MiR-490-3p modulates the proliferation of vascular smooth muscle cells induced by ox-LDL through targeting PAPP-A

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Aims

Oxidized low-density lipoprotein (ox-LDL) can induce vascular smooth muscle cell (VSMC) proliferation and differentiation, which is the important mechanism of the pro-atherogenic effects of ox-LDL. Although miRNAs are crucial regulators of the ox-LDL effects on VSMC, the detailed mechanisms are not fully elucidated. So, we investigated the miRNAs changes in the proliferation of human coronary artery smooth muscle cells (hCASMCs) induced by ox-LDL and the functional significance of some miRNAs, which were regulated significantly.

Methods and results

Using miRNA microarray, real-time PCR, and luciferase reporter assay, our study showed that miR-490-3p and insulin-like growth factor-1 (IGF1) expression were down-regulated, whereas pregnancy-associated plasma protein A (PAPP-A) and IGF2 expression were up-regulated significantly in hCASMC treated by ox-LDL and/or in human atherosclerotic plaque. Further studies indicated PAPP-A was the target gene of miR-490-3p. The miR-490-3p mimic could inhibit the up-regulation of PAPP-A induced by ox-LDL in hCASMC, which resulted in the suppression of the metalloprotease effect of PAPP-A on IGF-binding protein-4 (IGFBP-4). All these effects led to the inhibition of the proliferation of hCASMC induced by ox-LDL.

Conclusion

Ox-LDL could inhibit the expression of miR-490-3p and IGF1, whereas increase IGF2 expression. The inhibition of miR0-490-3p up-regulated its target gene PAPP-A and then increased the proteolysis of IGFBP-4. The increased expression of IGFBP-4 might activate pathways independent or dependent on IGF axis by autocrine and paracrine mechanisms and resulted in the VSMC proliferation. Our results could help us to understand the mechanisms of the pro-atherogenic effects of ox-LDL and the effects of PAPP-A on atherosclerosis development.

Keywords

miRNA • Vascular smooth muscle cells • Ox-LDL • Atherosclerosis

1. Introduction

Atherosclerosis (AS) is a complex polygenic disease with a long course and its pathogenesis has not been fully understood. However, it has been accepted that hyperlipidaemia (high cholesterol and high-low density lipoprotein) is one of the major risk factors for AS. Low-density lipoprotein (LDL) is susceptible to oxidation to oxidized LDL (ox-LDL) in the presence of large amounts of oxygen-free radicals induced by many other risk factors, such as aging, smoking, hypertension, and diabetes. Ox-LDL can promote the formation of foam cells derived from vascular smooth muscle cells (VSMCs) and regulate the proliferation, apoptosis, migration, and differentiation of VSMC, which play key roles in the development of AS.1 Ox-LDL can also regulate the expression and activity of many vasoactive substances including endothelin-1, platelet-derived growth factor, and insulin-like growth factor (IGF).2,3 However, because of the complexity of the structure and effect of ox-LDL, the detailed mechanism of how ox-LDL impacts the expression of vasoactive substances resulting in the proliferation of VSMC has not yet been fully elucidated.
Pregnancy-associated plasma protein A (PAPP-A) is a metalloproteinase and was originally found in placenta and also abundantly expresses in VSMCs. PAPP-A can activate local IGF through cleavage of the inhibitory IGF-binding protein-4 (IGFBP-4), which is the main substrate of PAPP-A. Recent studies have shown that over-expression of PAPP-A in VSMC can promote the proliferation of VSMC and over-expression of PAPP-A in mouse can promote the development of AS significantly.1–4 Furthermore, the PAPP-A secreted by VSMC plays critical roles in the development of AS through the local PAPP-A/IGFBP-4/IGF system that acts on VSMC, endothelium, macrophages and other related cells.5 Many clinical studies indicated that PAPP-A was highly expressed in ruptured plaques and the plasma of patients with acute coronary syndromes.9–11 However, some studies did not support this conclusion.12,13 Moreover, although in vitro studies showed that IGF activated by PAPP-A had pro-inflammatory and pro-atherogenic roles, more recent clinical and experimental studies indicated that IGF, particularly IGFI, might exert anti-inflammatory and anti-oxidant effects.14,15 So, the question of whether PAPP-A is pro- or anti-atherosclerotic has still been debated. For defining the roles of PAPP-A in the development of AS, more works on the regulation mechanism of PAPP-A in different vascular cells, especially in VSMCs, were required, and these works also could help us to understand the mechanisms of the effects of ox-LDL on the proliferation of VSMC.

Many studies have shown that miRNAs play critical roles in the pro-atherosclerotic effects of ox-LDL and the functional regulation of VSMCs. For instance, ox-LDL could up-regulate miR-29b, whereas increase IGF2 expression. The inhibition of miR-0-490-3p could help us to understand the mechanisms of the effects of ox-LDL on the proliferation of VSMC.

2. Methods

2.1 Ethics statement and tissue samples
This study has been approved by the Ethics Committee of the Chinese PLA General Hospital and conforms to the Declaration of Helsinki. All atherosclerotic plaques in coronary artery and normal coronary artery tissue samples were collected from the autopsy specimens in the Chinese PLA General Hospital.

2.2 Vectors, cell culture, and treatment
The siRNA and expression vector of PAPP-A were purchased from Origene. The mutation (MUT) and wild-type (WT) vector of miR-490-3p sponge were constructed by GenePharma (Shanghai, China). All chemical reagents were bought from Sigma-Aldrich. The mimic and inhibitor of miRNAs were synthesized by GenePharma. The HepG2 cell, hCASMC, and human coronary artery endothelial cell (hCAEC) were obtained from ATCC and LONZA and cultured in the recommended conditions, respectively. The cells were transfected by vectors, mimic/inhibitor of miR-490-3p or miR-195, and/or treated with ox-LDL (Kalen Biomedical, Montgomery Village, MD, USA), native LDL, or high-density lipoprotein (HDL) at different concentrations for different time. The final concentration of random sequence, mimic, and inhibitor of miRNAs was 50, 50, and 100 nM, respectively. The cells and culture mediums were also collected and stored in liquid nitrogen for further analysis. The cell numbers were determined using a Coulter Counter.

2.3 miRNA extraction and microarray analysis
The miRNAs were extracted from cells or tissue samples and purified using the mirVana miRNA Isolation Kit, following the manufacturer’s instructions. After the quality evaluation, the same amount of miRNA samples from ox-LDL-treated and -untreated hCASMCs were mixed as the common reference and labelled by Hy3. The miRNAs of ox-LDL-treated and -untreated hCASMCs were labelled by Hy5 and were also hybridized with the miRCURY LNA Array (v.14.0), respectively. After washing and scanning, the expression data of miRNAs were obtained. The raw data were normalized and analysed by the Gene Spring Software 12.0.

2.4 Real-time quantitative PCR
Total RNA was extracted from cells or tissue samples using the RNeasy Mini Kit. The reverse transcription was performed using TaqMan RT reagents according to the manual. The primers and Taqman probes of ERGIC3, PAPP-A, and the selected miRNAs (miR-490-3p, miR-145, miR-195, miR-29b, miR-150-3p, miR-362-5p, and miR-33b-3p) were bought from Applied Biosystems.

2.5 Western blot and ELISA
The hCASMCs or HepG2 cells were treated by mimic/inhibitor of miRNAs for 24 h, respectively, with or without ox-LDL treatment and were harvested. The total protein of the cells and tissue samples were prepared. Aliquots of them were used for western blot analysis with antibodies to ERGIC3, PAPP-A, and β-actin (Abcam, Cambridge, MA, USA). The culture mediums of the treated and untreated hCASMCs were collected, and the PAPP-A, IGFI, IGF2, and IGFBP-4 levels in them were measured using ELISA kits (Diagnostic Systems Laboratories, Inc., Abcam, Inc., and Abnova, Inc.), respectively, according to the manual.

2.6 IGFBP-4 Proteolysis ELISA assay
The cell-free IGFBP-4 proteolysis was assayed using the IGFBP-4 Proteolysis ELISA kit (Eagle Biosciences, Nashua, NH, USA) according to the manual. Briefly, the hCASMCs were transfected by PAPP-A siRNA or empty vector and treated with 20 and 60 μg/mL ox-LDL for 24 h. Then, the culture medium was concentrated and incubated with 0.5 μg of biotinylated IGFBP-4 for 2 h at 37°C. After stopping reaction, the concentrations of the non-proteolyzed IGFBP-4 were measured by microplate reader.

2.7 The prediction of targeted genes of miR-490-3p
Three different miRNA target prediction algorithms (miRanda, TargetScan, and PicTar) were used to determine the potential target genes of miR-490-3p. The sequences of PAPP-A mRNA and miR-490-3p were obtained from the NCBI and miRBase database, respectively. The RNA Hybrid program was used to predict the secondary structure and total free energy of the duplex formation between the miR-490-3p binding sites in the mRNA of PAPP-A 3'-UTR and miR-490-3p sequence.
2.8 Chromatin immunoprecipitation-quantitative PCR

Chromatin immunoprecipitation (ChIP) was performed using the ChIP-specific anti-RNA polymerase II antibody and anti-H3K9me2 antibody as well as the EZ-ChIP kit (Millipore, MA, USA), as described in the manufacturer’s protocol. According to the core sequence of the PAPP-A promoter validated experimentally in the Eukaryotic Promoter Database, the primers for quantitative PCR (qPCR) were designed: F: CAGATTAGCTACGCTTGTT; R: TTAAGACTGATCCGCTGAAT. The qPCR was conducted using the Power SYBR Green PCR Master Mix according to the manual.

2.9 Luciferase reporter assay

The luciferase reporter vectors were constructed by cloning either the target sequence of miR-490-3p in PAPP-A 3′-UTR or its mismatched version into pmiR-GLO Vector. hCASMCs were transiently transfected with above-mentioned vectors and miR-490-3p mimic/inhibitor or random sequence, respectively, and then treated by 60 μg/mL ox-LDL for 24 h. The firefly and renilla luciferase activity of the lysates were assayed using the Dual-Luciferase Reporter Assay System according to the manufacturer’s manual. The normalized firefly luciferase activity (firefly luciferase activity/Renilla luciferase activity) for each vector was compared with that of the non-transfected control group. For each transfection, luciferase activity was averaged from five replicates.

2.10 Statistical analysis

The Benjamini–Hochberg procedure was used for controlling the false positive rate in multiple comparisons in microarray data. The functional enrichment and pathway analysis was performed by the Pathway Studio software. The data were expressed as means ± SEM, and compared using Student’s t-tests or one-way analysis of variance with appropriate post-tests by SPSS 15.0. A P-value of <0.05 was considered statistically significant.

3. Results

3.1 MiR-490-3p was down-regulated in hCASMCs treated by ox-LDL

To investigate the role of miRNAs in the proliferation of VSMC induced by ox-LDL, we first profiled the miRNA expression of hCASMC treated by 60 μg/mL ox-LDL for 24 h using miRNA microarray. The results showed that the expression of 25 miRNAs were changed more than two-fold, and the P-values (Benjamini–Hochberg multiple testing correction) were <0.05 (Figure 1A and Supplementary material online, Table S1). Seven miRNAs, which were regulated more significantly, were selected for verification. The results of qPCR showed that the tendency of the change of six miRNAs, except for miR-33b-3p, was consistent with the microarray results (Figure 1B). We focus on the miR-490-3p because it was regulated...
more significantly than others, and the studies about its roles in functional regulation of VSMC have not been reported until now. Additionally, the hCASMCs were treated with 20 and 60 μg/mL native LDL and HDL, respectively, and the expression of miR-490-3p were also detected by qPCR. The results showed that the expression change of miR-490-3p was not significant and implied that the down-regulation of MiR-490-3p in hCASMCs was, to some extent, selective for ox-LDL (Figure 1C). The functional enrichment analysis of the 767 predicted target genes of miR-490-3p indicated that miR-490-3p were related to cell cycle, proliferation, and apoptosis. Moreover, the PAPP-A–IGFBP–IGF signalling axis was one of the key nodes in the whole network of the target genes of miR-490-3p. All these results suggested that miR-490-3p might play important roles in the effects of ox-LDL on hCASMCs.

3.2 PAPP-A was up-regulated in hCASMCs treated by ox-LDL

In order to reveal the relationship between miR-490-3p and PAPP-A, the expression of PAPP-A mRNA and protein as well as its secretion were assayed in hCASMCs treated by ox-LDL (20 and 60 μg/mL) for 12, 24, 48, and 72 h, respectively. The results showed that the expression of PAPP-A mRNA and protein as well as its secretion were significantly increased with the extension of the time of ox-LDL treatment (Figure 2A–C). Additionally, the concentration assay of IGF1, IGF2, and IGFBP-4 in the culture medium of hCASMCs demonstrated that ox-LDL increased the secretion of IGF2, but decreased the secretion of IGF1 and IGFBP-4, did not change significantly (Figure 2D). Moreover, there was a significant positive correlation between the levels of secreted PAPP-A and the cellular PAPP-A as well as its mRNA (Supplementary material online, Figure S1A). The assay result of the proteolysis of IGFBP-4 by PAPP-A indicated that the more hCASMCs secreted PAPP-A induced by ox-LDL, the more was IGFBP-4 proteolyzed. Moreover, the expression changes of mRNA, protein and secretion of PAPP-A, and the proteolysis of IGFBP-4 protein induced by ox-LDL were abolished after inhibition of PAPP-A expression using siRNA (Figure 2E and Supplementary material online, Figure S1B–D).

3.3 PAPP-A was the target gene of miR-490-3p

PAPP-A was likely to be one of the target genes of miR-490-3p predicted by TargetScan and other commonly used software. The predicted core

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**Figure 2** PAPP-A and IGF2 were up-regulated in hCASMCs treated by ox-LDL. (A–C) The mRNA and protein expression as well as the secretion of PAPP-A in hCASMCs were significantly increased with the extension of treatment time of 20 or 60 μg/mL ox-LDL compared with untreated hCASMC, i.e. the control group (A and B: n = 3 per group; C: n = 8 per group). *P < 0.01 compared with the control group. There are also significant differences between any of the hCASMC treated by ox-LDL for 24, 48, and 72 h (P < 0.01). (D) hCASMCs was treated with 20 and 60 μg/mL ox-LDL for 24 h. The concentrations of IGF1, IGF2, and IGFBP-4 in the culture medium were assayed, respectively. The untreated hCASMCs were used as control (n = 6). *P < 0.001 compared with the control group; ▲P < 0.05 compared with control, IGF2, and IGFBP-4 groups. (E) The metalloprotease activity of PAPP-A protein in the culture medium of hCASMCs treated by 20 and 60 μg/mL ox-LDL for 24 h was assayed. The treatment of ox-LDL significantly increased the proteolysis of IGFBP-4 protein catalysed by PAPP-A. The increased proteolysis of IGFBP-4 protein by PAPP-A induced by ox-LDL were abolished after inhibition of PAPP-A expression using siRNA. *P < 0.001 compared with untreated hCASMCs. ▲P < 0.001 compared with hCASMC treated with 20 μg/mL ox-LDL.
binding sequence of miR-490-3p in the 3′-UTR of PAPP-A was conserved in various species. The minimum free energy (MFE) between the putative binding site of miR-490-3p in PAPP-A and miR-490-3p was −24.9 kcal/mol and was much lower than many other MFE between miRNAs and their target genes that had been confirmed by experiments (Figure 3A), for example, the MF between miR-21 and its target gene CDK2AP1 is only −19.9 kcal/mol. Moreover, although the miR-SVR scores of miR-195 (0.37) predicated it might be a better candidate for targeting PAPP-A than miR-490-3p, the experimental results showed that miR-195 did not significantly affected the changes of PAPP-A mRNA, protein expression, and its secretion in hCASMCs induced by ox-LDL (Supplementary material online, Figure S2A). Thus, PAPP-A was very likely to be the target gene of miR-490-3p. To confirm this conclusion, we cloned the predicted miR-490-3p binding site in PAPP-A and its mutation into pmirGLO Dual-Luciferase miRNA target expression vector, respectively. Furthermore, the

![Figure 3](https://academic.oup.com/cardiovascres/article-abstract/100/2/272/413974)

**Figure 3** PAPP-A was predicted and verified to be the target gene of miR-490-3p. (A) Left panel, comparison of the predicted core binding sequence of miR-490-3p in the 3′-UTR of PAPP-A in various species. Right panel, miR-490-3p could putatively form a strong secondary structure with the target sequence of 3′-UTR of PAPP-A. (B) Upper panel, the sequence alignment between miR-490-3p and human PAPP-A 3′-UTR WT and MUT. The bold letters indicated the mutation sequence. Lower panel, hCASMCs were co-transfected by either luc-papp-a-wt or luc-papp-a-mut along with miR-490-3p mimic and/or inhibitor, random sequence, or no-insert empty vector. The non-transfected hCASMC was used as control (n = 5). *P < 0.05 compared with control and all other groups. (C) hCASMCs were treated according to the treatment combination shown in the figure for 24 h. The untreated hCASMCs were used as control (n = 4). *P < 0.05 compared with control. **P < 0.001 compared with all other ox-LDL-treated hCASMC groups. (D) The representative result of western blot of PAPP-A protein in hCASMCs. All experimental conditions were the same as description in C.
MiR-490-3p targets PAPP-A and regulates VSMC

3.4 MiR-490-3p did not regulate the PAPP-A through chromatin-level silencing

Some miRNAs could regulate target genes through chromatin-level silencing. To clarify whether miR-490-3p possessed this mechanism, according to the transcription start site of PAPP-A, the region of from −3000 to +100 bp was considered as the sequence in which the PAPP-A promoter might be included. The minimum binding energy between this region and miR-490-3p was −22.6 kcal/mol. This result predicted that the binding was not stable, i.e. miR-490-3p may not bind to the promoter of PAPP-A. Then, the ChIP was performed using the anti-RNA polymerase II or anti-H3K9me2 antibody. The qPCR results showed that there was no difference in the amount of PAPP-A promoter compared with that in untreated control. Moreover, the WT vector of miR-490-3p sponge, not the MUT vector of miR-490-3p sponge, inhibited the action of miR-490-3p resulting in the up-regulation of PAPP-A protein and mRNA expression (Figure 3C and D). Additionally, the miR-490-3p mimic could also significantly inhibit the up-regulation of PAPP-A induced by tumor necrosis factor (TNF)-α in hCAECs (Supplementary material online, Figure S2D). All these results indicated that PAPP-A was one of the target genes of miR-490-3p, and miR-490-3p might regulate it in a similar manner in hCAECs and hCASMCs. However, the regulation manner of miR-490-3p in hCASMCs (Supplementary material online, Figure S2D). These results indicated that the abnormal expression of miR-490-3p and its target gene PAPP-A might be involved in the pathogenesis of AS.

3.5 MiR-490-3p inhibited the proliferation of hCASMCs induced by ox-LDL

For revealing the effects of miR-490-3p on the proliferation of VSMC, the hCASMCs were transfected with miR-490-3p mimic, inhibitor, and random sequence, respectively, and were also treated by 60 μg/mL ox-LDL for 24 h. Moreover, the hCASMCs were also transfected with empty vector, PAPP-A siRNA, and expression vector, respectively, without the ox-LDL treatment. The results of cell counts of hCASMCs showed that miR-490-3p mimic significantly inhibited the proliferation of VSMC induced by ox-LDL, compared with that in untreated control while the miR-490-3p inhibitor eliminated this effect of miR-490-3p mimic (Figure 5A). The silencing or over-expression of PAPP-A in hCASMC (independent of ox-LDL) could mimic the effects of miR-490-3p on the proliferative capacity of hCASMC (Figure 5B). These results demonstrated that miR-490-3p was able to inhibit the proliferation of hCASMCs induced by ox-LDL by inhibiting the expression of its target genes PAPP-A. In other words, ox-LDL could induce the proliferation of hCASMCs by inhibiting the expression of miR-490-3p and by up-regulating the expression of its target genes PAPP-A.

3.6 PAPP-A was up-regulated, while miR-490-3p was down-regulated in atherosclerotic plaque

To further reveal the relationship between the decreased expression of PAPP-A by miR-490-3p and AS as well as its clinical significance, the expression of PAPP-A at mRNA and protein level and miR-490-3p in six atherosclerotic plaques were detected. The results of qPCR and western blot indicated that the expression of PAPP-A mRNA and protein was up-regulated, whereas the expression of miR-490-3p was down-regulated significantly in human atherosclerotic plaque compared with the normal vessel wall of the coronary artery (Figure 6). These results indicated that the abnormal expression of miR-490-3p and its target gene PAPP-A might be involved in the pathogenesis of AS.

4. Discussion

Ox-LDL is a key factor which induced vascular damage and promotes the pathogenesis of AS. In early stage of AS, ox-LDL permeates into sub-endothelial layer through injured endothelium and triggers a series of pathological events. After the formation of the fibrous cap and lipid core, ox-LDL in circulation and lipid core can directly stimulate the normal and abnormal VSMCs. Many studies of AS focused on how VSMCs stimulated by ox-LDL change from normal to abnormal.
Ox-LDL can induce the proliferation of VSMC while ox-LDL in high concentration can induce VSMC apoptosis after a long-term treatment.\textsuperscript{20,21} This might be one of the reasons that ox-LDL promotes the development of atherosclerotic plaques in the early stage and increases its vulnerability in the late stage. Additionally, IGF axis is an important growth factor pathway. IGF1/2 and IGFBP1–6 are the major components of IGF axis and are widely expressed in cardiovascular cells including VSMCs.\textsuperscript{22–25} The type and amount of IGF1/2 and IGFBPs in local extracellular microenvironment are different from those in circulation, and they could function locally by autocrine and paracrine mechanisms in AS.\textsuperscript{26–28} Some studies indicated that the local IGF1 and IGF2 might play different roles in the proliferation of VSMC induced by different stimuli.\textsuperscript{22–25} Our results showed that ox-LDL increased the secretion of IGF2 while decreased that of IGF1 significantly in VSMC. These implied that IGF2 might have more important roles in the VSMC proliferation induced by ox-LDL than IGF1. However, more in-depth research was needed to clarify the detailed mechanism of the effects of ox-LDL and IGF1/2 on the proliferation of VSMC in the AS development.

PAPP-A can proteolyze IGFBP-4 and the vascular cell-derived PAPP-A may play key roles in the development of atherosclerotic plaque. IGFBP-4, the substrate of PAPP-A, can bind IGF1 and IGF2 with higher affinity and has the function dependent or independent on IGF1/2. IGFBP-4 locally secreted by VSMCs had more important roles in the function regulation of VSMC than them distributed in circulation.\textsuperscript{25,29} Our study showed that ox-LDL increased the expression of PAPP-A and the proteolysis of IGFBP-4 resulting in the proliferation of hCASMCs. The study of mice that over-express PAPP-A in VSMC was consistent with our results.\textsuperscript{4} Furthermore, the secretion of IGFBP-4 in hCASMCs treated with ox-LDL did not significantly change. So, PAPP-A secreted by VSMCs may locally act on the IGFBP-4 surrounding VSMCs that is permeated from circulation or secreted by VSMC and other cells and play its regulatory roles by autocrine and paracrine mechanisms in AS. However, some other studies indicated that the correlation between PAPP-A and AS was poor.\textsuperscript{12,13} Whether PAPP-A is pro-atherosclerotic or anti-atherosclerotic needs to be further investigated. Moreover, it is also necessary to further study the detailed

\[\text{Figure 5}\] miR-490-3p inhibited the proliferation of VSMC induced by ox-LDL. (A) The hCASMCs proliferation was measured after treatment as shown in the figure for 24 h. The untreated hCASMCs were used as control \((n = 4)\). \(* P < 0.05\) compared with control. \(**P < 0.001\) compared with all other ox-LDL-treated groups. (B) The empty vector, siRNA, or expression vector of PAPP-A was transfected, and the hCASMCs proliferation was also measured 24 h after transfection. The results showed the silencing or over-expression of PAPP-A could mimic the effects of miR-490-3p on the proliferative capacity of hCASMC and it is independent of ox-LDL. The untreated hCASMCs were used as control \((n = 4)\). \(*P < 0.05, **P < 0.001\) compared with control.

\[\text{Figure 6}\] The abnormal expression of PAPP-A and miR-490-3p in atherosclerotic plaque. (A) The representative result of western blot of PAPP-A protein in human atherosclerotic plaques in the coronary artery (AP) and normal vessel walls of coronary artery (CAWW) \((n = 6)\). (B) The qPCR analysis of PAPP-A mRNA and miR-490-3p in atherosclerotic plaques. The CAWW were used as control \((n = 6)\). \(*P < 0.01, **P < 0.001\) compared with control.
mechanism of the autocrine and paracrine effects of PAPP-A on the development of AS. Ox-LDL can induce the expression of PAPP-A in VSMC, but the mechanism involved in this effect is not fully clear. miRNA is a class of small non-coding RNAs (19–25 nt) that can regulate the expression of its target genes through binding to the 3′-UTR of target genes. Many studies have shown that miRNAs play key roles in the development of AS. Our results revealed that the down-regulation of miR-490-3p was one of the important mechanisms by which ox-LDL increased the expression of PAPP-A in VSMC. Currently, the studies of miR-490-3p are very few. Some study showed that the expression of miR-490-3p was up-regulated in HCC and promoted the proliferation, migration, and invasion of HCC cells. MiR-490-3p might be an oncogene. On the contrary, another study revealed that the expression of miR-490-3p was significantly down-regulated in colorectal cancer, and it might have the function of tumour suppressor gene.

Many studies have shown that miRNAs play key roles in the development of AS. A30 Our results revealed that the down-regulation of miR-490-3p was one of the important mechanisms by which ox-LDL increased the expression of PAPP-A in VSMC. Currently, the studies of miR-490-3p are very few. Some study showed that the expression of miR-490-3p is one of the important mechanisms by which ox-LDL might activate a series of signalling pathways independent or dependent on IGFBPs, leading to the proliferation of hCASMCs. These results could help us to understand the mechanisms of the pro-atherogenic effects of ox-LDL. However, the detailed mechanism of autocrine and paracrine regulation in the proliferation of hCASMCs induced by ox-LDL need to be further investigated.

Supplementary material

Supplementary material is available at Cardiovascular Research online.

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