (χ^2) was used for comparison. $P < 0.05$ was considered statistically significant.

Results

miR-98-5p target inhibition of ADAM15

There were binding sites found between miR-98-5p and ADAM15 according to the bioinformatics software of Targetscan, see **Figure 1A**. The results showed that compared with co-transfection of mimic NC and ADAM15-3'UTR-WT, the luciferase activity in the plasmid co-

miR-98-5p targets ADAM15 through p38MAPK

A

	Predicted consequential pairing of target region (top) and miRNA (bottom)	Site type
Position 47-53 of ADAM15 3' UTR	5' ... UGCAGCAUCUUGGCUUACCUU... 3' UUGUUAUGUUGAAUGAUGAGU	7mer-m8
mmu-miR-98-5p		

B

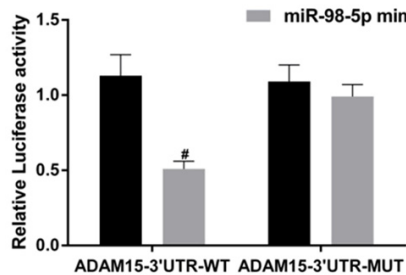


Figure 1. Verification of the targeting relationship between miR-98-5p and ADAM15 (n=3). Compared with the co-transfection of mimic NC and ADAM15-3'UTR-WT group, [#]P<0.05. A. Prediction results by Targetscan; B. Experimental results by dual-luciferase reporter assay. ADAM15: a disintegrin and metalloproteinase 15.

transfected with miR-98-5p mimic and ADAM15-3'UTR-WT was significantly inhibited (P<0.05), see **Figure 1B**. The results indicated that miR-98-5p directly acted on the predicted target site of ADAM15-3'UTR.

ELISA results

IL-6, IL-10, TNF- α and VEGF levels in mouse serum were detected by ELISA. Compared with the normal group, IL-6, TNF- α and VEGF levels increased in the model group, while the expression of IL-10 decreased (all P<0.05). Compared with the model group, IL-6, TNF- α and VEGF levels in the miR-98-5p agomir group and si-ADAM15 group decreased, while IL-10 level increased (all P<0.05). IL-6, TNF- α and VEGF levels in the miR-98-5p antagomir group increased, while IL-10 level decreased (all P<0.05). The indicators in the miR-98-5p-NC group and ADAM15-NC group had no statistically significant difference from those in the model group (all P>0.05). IL-6, TNF- α and VEGF levels in the si-ADAM15 group were reduced compared with those in the miR-98-5p antagomir + si-ADAM15 group, while IL-10 level increased (all P<0.05). IL-6, TNF- α and VEGF in the miR-98-5p antagomir group increased, while IL-10 level decreased (all P<0.05). See **Figure 2**.

IVUS results

The IVUS results showed that compared with the normal group, LA, EEMA and PA in the model group increased obviously, and PB was higher (all P<0.05). Compared with the model group, LA, EEMA, PA and PB in the miR-98-5p

agomir group and si-ADAM15 group were lower (all P<0.05); LA, EEMA and PA in the miR-98-5p antagomir group increased, and PB was higher (all P<0.05). The indicators in the miR-98-5p-NC group and ADAM15-NC group showed no significant difference from those in the model group (all P>0.05). Compared with the miR-98-5p antagomir + si-ADAM15 group, LA, EEMA and PA in the miR-98-5p antagomir group increased, and PB was higher (all P<0.05); LA,

EEMA and PA in the si-ADAM15 group decreased, and PB was lower (all P<0.05). See **Figure 3**.

Histopathological observation of tissue

H&E staining results showed a thinner aorta, smooth and orderly intima, clear vascular tissue structure, neatly arranged cells and complete cell morphology in the normal group; no obvious pathological changes were noted. The aortic intima in the model group was obviously thickened and irregular, and visible plaques were formed due to lipid deposition. Compared with the model group, the conditions in the miR-98-5p agomir group and si-ADAM15 group improved, with decreased plaque and thinner intima; in the miR-98-5p antagomir group, the plaques increased and the intima became thicker; the changes in the miR-98-5p-NC group and ADAM15-NC group were not significantly different from those in the model group. Compared with miR-98-5p antagomir + si-ADAM15 group, aortic intima in the miR-98-5p antagomir group had more plaques and thickened intima, while those in the si-ADAM15 group had fewer plaques and thinner intima. See **Figure 4**.

Overexpression of miR-98-5p or interference with ADAM15 could reduce the mRNA expressions of p38MAPK, Bax, Fas and p53

Compared with the normal group, the mRNA expressions of miR-98-5p in the model group decreased, whereas the mRNA expressions of ADAM15, p38MAPK, Bax, Fas and p53 all increased (all P<0.05). Compared with the model group, the mRNA expressions of ADAM15, p38MAPK, Bax, Fas and p53 in the miR-98-5p

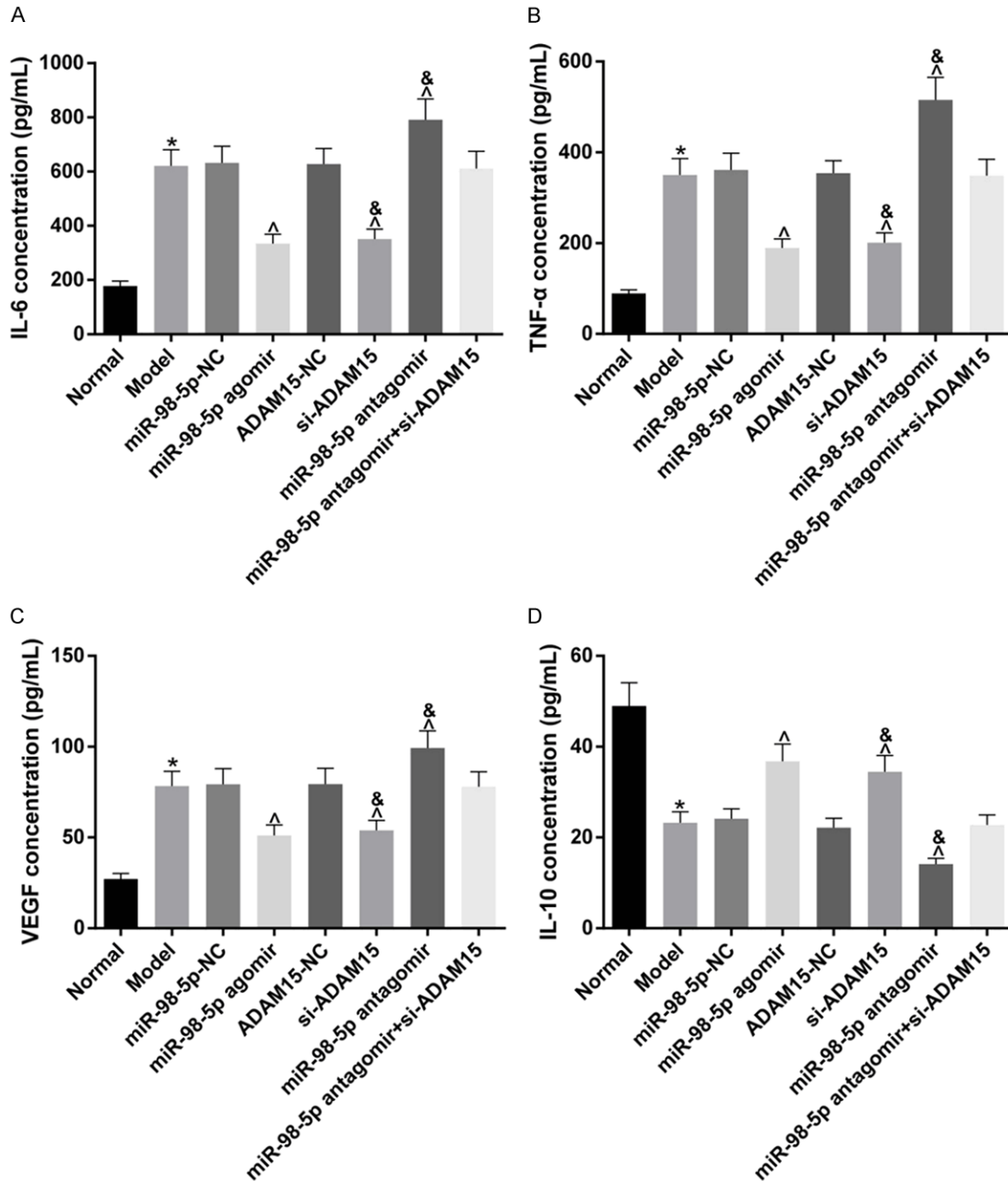


Figure 2. Results of ELISA (n=6). A. IL-6 level in mouse serum of each group; B. TNF-α level in mouse serum of each group; C. VEGF level in mouse serum of each group; D. IL-10 in mouse serum of each group. Compared with normal group, *P<0.05; compared with model group, ^P<0.05; compared with miR-98-5p antagonist + si-ADAM15 group, &P<0.05. ADAM15: a disintegrin and metalloproteinase 15; IL: interleukin; TNF-α: tumor necrosis factor-α; VEGF: vascular endothelial growth factor; ELISA: enzyme-linked immunosorbent assay.

agomir group and si-ADAM15 group were significantly reduced (all P<0.05); whereas the mRNA expressions in the miR-98-5p antagonist group significantly increased (all P<0.05). The indicators in the miR-98-5p-NC group and ADAM15-NC group were not significantly differ-

ent from those in the model group (all P>0.05). Compared with miR-98-5p antagonist + si-ADAM15 group, the mRNA expressions of ADAM15, p38MAPK, Bax, Fas and p53 increased in the miR-98-5p antagonist group (all P<0.05); the mRNA expressions of ADAM15,

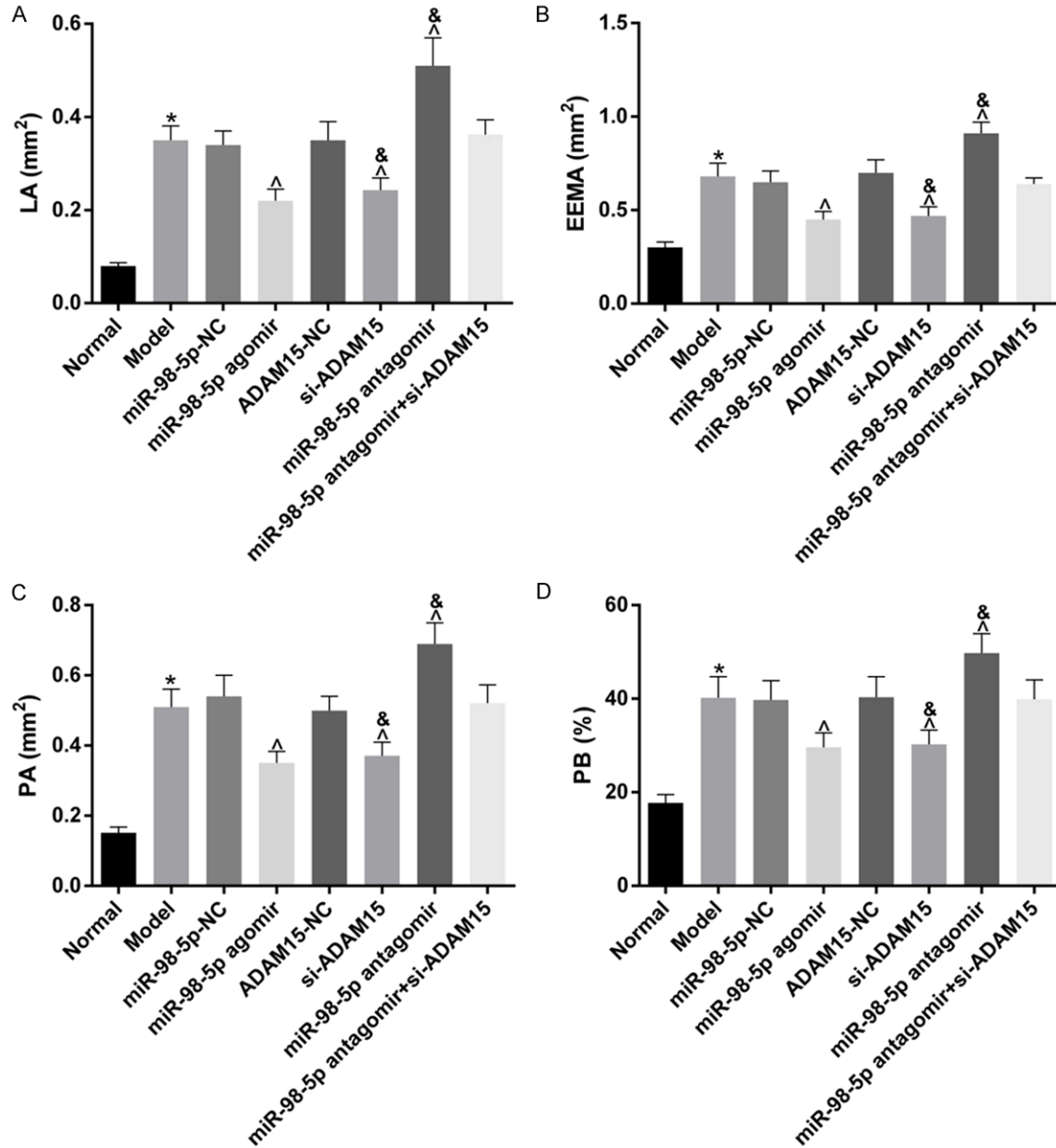


Figure 3. IVUS results in each group (n=6). A. Comparison of LA in each group; B. Comparison of EEMA in each group; C. Comparison of PA in each group; D. Comparison of PB in each group. Compared with normal group, *P<0.05; compared with model group, ^P<0.05; compared with miR-98-5p antagonist + si-ADAM15 group, &P<0.05. LA: luminal area; EEMA: external elastic membrane area; PA: plaque area; PB: plaque burden; ADAM15: a disintegrin and metalloproteinase 15.

p38MAPK, Bax, Fas and p53 in the si-ADAM15 group decreased (all P<0.05). See **Figure 5**.

Overexpression of miR-98-5p or interference with ADAM15 inhibited the p38MAPK phosphorylated protein, as well as the protein expression of Bax, Fas and p53

Compared with the normal group, the p38MAPK phosphorylated protein, as well as the pro-

tein expression of ADAM15, Bax, Fas and p53 in the model group all increased (all P<0.05). Compared with the model group, p38MAPK phosphorylated protein, as well as the protein expressions of ADAM15, Bax, Fas and p53 in the miR-98-5p agomir group and si-ADAM15 group were significantly reduced (all P<0.05); whereas the above indicators in the miR-98-5p antagonist group significantly increased (all P<0.05). The indicators in the miR-98-5p-NC

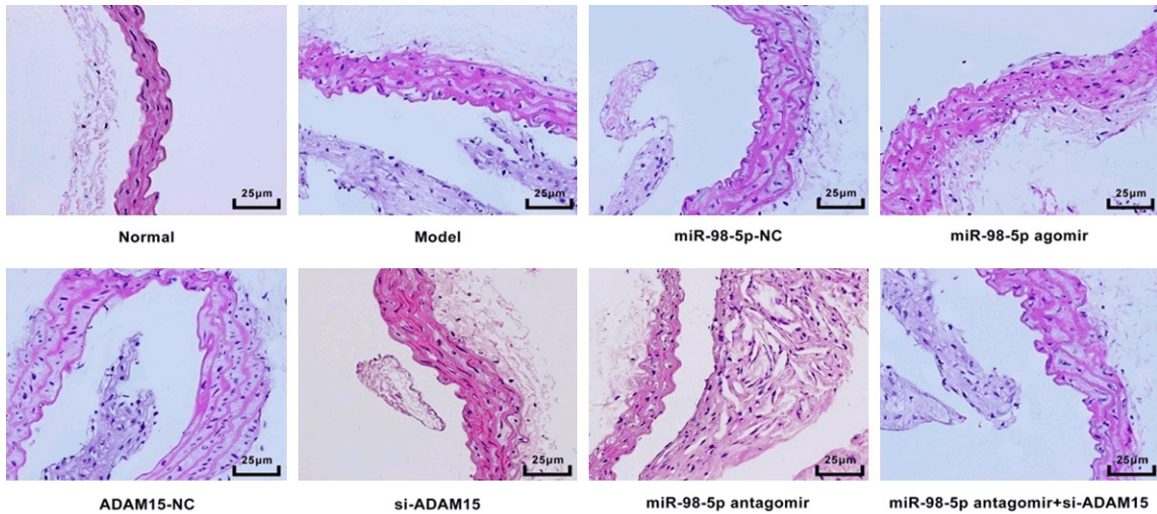


Figure 4. H&E staining results (400 $\times$, n=6). ADAM15: a disintegrin and metalloproteinase 15.

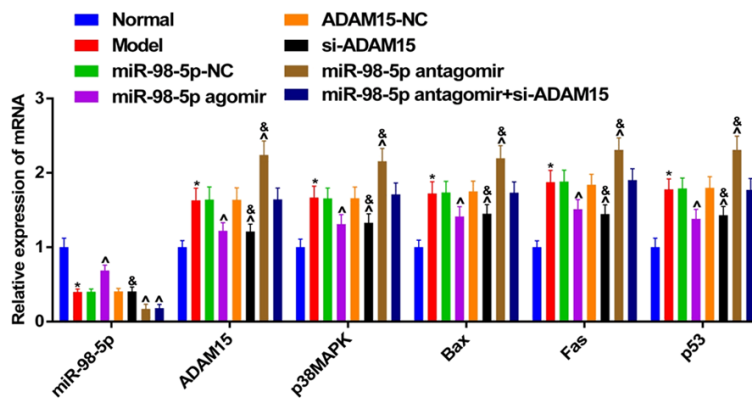


Figure 5. qRT-PCR results in each group (n=6). Compared with normal group, *P<0.05; compared with model group, ^P<0.05; compared with miR-98-5p antagonist + si-ADAM15 group, &P<0.05. qRT-PCR: quantitative real-time polymerase chain reaction; ADAM15: a disintegrin and metalloproteinase 15; p38MAPK: p38 mitogen-activated protein kinase.

group and ADAM15-NC group were not significantly different from those in the model group (all P>0.05). Compared with miR-98-5p antagonist + si-ADAM15 group, p38MAPK phosphorylated protein, as well as the protein expression of ADAM15, Bax, Fas and p53 increased in the miR-98-5p antagonist group (all P<0.05); whereas the above indicators decreased in the si-ADAM15 group (all P<0.05). See Figure 6.

Discussion

ACS is a syndrome caused by incomplete or complete coronary occlusion. The main cause of the disease is acute hemorrhage and th-

rombosis induced by plaque rupture. Recent studies have found that there are a large number of inflammatory cells in ACS plaques, and most drugs for the treatment of ACS mainly focus on anti-inflammatory effects [18-20]. In this study, it was found through the detection of inflammatory factors that the expression of serum inflammatory factors (IL-6, IL-10 and TNF- α) and VEGF in mice with ACS was abnormal, whereas the over-expression of miR-98-5p or silence of ADAM15 could effectively inhibit the expression of inflammatory factors. In

addition, when discussing the relationship between ADAM15 and retinal-related diseases, it was found that ADAM15 was positively correlated with VEGF expression, and VEGF expression was also inhibited after ADAM15 inhibition [21], indicating that the regulation of miR-98-5p/ADAM15 could exert the possibility of anti-inflammatory and angiogenesis regulation.

As one of many microRNAs, miR-98-5p is involved in the occurrence of various diseases, and its expression is abnormal in Alzheimer's disease and lung cancer [22-24]. Besides, Chen's study has confirmed that the overexpression of miR-98-5p can improve blood li-

miR-98-5p targets ADAM15 through p38MAPK

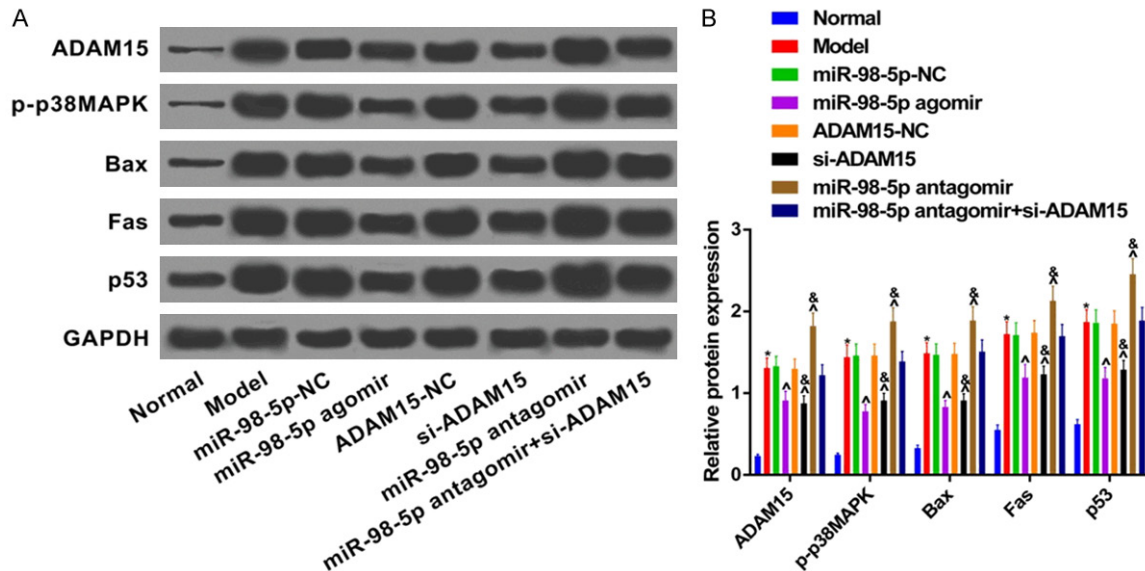


Figure 6. Western blot detection results in each group (n=6). A. Protein bands in each group; B. Quantification of protein expression in each group. Compared with normal group, *P<0.05; compared with model group, ^P<0.05; compared with miR-98-5p antagonist + si-ADAM15 group, &P<0.05. ADAM15: a disintegrin and metalloproteinase 15; p38MAPK: p38 mitogen-activated protein kinase; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

pids in mice with hyperlipidemia induced by high-fat diet and play a role in lowering blood lipids [25]. Other studies have also found that miR-98 is involved in the pathogenesis of AS [8]. This study further confirmed by qRT-PCR and Western blot that the expression of miR-98-5p decreased in mice with ACS, and it could regulate the downstream target genes of ADAM15 and p38MAPK pathway.

ADAM15, a member of ADAMs family, participates in the regulation of cell adhesion, degradation of extracellular matrix, influence of cell signaling and other important physiological processes. Studies have shown that the expression of ADAM15 in AS significantly increases [13, 26, 27]. As a member of the MARK family, p38MAPK is an important signal transduction pathway in cells, which can be activated by various factors such as pro-inflammatory cytokines, hyperlipidemia and hyperglycemia. The activated p38MAPK can promote the expression of inflammatory cytokines, thereby directly or indirectly leading to the production of inflammatory cytokines such as IL-1, TNF- α , etc. [28-31]. Kristian's study has found that inhibiting the expression of p38MAPK is conducive to the improvement of ACS [32]. In this study, through regulating the expression of related factors in mice with ACS, it was found that

the overexpression of miR-98-5p or silence of ADAM15 could promote the improvement of intravascular ultrasound-related indicators (LA, EEMA, PA, PB) in mice with ACS. In contrast, the experimental change was opposite by inhibition of miR-98-5p. The effect of miR-98-5p antagonist on ACS could be rescued by si-ADAM15.

Fas is a member of the TNF family, which exists on the surface of mammalian cells in the form of membrane molecules or soluble molecules and promotes cell apoptosis [33]. p53 inhibits cell proliferation by regulating the cell growth cycle, and is closely related to the stagnation of cell growth and apoptosis. Studies have proved that p53 can promote the apoptosis of vascular smooth muscle cells in AS [34]. In this study, the mRNA and protein expression of ADAM15, p38MAPK, Bax, Fas and p53 were significantly reduced after the overexpressing of miR-98-5p or silencing of ADAM15 by qRT-PCR and Western blot detection, further indicating that the effect of miR-98-5p/ADAM15 on the progress of ACS might be achieved by regulating the p38MAPK signaling pathway, while the effect of miR-98-5p antagonist on ACS could be rescued by si-ADAM15.

However, the effects of other miR-98-5p target proteins were not detected in this model. The

activity of p38MAPK was also not inhibited to further observe whether the correlations of miR-98-5p/ADAM15 depended on p38MAPK signal. The specific mechanism needs to be verified through more experiments, which is the focus of our subsequent research.

In summary, this study indicated that overexpression of miR-98-5p played a protective role in mice with ACS, which could inhibit the expression of ADAM15 and thus inhibit the activation of the p38MAPK pathway, thereby reducing the inflammatory response in mice with ACS, inhibiting plaque formation and vascular remodeling. With the continuous deepening of the research on the function of miR-98-5p, this study is believed to be of great significance in the prevention and treatment of ACS in combination with clinical and drug treatment.

Disclosure of conflict of interest

None.

Address correspondence to: Ping Liu, Department of Pharmacy, Qinghai University Affiliated Hospital, No.29 Tongren Road, Xining 810001, Qinghai Province, China. Tel: +86-13897261215; E-mail: liuping6q8h@163.com

References

- [1] Kvisvik B, Mørkrid L, Røsjø H, Cvancarova M, Rowe AD, Eek C, Bendz B, Edvardsen T and Gravning J. High-sensitivity troponin T vs I in acute coronary syndrome: prediction of significant coronary lesions and long-term prognosis. *Clin Chem* 2017; 63: 552-562.
- [2] Benson TW, Weintraub NL, Kim HW, Seigler N, Kumar S, Pye J, Horimatsu T, Pellenberg R, Stepp DW, Lucas R, Bogdanov VY, Litwin SE, Brittain JE and Harris RA. A single high-fat meal provokes pathological erythrocyte remodeling and increases myeloperoxidase levels: implications for acute coronary syndrome. *Lab Invest* 2018; 98: 1300-1310.
- [3] Badertscher P, Boeddinghaus J, Nestelberger T, Twerenbold R, Wildi K, Sabti Z, Puelacher C, Giménez MR, Pfäffli J, Flores D, du Fay de Lavallaz J, Miró O, Martin-Sanchez FJ, Morawiec B, Lohrmann J, Buser A, Keller DI, Geigy N, Reichlin T and Mueller C. Effect of acute coronary syndrome probability on diagnostic and prognostic performance of high-sensitivity cardiac troponin. *Clin Chem* 2018; 64: 515-525.
- [4] Cornara S, Crimi G, Somaschini A, Cattaneo M, Angiolillo DJ, Palmerini T and De Servi S. Systemic inflammatory status is associated with increased platelet reactivity in the early period after acute coronary syndromes. *Platelets* 2018; 29: 528-530.
- [5] Li SL, Sui Y, Sun J, Jiang TQ and Dong G. Identification of tumor suppressive role of microRNA-132 and its target gene in tumorigenesis of prostate cancer. *Int J Mol Med* 2018; 41: 2429-2433.
- [6] Xue MY and Cao HX. LINC01551 promotes metastasis of nasopharyngeal carcinoma through targeting microRNA-132-5p. *Eur Rev Med Pharmacol Sci* 2020; 24: 3724-3733.
- [7] Wang W, Li T, Gao L, Li Y, Sun Y and Yao HC. Diagnostic and prognostic impact of circulating microRNA-208b and microRNA-499 in patients with acute coronary syndrome. *Biomark Med* 2020; 14: 87-95.
- [8] Chen ZB, Wang M, He Q, Li ZL, Zhao Y, Wang WJ, Ma JY, Li YX and Chang GQ. MicroRNA-98 rescues proliferation and alleviates ox-LDL-induced apoptosis in HUVECs by targeting LOX-1. *Exp Ther Med* 2017; 13: 1702-1710.
- [9] Seo T, Sakon T, Nakazawa S, Nishioka A, Watanabe K, Matsumoto K, Akasaka M, Shioi N, Sawada H and Araki S. Haemorrhagic snake venom metalloproteases and human ADAMs cleave LRP5/6, which disrupts cell-cell adhesions in vitro and induces haemorrhage in vivo. *FEBS J* 2017; 284: 1657-1671.
- [10] Seipold L, Altmeyden H, Koudelka T, Tholey A, Kasperek P, Sedlacek R, Schweizer M, Bär B, Mikhaylova M, Glatzel M and Saftig P. In vivo regulation of the A disintegrin and metalloproteinase 10 (ADAM10) by the tetraspanin 15. *Cell Mol Life Sci* 2018; 75: 3251-3267.
- [11] Kelwick R, Desanlis I, Wheeler GN and Edwards DR. The ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) family. *Genome Biol* 2015; 16: 113.
- [12] Charrier-Hisamuddin L, Laboisie CL and Merlin D. ADAM-15: a metalloprotease that mediates inflammation. *FASEB J* 2008; 22: 641-653.
- [13] Sun CX, Wu MH, Lee ES and Yuan SY. A disintegrin and metalloproteinase 15 contributes to atherosclerosis by mediating endothelial barrier dysfunction via Src family kinase activity. *Arterioscler Thromb Vasc Biol* 2012; 32: 2444-2451.
- [14] Zhang WJ, Zha CJ, Lu XM, Jia RC, Gao F, Sun Q, Jin ML and Liu YH. Anti-β2GPI/β2GPI complexes induce platelet activation and promote thrombosis via p38MAPK: a pathway to targeted therapies. *Front Med* 2019; 13: 680-689.
- [15] Ren HM, Guo X, Wang XT and Cui ZH. Adenosine A2A receptor deficiency prevents p38-MAPK activation and apoptosis of mouse hippocampal cells in the chronic hypoxic-hyper-

- capnia model. *Biosci Biotechnol Biochem* 2019; 83: 1837-1842.
- [16] Zhou Y, Wang ZF, Li W, Hong H, Chen J, Tian Y and Liu ZY. Protective effects of microRNA-330 on amyloid β -protein production, oxidative stress, and mitochondrial dysfunction in Alzheimer's disease by targeting VAV1 via the MAPK signaling pathway. *J Cell Biochem* 2018; 119: 5437-5448.
- [17] Cozzi E, Hazarika S, Stallings HW 3rd, Cascio WE, Devlin RB, Lust RM, Wingard CJ and Van Scott MR. Ultrafine particulate matter exposure augments ischemia-reperfusion injury in mice. *Am J Physiol Heart Circ Physiol* 2006; 291: H894-H903.
- [18] Altaf A, Qu P, Zhao Y, Wang HY, Lou DY and Niu N. NLRP3 inflammasome in peripheral blood monocytes of acute coronary syndrome patients and its relationship with statins. *Coron Artery Dis* 2015; 26: 409-421.
- [19] Robertson S, Martínez GJ, Payet CA, Barraclough JY, Celermajor DS, Bursill C and Patel S. Colchicine therapy in acute coronary syndrome patients acts on caspase-1 to suppress NLRP3 inflammasome monocyte activation. *Clin Sci* 2016; 130: 1237-1246.
- [20] Sepehri ZS, Masoomi M, Ruzbehi F, Kiani Z, Nasiri AA, Kohan F, Fathollahi MS, Arababadi MK, Kennedy D and Asadikaram GA. Comparison of serum levels of IL-6, IL-8, TGF- β and TNF- α in coronary artery diseases, stable angina and participants with normal coronary artery. *Cell Mol Biol* 2018; 64: 1-6.
- [21] Xie B, Shen JK, Dong A, Swaim M, Hackett SF, Wyder L, Worpenberg S, Barbieri S and Campochiaro PA. An Adam15 amplification loop promotes vascular endothelial growth factor-induced ocular neovascularization. *FASEB J* 2008; 22: 2775-2783.
- [22] Li QS, Li XD, Wang L, Zhang YH and Chen L. miR-98-5p acts as a target for Alzheimer's disease by regulating $\alpha\beta$ production through modulating SNX6 expression. *J Mol Neurosci* 2016; 60: 413-420.
- [23] Tan L, Yu JT, Tan MS, Liu QY, Wang HF, Zhang W, Jiang T and Tan L. Genome-wide serum microRNA expression profiling identifies serum biomarkers for Alzheimer's disease. *J Alzheimers Dis* 2014; 40: 1017-1027.
- [24] Tang YF, Wu LJ, Zhao MJ, Zhao GD, Mao ST, Wang LL, Liu S and Wang XG. LncRNA SNHG4 promotes the proliferation, migration, invasiveness, and epithelial-mesenchymal transition of lung cancer cells by regulating. *Biochem Cell Biol* 2019; 97: 767-776.
- [25] Chen SY, Wen XA, Zhang WX, Wang C, Liu J and Liu C. Hypolipidemic effect of oleanolic acid is mediated by the miR-98-5p/PGC-1 β axis in high-fat diet-induced hyperlipidemic mice. *FASEB J* 2017; 31: 1085-1096.
- [26] Mattern J, Roghi CS, Hurtz M, Knäuper V, Edwards DR and Poghosyan Z. ADAM15 mediates upregulation of Claudin-1 expression in breast cancer cells. *Sci Rep* 2019; 9: 12540.
- [27] Janczi T, Böhm BB, Fehrl Y, DeGiacomo P, Kinne RW and Burkhardt H. ADAM15 in apoptosis resistance of synovial fibroblasts: converting Fas/CD95 death signals into the activation of prosurvival pathways by calmodulin recruitment. *Arthritis Rheumatol* 2019; 71: 63-72.
- [28] Zhao YG, Li W, Chen X, Dai JY, Tan Y and Cai L. SDF-1 β protects cardiac cells from high fat-induced fibrosis through its receptor cxc7-mediated AMPK/P38 MAPK activation and IL-6 excretion. *J Am Coll Cardiol* 2014; 63: A855.
- [29] Plastira I, Bernhart E, Joshi L, Koyani CN, Strohmaier H, Reicher H, Malle E and Sattler W. MAPK signaling determines lysophosphatidic acid (LPA)-induced inflammation in microglia. *J Neuroinflamm* 2020; 17: 127.
- [30] Li W, Wu Z, Ma QY, Liu JB, Xu QH, Han L, Duan WX, Lv YF, Wang FF, Reindl KM and Wu E. Hyperglycemia regulates TXNIP/TRX/ROS axis via p38 MAPK and ERK pathways in pancreatic cancer. *Curr Cancer Drug Targets* 2014; 14: 348-356.
- [31] Gee MS, Son SH, Jeon SH, Do J, Kim N, Ju Y, Lee SJ, Chung EK, Inn K, Kim N and Lee JK. A selective p38 α/β MAPK inhibitor alleviates neuropathology and cognitive impairment, and modulates microglia function in 5XFAD mouse. *Alzheimers Res Ther* 2020; 12: 45.
- [32] Kragholm K, Newby LK and Melloni C. Emerging treatment options to improve cardiovascular outcomes in patients with acute coronary syndrome: focus on losmapimod. *Drug Des Devel Ther* 2015; 9: 4279-4286.
- [33] Nistor GI and Dillman RO. Cytokine network analysis of immune responses before and after autologous dendritic cell and tumor cell vaccine immunotherapies in a randomized trial. *J Transl Med* 2020; 18: 176.
- [34] von der Thüsen JH, van Vlijmen BJ, Hoebe RC, Kockx MM, Havekes LM, van Berkel TJ and Biessen EA. Induction of atherosclerotic plaque rupture in apolipoprotein E-/- mice after adenovirus-mediated transfer of p53. *Circulation* 2002; 105: 2064-2070.