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Liraglutide improves atherosclerosis by regulating long non-coding RNA RMRP/ miR-128-1-5P/Gadd45g axis

J.-H. AN, Z.-Y. CHEN, Q.-L. MA, Y.-B. LI, F.-W. SHI

Department of Cardiac Surgery, Second Hospital Affiliated to Hebei Medical University, Shijiazhuang, Hebei Province, China

Abstract. – OBJECTIVE: Recent studies indicated that long non-coding RNA is involved in the formation of atherosclerosis, which is the pathological basis of coronary heart disease. Here, we reported the function and regulatory mechanism of RMRP in coronary atherosclerosis.

MATERIALS AND METHODS: qPCR was used to investigate the expression of IL-6, IL-8, RMRP, and miR-128-1-5P in coronary atherosclerosis and human vascular smooth muscle cells. Luciferase reporter assay confirmed the direct target effect of RMRP with miR-128-1-5P and miR-128-1-5P with Gadd45g on HEK293T. Western blot was used to detect protein expression in coronary atherosclerosis and human vascular smooth muscle cells.

RESULTS: RMRP expression and Gadd45g protein level were up-regulated in coronary atherosclerosis and human vascular smooth muscle cells, while miR-128-1-5P was down-regulated. RMRP downregulation remarkably inhibited the expression of IL-6, IL-8, and apoptosis related protein in human vascular smooth muscle cells after ox-LDL treatment. In addition, bioinformatics analysis and Luciferase report experiments confirmed that RMRP was the direct target of miR-128-1-5P. Moreover, miR-128-1-5P inhibitor reserved evidently the effect of IL-6, IL-8, and apoptosis related protein induced RMRP-si after treatment of human vascular smooth muscle cells with ox-LDL, implying RMRP negatively and directly regulated miR-128-1-5P in coronary atherosclerosis. More importantly, RMRP silencing increased Gadd45g protein level in human vascular smooth muscle cells. The same results were found when miR-128 was upregulated. Meanwhile, Gadd45g-si extremely reversed the result of IL-6, IL-8, and apoptosis related protein induced miR-128-1-5P inhibitor after treatment of human vascular smooth muscle cells with ox-LDL and Luciferase report experiments showed that Gadd45g was a direct target of miR-128-1-5P, implying Gadd45g negatively and directly regulated miR-128-1-5P in coronary atherosclerosis. Furthermore, liraglutide restrained evidently the expression of IL-6, IL-8, and apoptosis related protein in coronary atherosclerosis. After all, these results showed that liraglutide could regulate RMRP/miR-128-1-5P/ Gadd45g signal pathway to improve coronary atherosclerosis.

CONCLUSIONS: Liraglutide could curb the expression of inflammatory cytokines and apoptosis related protein in coronary atherosclerosis by regulating RMRP/miR-128-1-5P/Gadd45g signaling pathway, providing a new potential strategy for the treatment of coronary atherosclerosis.

Key Words:
Liraglutide, Gadd45g, Atherosclerosis, MiR-128-1-5P, LncRMRP.

Introduction

Atherosclerosis, the most common type in cardiovascular diseases, remains a chronic inflammatory reaction induced by genetic and environmental factors[1,2]. Inflammation is responsible for the formation of atherosclerotic plaque and intervention in the inflammatory response will delay atherosclerotic plaque formation and rupture[3,4]. The main causes of arterial wall thickening and sclerosis are the accumulation of lesions on the arterial wall, the change of sphenoidal type of vascular smooth muscle cells, the deposition of extracellular matrix, and the formation of fibrous cap[5]. The pathogenesis of atherosclerosis is related to the dysfunction or activation of various cells in the arterial wall under stress[6,7] and activated vascular smooth muscle cells play a vital role in the progression and stability of atherosclerotic plaques[8-10].

GLP-1 agonists have anti-inflammatory and anti-oxidative effects, mediate functional changes of monocytes, reduce macrophage infiltration, and subsequently inhibit pro-inflammatory path-

Corresponding Author: FengWu Shi, MD; e-mail: drjinghuian@protonmail.com

ways. In addition, GLP-1 agonists have beneficial effects on memory and direct neuroprotective effects on the central nervous system and their neuroprotective effects are independent from their glycemic effects[11-15]. Liraglutide, a long-acting synthetic GLP-1R agonist, was mainly used in the treatment of type 2 diabetes mellitus by regulating inflammatory cytokines, such as IL-6 and has beneficial effects on blood sugar homeostasis and weight control. However, the role of liraglutide in coronary atherosclerosis is unclear[16].

Several studies[17-22] have indicated that both miRNAs and lncRNAs play an important role in the occurrence and development of cardiovascular diseases. LncRNA HOTAIR regulates autophagy in ischemia/reperfusion injury through miR-20b-5P/ATG7 signal pathway[23]. Furthermore, authors showed that by regulating the function of vascular endothelial cells[24,25], miR-126-5P plays an anti-atherosclerosis role in promoting endothelial repair. LncRNA RMRP, a new RNA, was first found in hypoplasia of cartilage and hair. Zhou et al[26] showed that RMRP is the role of ceRNA as miR-613 in the regulation of HCC tumorigenesis; further, silence of RMRP improves OGD/R-induced neuronal damage by inhibiting autophagy and apoptotic pathways[27]. However, the biological role and molecular mechanism of RMRP in coronary atherosclerosis remain unclear.

The purpose of this study was to explore the mechanism of liraglutide-mediated RMRP/miR-128-1-5P/Gadd45g signaling pathway in coronary atherosclerosis to further understand the molecular mechanism of liraglutide, providing a novel perspective for the treatment of coronary atherosclerosis.

Methods and Methods

Animals

Wistar rats were purchased from Shanghai Jiake Biotechnology Co., Ltd. (Shanghai, China). This animal experiment was approved by the Faculty of Medicine's Ethics Committee of our hospital. Rats were randomly divided into four groups: (1) normal group and (2) model group; (3) negative control model group and (4) liraglutide model group. 0.09 mg/kg liraglutide (HY-P0014, Novo Alle, Kalundborg, Denmark) was injected subcutaneously into liraglutide model rats. The negative control group was given the same dose of saline. Liraglutide or saline was treated for 16 weeks. Coronary artery tissue was collected for detection.

Animal Models

A rat model of coronary atherosclerosis was established according to the Ganzetti et al model[8,9]. The rats in the model group were injected with vitamin D3 from the right lower limb 30 days before the establishment of the model, and fed with high-fat diet (formula: 0.2% propylthiouracil, 10% lard, 1.5% sodium cholate, 4% cholesterol, 84.3% basic diet) provided by Guangzhou Sai Bian Biotechnology Co., Ltd. (Guangzhou, China). After three and six weeks of feeding, 10% bovine serum albumin (BSA; 250 mg/kg) was injected into tail vein to induce immune injury. The control group was fed with normal diet. Rats were euthanized 15 weeks after feeding. Coronary artery plaque tissue was taken for cardiac examination in model group. The existence of plaque suggests that the model is successful.

Cell Culture and Treatment

Human vascular smooth muscle cells (CellBio, Shanghai, China) were cultured in Roswell Park Memorial Institute-1640 (RPMI-1640) medium (Invitrogen, Carlsbad, CA, USA) with 10% fetal bovine serum (FBS). Ox-LDL acts as a stimulator for cultured human vascular smooth muscle cells. The cells treated with ox-LDL at 80 μg/ml concentration (Yiyuan Biology, Guangzhou, China) were treated for 48 h. Cells without ox-LDL treatment were used as control group.

SiRNA Transfection

Small interfering RNA (siRNA) and negative control siRNA (NC-si) were designed and synthesized by RiboBio company (Guangzhou. China). According to the manufacturer's instructions, human vascular smooth muscle cells were transfected with lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) and opti-mem siRNA mixture for 4 h and then replaced with normal medium. After 48 h of treatment, the transfection efficiency was detected by Western blot.

Luciferase Reporter Assay

HEK293T cells inoculated on 96-well plate were co-transfected with 60 ng miR-128-1-5P plasmid or control plasmid with 100 ng RMRP (Gadd45g) 3'-UTR-WT or RMRP (Gadd45g) 3'-UTR-MUT (mutant miR-128-1-5P target in RMRP (Gadd45g) 3'-UTR) by lipofectamine 2000 (Invitrogen). After 48 h of treatment, cells were collected according to Dual-Luciferase assay kit (Promega, Madison, WI, USA). MiR-128-1-5P inhibitor, miR-128-1-5P mimic, plasmid, Luciferase structure, RMRP (Gadd45g) 3'-UTR or

3'-UTR-MUT were purchased from Gene Pharmaceutical Co., Ltd. (Shanghai, China).

Isolation of RNA and Real Time PCR

Total RNA was isolated from the prepared tissues and human vascular smooth muscle cells using TRIzol reagent (Cwbio, Beijing, China). RNA of each sample was used for reverse transcription and DNA synthesis by PrimeScript RT Kit (Perfect real time) (TaKaRa, Otsu, Shiga, Japan) following the manufacturer's information. qPCR was performed using iTaq™ universal SYBR®Green supermix (Bio-Rad, Hercules, CA, USA). GAPDH expression as internal control. The primer sequences involved were listed in Table I.

Western Blot Analysis

The cellular proteins were fractionated by 12% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) gel and transferred onto Immobilon® polyvinylidene difluoride (PVDF) membranes (Merck KGaA, Darmstadt, Germany). Blocked with 5% skimmed milk, the membranes were cultured overnight at 4°C with primary antibodies against caspase-3 (1:1000, Cell Signaling Tech., Danvers, MA, USA), cleaved-caspase3 (1:1000, Cell Signaling Tech), caspase-9 (1:1000, Cell Signaling Tech), cleaved-caspase9 (1:1000, Cell Signaling Tech), PARP (1:1000, Cell Signaling Tech), cleaved-PARP (1:1000, Cell Signaling Tech), Bax (1:1000, Cell Signaling Tech), Gadd45g (1:1000, Cell Signaling Tech). After incubation with horseradish peroxidase (HRP)-conjugated secondary antibodies (Goat anti-human IgG, Proteintech, Wuhan, Hebei, China), membranes were then developed using an enhanced chemiluminescent (ECL) detection kit (Cwbio, Beijing, China) and results were detected using the Gel Imaging System.

Statistical Analysis

The results were showed as mean±SD. Statistical analysis of the data was performed using Student's *t*-test and Bonferroni detection method was used to detect the comparison between two pairs after multiple comparisons. All analyses were completed using GraphPad Prism 6 (GraphPad Software, San Diego, CA, USA). $p < 0.05$ was considered statistically significant.

Results

RMRP Downregulation Distinctly Repressed the Expression of IL-6, IL-8, and Apoptosis Related Protein In Human Vascular Smooth Muscle Cells

LncRNA plays an important role in coronary atherosclerosis progression. Therefore, we detected the expression of RMRP in ox-LDL treated human vascular smooth muscle cells and coronary atherosclerosis. The experimental results showed that the expression of RMRP in coronary atherosclerosis and ox-LDL treated human vascular smooth muscle cells was up-regulated (Figure 1A-1B). Compared with the negative control group, the expression of RMRP in human vascular smooth muscle cells was clearly decreased by transfection with si-RMRP into vascular smooth muscle cells and the transfection efficiency of human vascular smooth muscle cells was detected by qPCR (Figure 1C).

Table I. Primers sequences used for qPCR.

Name	Sequences
RMRP-F	5'-ACTCCAAAGTCCGCCAAGA-3'
RMRP-R	5'-TGCGTAACTAGAGGGAGCTGAC-3'
IL-6-F	5'-CTCTCCGCAAGAGACTTCCA-3'
IL-6-R	5'-TGGTCTTCTGGAGTTCCGTT-3'
IL-8-F	5'-CTTTGTCCATTCCCACTTCTGA-3'
IL-8-R	5'-TCCCTAACGGTTGCCTTTGTAT-3'
miR-128 sense	5'-ACACTCCAGCTGGGTCACAGTGAACCGGTC-3'
miR-128 anti-sense	5'-TGGTGTCGTGGAGTCG-3'
U6 sense	5'-CTCGCT TCGGCAGCACA-3'
U6 anti-sense	5'-AACGCTTCACGAATTTGCGT-3'
GAPDH-F	5'-GATTCCACCCATGHCCAAATTC-3'
GAPDH-R	5'-CTGGAAGATGGTGATGGGATT-3'
Gadd45g-F	5'- AACGTCTTGCTGACATCGTG-3'
Gadd45g-R	5'- GCCCAATTTCTCAAGAGCAG-3'

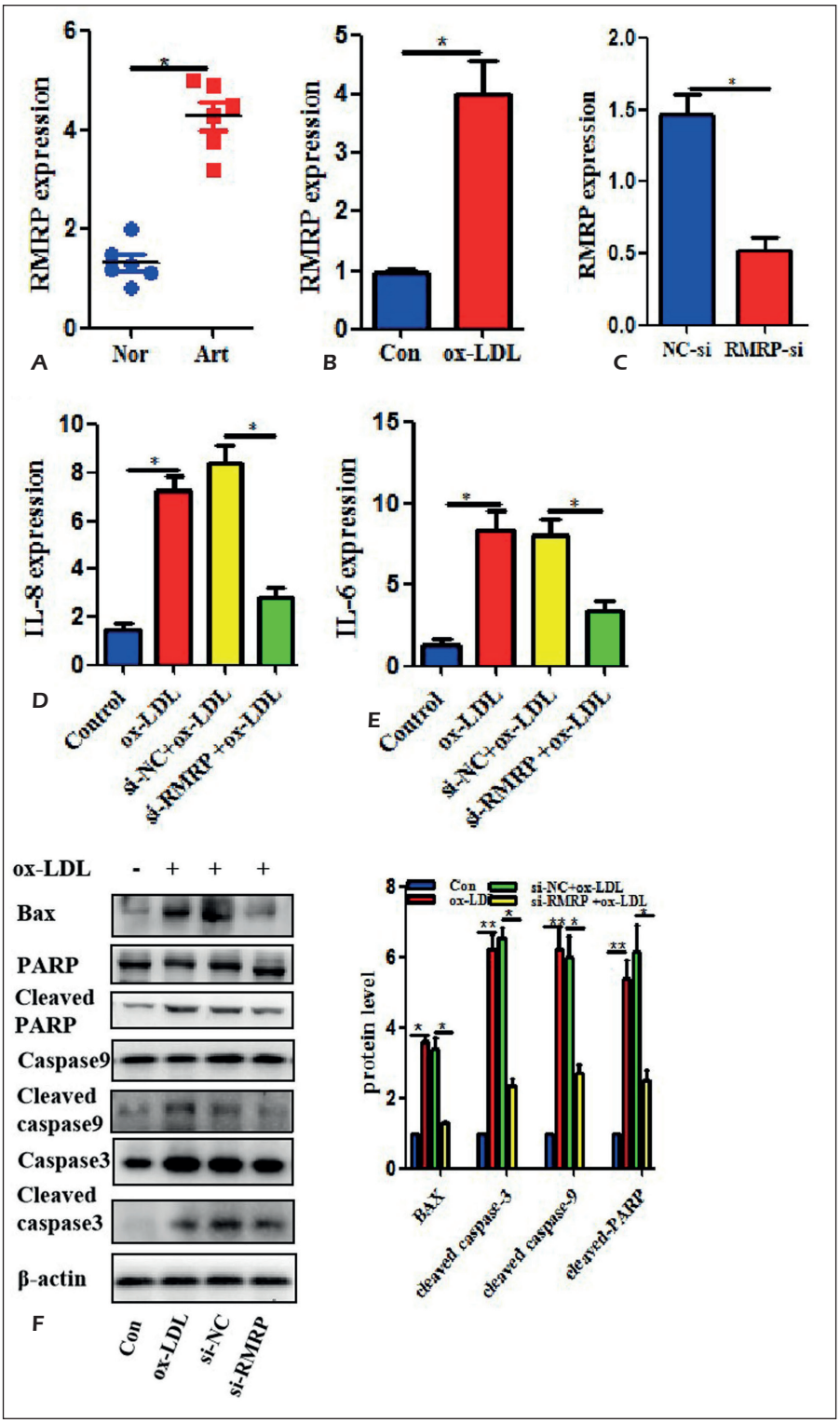

Figure 1. RMRP downregulation distinctly repressed the expression of IL-6, IL-8, and apoptosis related protein in human vascular smooth muscle cells. **A-B**, The mRNA expression level of RMRP was measured by qPCR in coronary artery tissues (n=6, *p<0.05) and human vascular smooth muscle cells treated with coronary ox-LDL. **C**, RMRP expression was measured by qPCR in human vascular smooth muscle cells. **D-E**, The RNA expression of IL-6 and IL-8 was checked using qPCR. **F,** Bax, cleaved-caspase3, cleaved-caspase9, and cleaved-PARP protein level after transfection with si-RMRP were tested by Western blot assay. The data are expressed as mean ± SD. n=3 *p<0.05.

Then, qPCR was used to detect the effect of RMRP dowregulation on the expression of IL-6 and IL-8 and apoptosis related protein of vascular smooth muscle cells. These results showed that RMRP was down-regulated in vascular smooth muscle cells treated with ox-LDL and coronary atherosclerosis and down-regulated RMRP in human vascular smooth muscle cells visibly repressed the expression of IL-6 and IL-8 (Figure 1D-1E) and the protein level of apoptosis related protein (Figure 1F).

RMRP Suppressed MiR-128-1-5P Expression by Direct Interaction

To explore the mechanism of RMRP in the progression of coronary atherosclerosis, the potential target miRNAs of RMRP were predicted by using miRanda, PITA and RNAhybrid databases. As a result, miR-128-1-5P was a potential regulator of RMRP expression. Potential target site of miR-128-1-5P interaction was rat RMRP 3'-UTR (Figure 2A). To verify whether miR-128-1-5P could directly target 3'-UTR of RMRP in a sequence-specific manner, we generated a Luciferase structure containing miR-128-1-5P potential binding site and produced a mutant structure with mutant potential target sites mutated (Figure 2B). The Dual-Luciferase report experiment showed that the over-expression of miR-128-1-5P significantly reduced the Luciferase activity of RMRP-WT reporter compared with miR-NC. Moreover, the Luciferase activity of RMRP-MUT reporter did not significantly decrease in HEK293T cells (Figure 2C), indicating miR-128-1-5P was a direct target of RMRP. We further confirmed the effect of RMRP on the expression of miR-128-1-5P in vascular smooth muscle cells. The expression of miR-128-1-5P in vascular smooth muscle cells transfected with si-RMRP prominently increased (Figure 2D). We further explored whether microRNA-128-1-5P was involved in coronary atherosclerosis and it was showed that the expression of miR-128-1-5P in human vascular smooth muscle cells treated with ox-LDL and coronary atherosclerosis tissues was down-regulated compared with the corresponding negative control (Figure 2E-2F). All in all, these results indicated that RMRP inhibited the expression of miR-128-1-5P through a direct interaction in coronary atherosclerosis.

MiR-128-1-5P Inhibitor Reversed the Effect of IL-6, IL-8, and Apoptosis Related Protein In Human Vascular Smooth Muscle Cells

In the above experiments, we examined the expression of miR-128-1-5P inhibitor on IL-6, IL-8, and apoptosis related protein expression of human vascular smooth muscle cells induced RMRP-si. Results showed that miR-128-1-5P inhibitor extremely reversed RMRP knockout effect of IL-6 and IL-8 (Figure 3A-3B), as well as the level of apoptosis related protein (Figure 3C) in human vascular smooth muscle cells after ox-LDL treatment. In general, these data showed RMRP negatively regulated miR-128-1-5P in coronary atherosclerosis.

MiR-128-1-5P Directly Suppressed Gadd45g In Human Vascular Smooth Muscle Cells

P38 signaling pathway is involved in the regulation of coronary atherosclerosis and Gadd45g is the key protein of P38 signaling pathway. Therefore, to further explore whether the role of miR-128-1-5P in coronary atherosclerosis was mediated by Gadd45g, miR-128-1-5P mimics and mimic NC were transfected into human vascular smooth muscle cells and Western blot was used to test the protein level of Gadd45g. Results showed that miR-128-1-5P mimic resulted in a significant decrease in Gadd45g mRNA expression and protein (Figure 4A-4C) in human vascular smooth muscle cells. Meanwhile, bioinformatics analysis and the Dual-Luciferase report experiment showed that Gadd45g was a potential target of miR-128-1-5P (Figure 4D-4E). In addition, the level of Gadd45g mRNA expression and protein in human vascular smooth muscle cells was sharply cut down by the decrease of RMRP (Figure 4F-4G). In all, Gadd45g was the target of miR-128-1-5P in vascular smooth muscle cells and RMRP plays a role in coronary atherosclerosis by possibly regulating miR-128-1-5P/Gadd45g signaling pathway.

Gadd45g Down-Regulation Reversed the Effect of IL-6, IL-8, and Apoptosis Related Protein Induced by MiR-128-1-5P Inhibitor In Human Vascular Smooth Muscle Cells

We investigated whether Gadd45g was involved in coronary atherosclerosis and its regulatory mechanism. Results showed that Gadd45g expression was up-regulated in atherosclerotic coronary artery and vascular smooth muscle cells treated with ox-LDL compared with the corresponding negative control group (Figure 5A-5B). The expression of Gadd45g in human vascular smooth muscle cells was evidently inhibited by si-Gadd45g and the transfection efficiency was detected by Western blot (Figure 5C). Then, we examined the expression of IL-6, IL-8 in vascular smooth muscle cells treated with ox-LDL and apoptosis related protein after co-transfection with miR-128-1-5P inhibitor and si-Gadd45g treatment. Results showed that down-regulated Gadd45g distinctly reversed the effect of IL-6, IL-8 (Figure 5D-5E), and apoptosis related protein induced miR-128-1-5P inhibitor (Figure 5F) in vascular smooth muscle cells treated with ox-LDL, implying miR-128-1-5P played a key role in atherosclerotic coronary artery by negative regulation of Gadd45g.

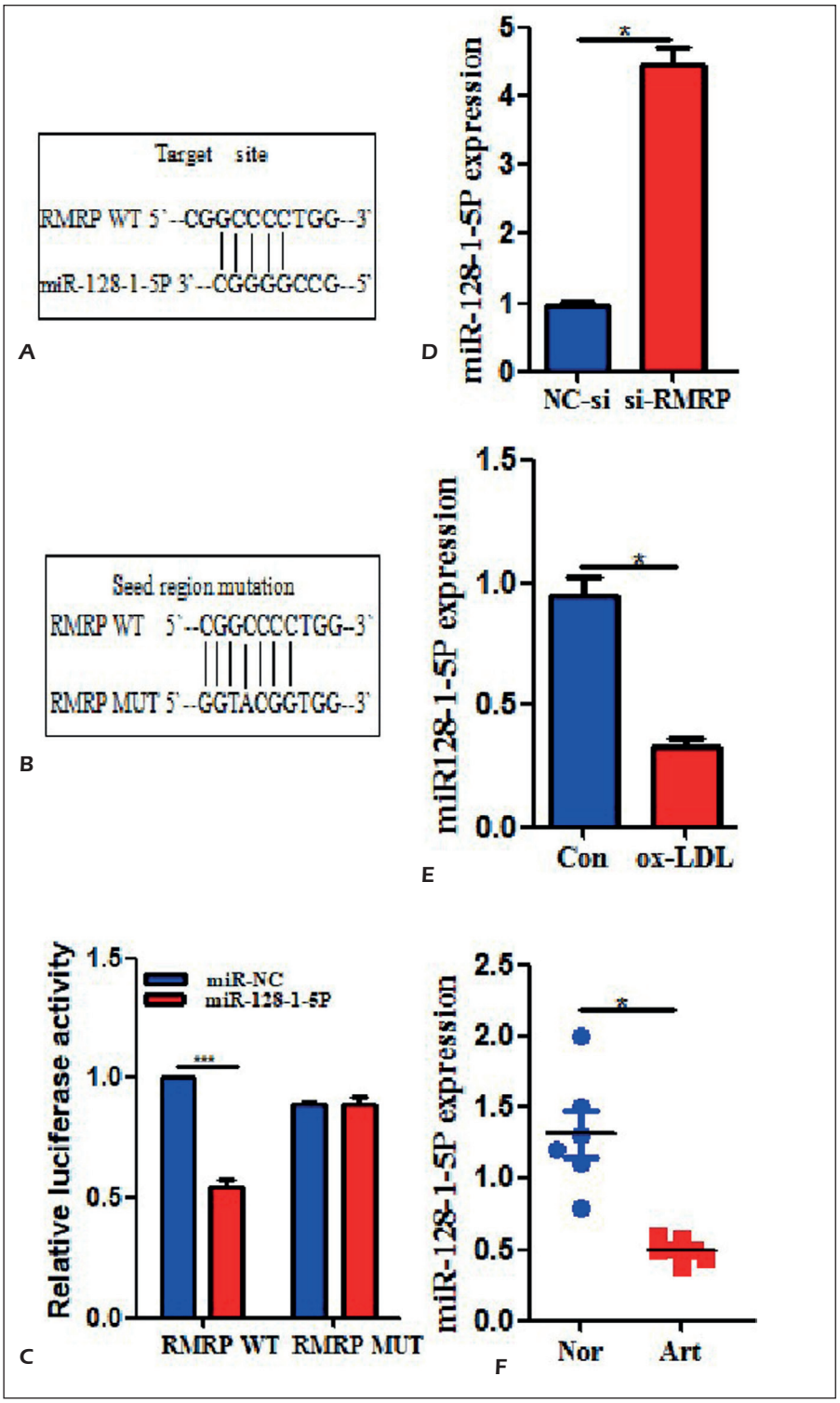

Figure 2. RMRP suppressed miR-128-1-5P expression by direct interaction. ***A***, Sequence alignment of miR-128 (miR-128-1-5P) and its target site in the 3'-UTR of RMRP. ***B***, Seed region of RMRP 3'-UTR was mutated as indicated. ***C***, Effect of miR-128 on the activity of RMRP-WT and RMRP-MUT reporter Luciferase in HEK293T cells was detected by Luciferase assay. ***D***, MiR-128 expression was determined after transfection with si-RMRP. ***E-F***, Expression levels of miR-128 were measured by qPCR in coronary artery tissues (n=6, *p<0.05) and human vascular smooth muscle cells after ox-LDL treatment. The data are expressed as mean ± SD. n=3. *p<0.05.

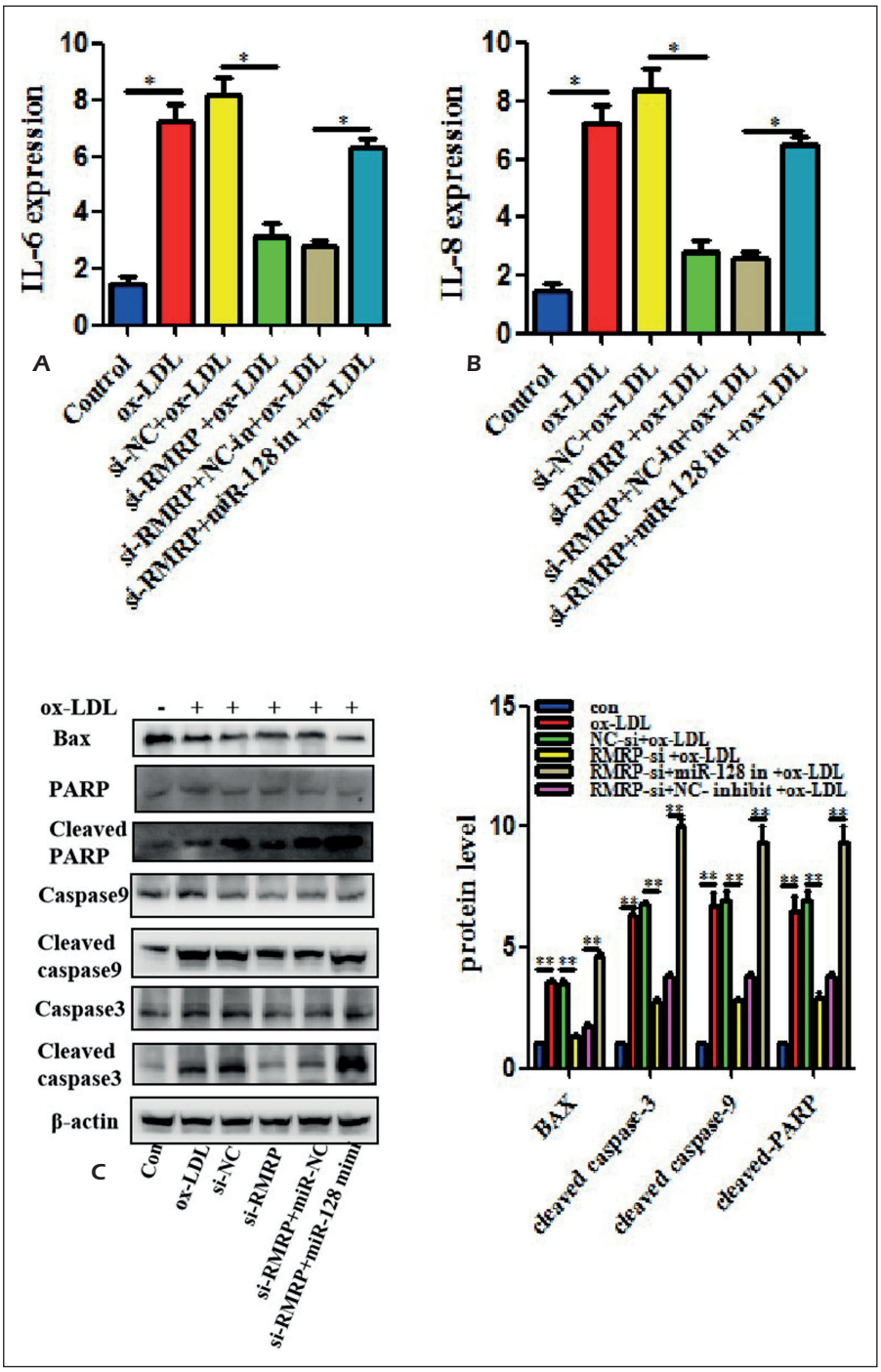

Figure 3. MiR-128-1-5P inhibitor reversed the effect of IL-6, IL-8, and apoptosis related protein in human vascular smooth muscle cells. **A**-**B**, Expression of IL-6 and IL-8 cells was detected by qPCR in human vascular smooth muscle cells. **C**, Bax, cleaved-caspase3, cleaved-caspase9, and cleaved-PARP protein level were detected by Western blot assay in human vascular smooth muscle cells. The data are expressed as mean ± SD. n=3. *p<0.05.

Liraglutide Blocked the Expression of IL-6, IL-8, and Apoptosis Via Regulating RMRP/MiRNA-128-1-5P/ Gadd45g In Coronary Atherosclerosis

According to reports, liraglutide has an anti-inflammatory effect and atherosclerosis is a progressive inflammatory disease. Therefore, the role of liraglutide in coronary atherosclerosis would be further explored. qPCR and Western blot were used to detect the expression of IL-6, IL-8, and apoptosis protein in coronary atherosclerosis after liraglutide treatment. The dates showed that compared with negative model group, liraglutide could significantly reduce the expression of IL-6, IL-8, and the level of apoptosis related protein (Figure 6A-6C).

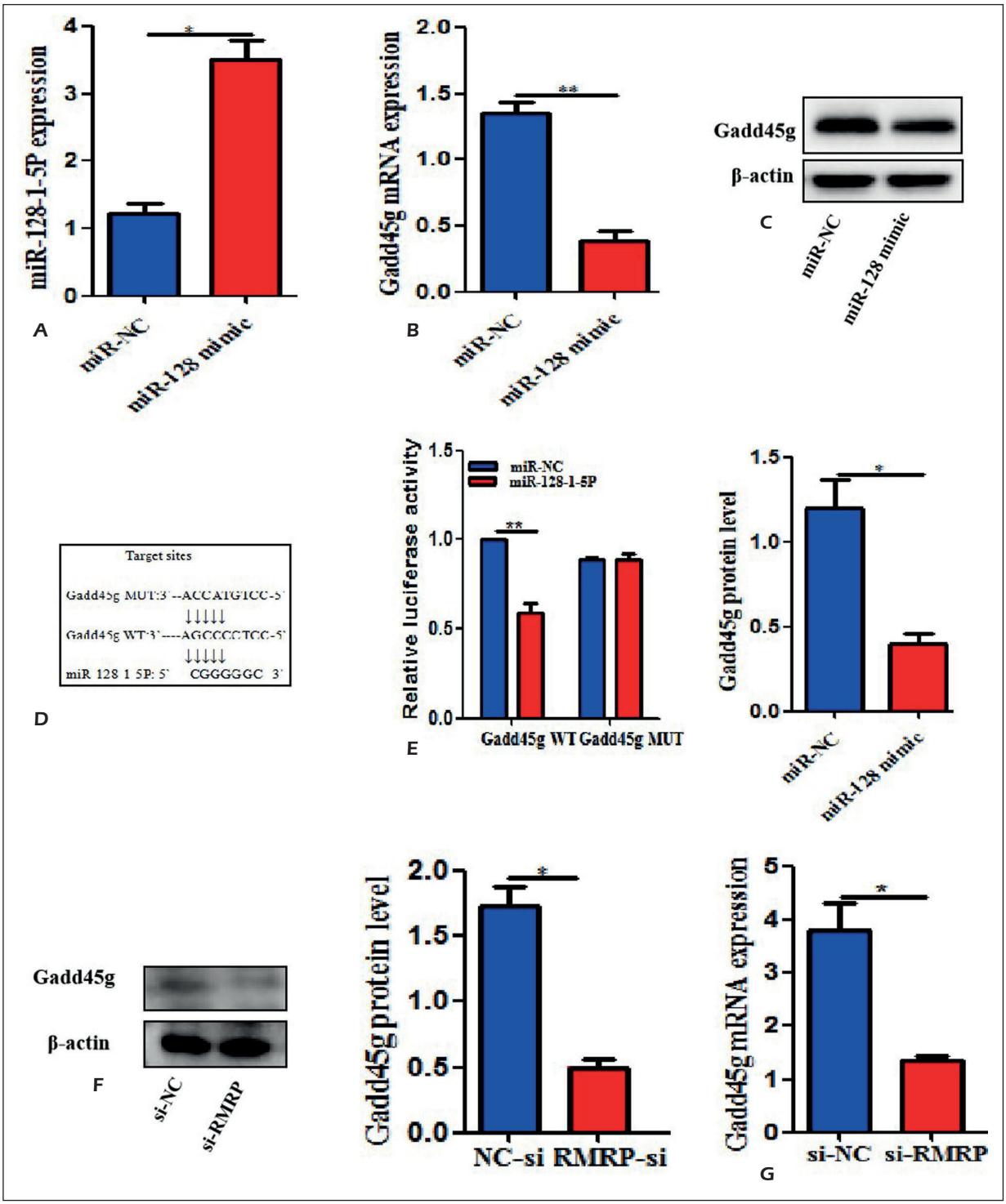

Figure 4. MiR-128-1-5P directly suppressed Gadd45g in human vascular smooth muscle cells. **A**-**B**, Expression of miR-128 and Gadd45g was detected by qPCR. **C**, Gadd45g protein level was tested by Western blot in human vascular smooth muscle cells. **D**, Sequences of MiR-128-1-5P and its target site in the 3'-UTR of Gadd45g. **E**, Effect of miR-128 on the activity of Gadd45g-WT and Gadd45gP-MUT was detected by Luciferase report assay. **F**, Gadd45g expression was checked by qPCR. **G**, Gadd45g protein level was checked by Western blot. The data are expressed as mean ± SD. n=3. *p<0.05.

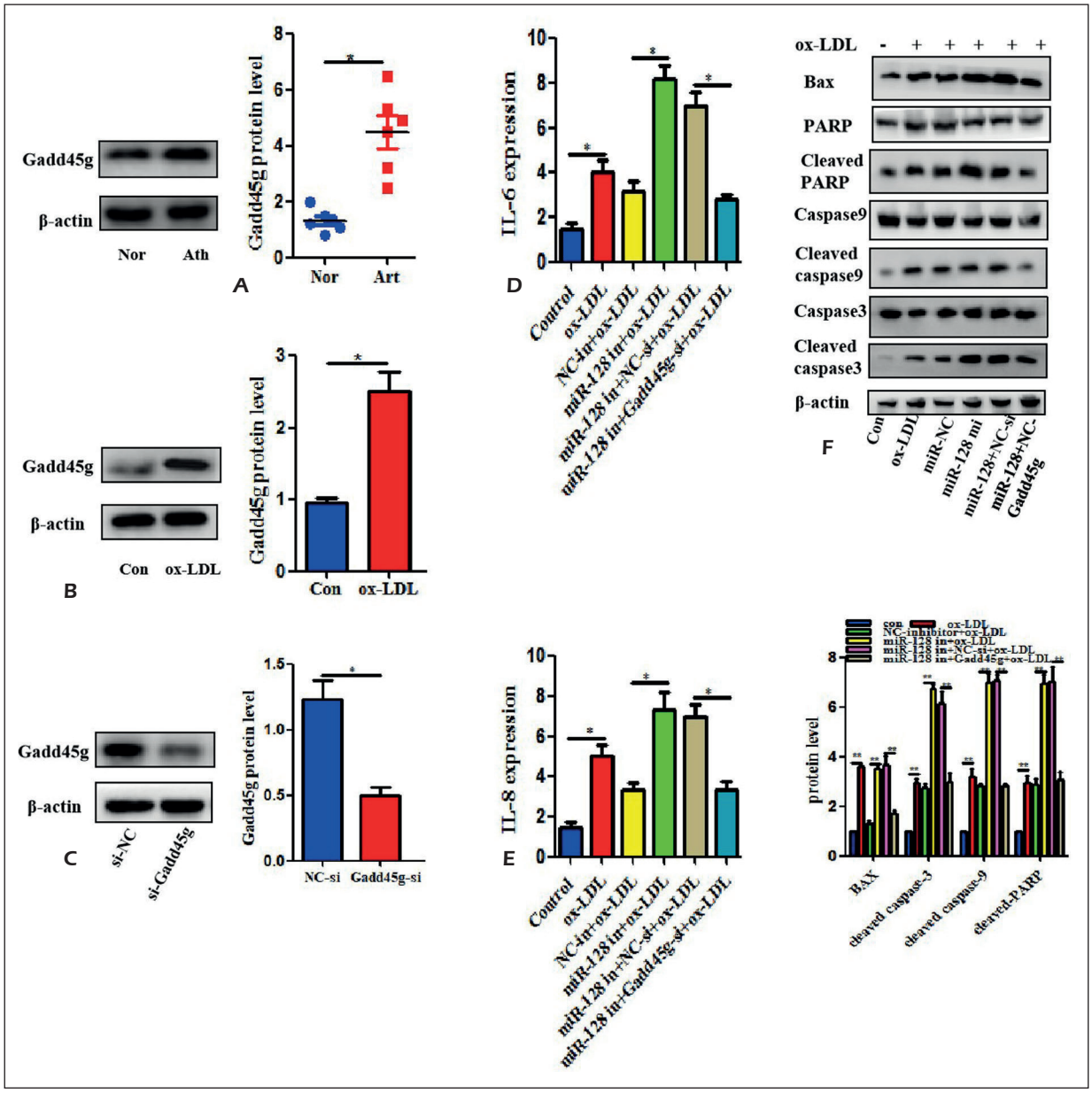

Figure 5. Gadd45g down-regulation reversed the effect of IL-6, IL-8, and apoptosis related protein induced by miR-128-1-5P inhibitor in human vascular smooth muscle cells. **A-C**, Gadd45g protein level was investigated by Western blot in coronary artery (n=6, *p<0.05) and human vascular smooth muscle cells. **D-E**, IL-6 and IL-8 expression were measured in human vascular smooth muscle cells. **F**, Bax, cleaved-caspase3, cleaved-caspase9, and cleaved-PARP protein level were detected by Western blot assay. The data are expressed as mean ± SD. n=3. *p<0.05.

Based on the above results, we next explored the possible regulatory mechanism of liraglutide in the progression of coronary atherosclerosis. qPCR and Western blot were used to detect the effect of liraglutide on the effect of RMRP, miR-128-1-5P, and Gadd45g in coronary atherosclerosis. Results showed that liraglutide extremely decreased the expression of RMRP and Gadd45g (Figure 6D-6E) and significantly increased the expression of miR-128-1-5P (Figure 6F) in coronary atherosclerosis. The above experimental data indicated that liraglutide regulates coronary atherosclerosis through RMRP/miRNA-128-1-5P/Gadd45g.

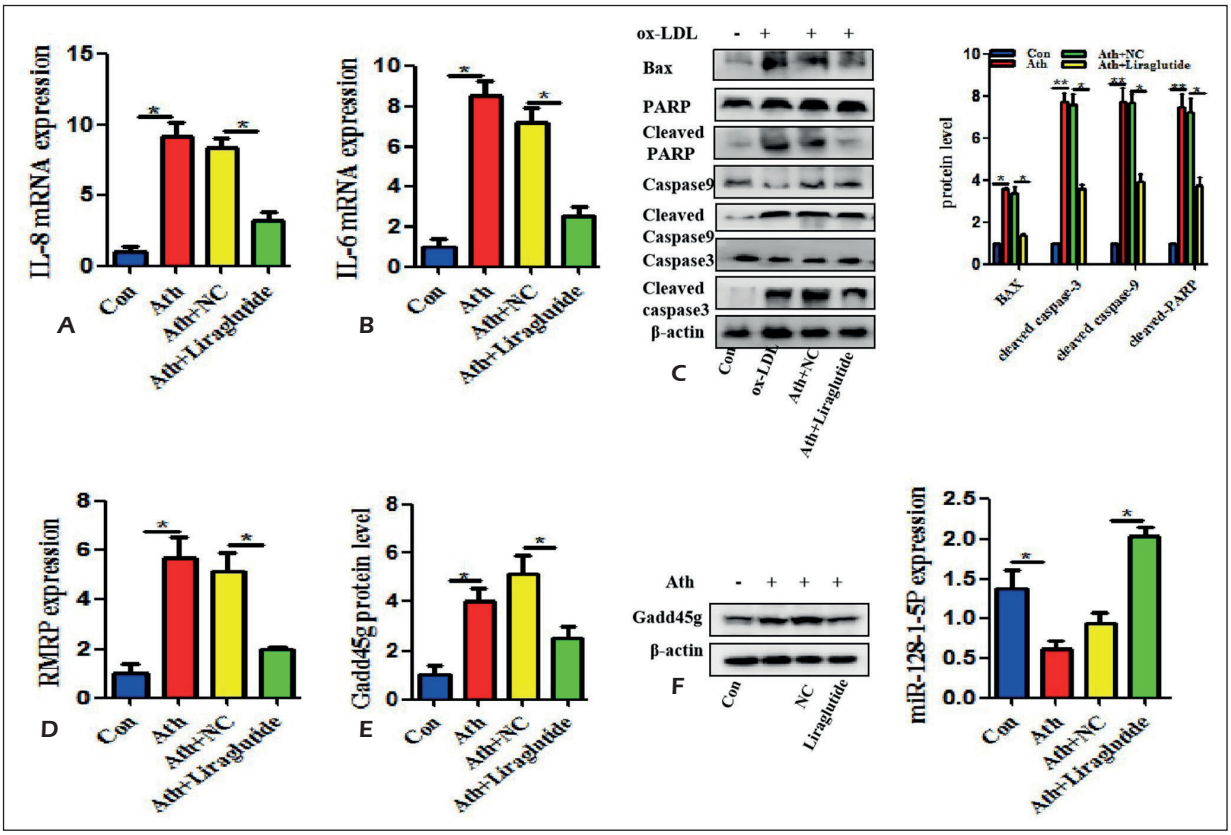

Figure 6. Liraglutide blocked the expression of IL-6, IL-8, and apoptosis via regulating RMRP/miRNA-128-1-5P/Gadd45g in coronary atherosclerosis. **A-B**, Expression of IL-6, IL-8 was measured after liraglutide treatment. **C**, Bax, cleaved-caspase3, cleaved-caspase9, and cleaved-PARP protein level were tested after treated with liraglutide in coronary atherosclerosis. **D-E**, Expression level of RMRP and miRNA-128 was measured by qPCR in coronary artery with liraglutide treatment. **F**, Gadd45g protein was detected after liraglutide treatment. The data are expressed as mean ± SD. n=6. *p<0.05.

Discussion

Increasing evidence suggests that lncRNAs play a key role in cardiovascular disease. Jin et al[28] elaborated lncRNA Malat1 reduced ischemia-induced apoptosis and pro-inflammatory cytokine expression in ischemic stroke[29]. LncRNA RMRP, located chr15, is highly expressed in cardiovascular disease and its expression level is a powerful predictor of prognosis. Kong et al[30] indicated lncRNA RMRP upregulation aggravated myocardial ischemia-reperfusion injury by sponging miR-206 to target ATG3 expression. Therefore, we discussed its role in the progression of coronary atherosclerosis. In the course of our research, RMRP expression was significantly elevated in coronary atherosclerosis compared with normal coronary artery. RMRP knocked down markedly repressed proinflammatory cytokines expression. The level of apoptosis relative protein in human vascular smooth muscle cells, which is similar to previous studies, showed that RMRP increased the level of apoptosis protein. Overall, these results suggested that RMRP may play an oncogene role in coronary atherosclerosis and the knockout of RMRP is beneficial to inhibit the occurrence and development of coronary atherosclerosis.

Several studies have shown that lncRNA interacts with miRNA in the way of ceRNA in some diseases[31]. For example, HOTAIR promotes osteosarcoma cell growth, migration, invasion, and cell apoptosis by sponging miR-217[32]. Silencing of long noncoding RNA XIST attenuated Alzheimer's disease through miR-124[33]. Additionally, lncRNA TUG1 contributes to ESCC progression via regulating miR-148a-3p *in vitro*[34]. In this study, to verify whether RMRP was involved in coronary atherosclerosis through this mechanism, we conducted Luciferase assay and bioinformat-

ics analysis to find its possible target. As a result, our data showed that miR-128-1-5P was a possible target of RMRP. Meanwhile, RMRP downregulation prominently increased the expression of miR-128-1-5P. Further experiments showed the expression of microRNA-128-1-5P was significantly lower in coronary atherosclerosis than normal coronary artery and microRNA-128-1-5P inhibitor could extremely reverse RMRP-si induced effect on inflammatory factors and apoptosis related protein expression in human vascular smooth muscle cells. Taken together, this information implied that RMRP played a key role by regulating miR-128-1-5P and may act as a ceRNA function in atherosclerosis.

Growth arrest and DNA damage 45G (GADD45G), a member of GADD45 family, is an important protein involved in P38 MAPK signaling pathway. Our data indicated that Gadd45g protein level in atherosclerosis was extremely higher than normal control group and miR-128-1-5P mimic sharply suppressed Gadd45g expression at protein level in human vascular smooth muscle cells. The same findings were discovered after treatment with si-RMRP in human vascular smooth muscle cells. Meanwhile, Luciferase assay showed Gadd45g was a target of miR-128-1-5P and the downregulation of Gadd45g could reverse miR-128-1-5P effect on inflammatory factors and apoptosis relative protein in human vascular smooth muscle cells. Collectively, these results suggested that RMRP/miR-128-1-5P/Gadd45g played an important role in the occurrence and development of coronary atherosclerosis.

Liraglutide, a long-acting synthetic GLP-1R agonist, has been used in cardiovascular diseases, such as type 2 diabetes mellitus. Further reports are needed to confirm the role of liraglutide in coronary atherosclerosis and searched its related mechanisms. In this study, the effects of liraglutide on pro-inflammatory factors and apoptosis related protein were observed. Results showed that liraglutide could visibly reduce the expression of inflammatory factors and the level of apoptosis related protein. We further probed the mechanism of liraglutide in coronary atherosclerosis. Experiments showed that liraglutide distinctly reduced the regulation of RMRP and Gadd45g and enhanced the regulation of miR-128-1-5P. All of these data suggested that liraglutide participated in the occurrence and development of coronary atherosclerosis through RMRP/miRNA-128-1-5P/Gadd45g signal pathway, which provided a theoretical basis for the clinical application of liraglutide in the treatment of coronary atherosclerosis.

Conclusions

To the best of our knowledge, this study was the first to detect high levels of RMRP and find new function of liraglutide, which improves coronary atherosclerosis by regulating RMRP/miRNA-128-1-5P/Gadd45g signal pathway. We also proposed a new mechanism of direct interaction between RMRP with miR-128-1-5P and miR-128-1-5P with Gadd45g. These results further deepen our understanding of the pharmacological effects of liraglutide and provided new information for further elucidation of its mechanism, which is helpful to understand the molecular mechanism of the progression of coronary atherosclerosis and provide new potential strategies for the treatment of coronary atherosclerosis.

Conflict of Interests

The authors declare that they have no conflict of interests.

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