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# Plin2 Involved in oxLDL Induced LOX1 Expression in Macrophages *via* NF-κB Pathway*

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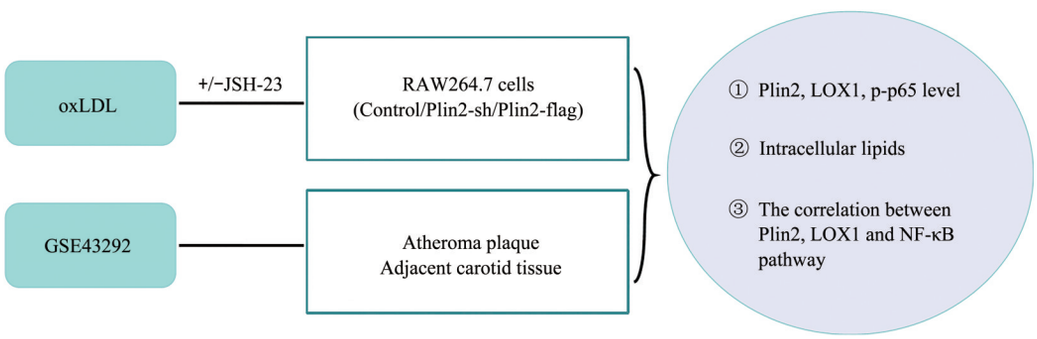
## Graphical abstract




**Abstract** **Objective** OxLDL can increase Plin2 expression, then promote the formation of foam cells. LOX1 was a scavenger receptor for oxLDL. Here, we investigate the relationship between Plin2 and LOX1 in the progress of atherosclerosis. **Methods** The data GSE43292 from GEO database were analyzed for Plin2 and LOX1 expressions and the correlation between Plin2, LOX1 and NF-κB pathway. RAW264.7 cells stimulated by oxLDL served as a cellular model of atherosclerosis. The Plin2, LOX1 and p-p65 expressions were analyzed by immunoblotting, the intracellular lipids were detected by BODIPY 493/503 staining. **Results** The Plin2 and LOX1 expressions in atheroma plaques were significantly higher than that in adjacent carotid tissues by analyzing

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GSE43292. The expressions of Plin2 and LOX1, the lipid droplets were increased obviously in RAW264.7 cells after treated with oxLDL for 24 h. And Plin2 overexpression significantly increased the expression of LOX1. This change was more obvious after oxLDL incubation. But knockdown Plin2 maked no difference on LOX1 when without oxLDL treatment. Furthermore, the GSEA plot showed that the expressions of Plin2 and LOX1 were positively related with NF-κB activation in atherosclerosis. Meanwhile, although oxLDL incubation, NF-κB inhibitor JSH-23 pretreatment significantly reduced Plin2 and LOX1 expressions and the amounts of intracellular lipids. In addition, the expressions of Plin2 and LOX1 were significantly inhibited by JSH-23 in spite of Plin2 overexpression plus oxLDL incubation. **Conclusion** Altogether, Plin2 can promote the intracellular lipids accumulation and may participate in pathophysiological process of atherosclerosis by increasing the expression of LOX1, which at least partly through the activation of NF-κB pathway.

**Key words** Plin2, atherosclerosis, lectin-like oxidized LDL receptor-1, nuclear factor-κB
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Atherosclerosis, relating to an unbalanced lipid metabolism and a maladaptive inflammatory response, is a complex process. It is the underlying cause of both myocardial infarction and stroke. In the process of atherogenesis, monocytes migrate into the vascular intima, take up modified low-density lipoproteins (LDL) especially oxidized LDL (oxLDL) through scavenger receptors (SRs) and then results in lipid droplets accumulation and foam cell formation. And oxLDL was a major risk factor in atherosclerosis[1].

Plin2 (adipose differentiation-related protein, ADRP), a member of the lipid droplet associated proteins family (PLINs), was firstly identified by Jiang *et al*. [2] Its absence severely restricted foam macrophage cell formation and attenuated atherosclerosis[3]. *In vivo*, its expression increased in symptomatic compared with asymptomatic carotid atherosclerosis[4] and in atherosclerosis-studded arteries of *ApoE*$^{-/-}$ mice compared with control healthy arteries[3,5]. And Plin2 inactivation in *ApoE*$^{-/-}$ mice protected against atherosclerosis[3]. Moreover, Plin2 augmented proinflammatory cytokines TNF-α, MCP-1, and IL-6 expression in THP-1 macrophage[6-7]. In turn, specific antibodies against IL-6, IL-1α, and IFN-β significantly suppressed the lipopolysaccharide (LPS) induced Plin2 expression. These results suggested that Plin2 may promote atherogenesis not only by promoting macrophage-derived foam cell formation but also by intensifying the inflammatory process through enhancement of cytokine expression. But the mechanism of Plin2 in lipid storage in the cells and its relevance to atherosclerosis mandates in-depth investigation.

Lectin-like oxidized LDL receptor-1 (LOX1) was initially identified as a major scavenger receptor for oxLDL, which is activated in many diseases, including atherosclerosis[8]. The importance of LOX1 in atherogenesis has been proven by deletion and overexpression studies[9]. LOX1 overexpression has been observed in endothelial cells, intimal smooth muscle cells, and macrophages in human atherosclerotic lesions[10]. In addition, *ApoE*$^{-/-}$ mice overexpressing LOX1 have been reported to show increasing in atheroma-like lesions, whereas macrophages from LOX1 knockout mice exhibited a marked reduction in migration compared to those from wild-type mice both *in vitro* and *in vivo*[11]. Thus, accumulating evidences implicate the involvement of LOX1 in the pathogenesis of atherosclerosis[12]. LOX1 has recently emerged as a promising biomarker and target for intervention in cardiovascular disease (CVD) [13]. Moreover, LPS or oxLDL not only enhanced expression of Plin2, but also induced of LOX1 expression[7,14-15], which involved in NF-κB[16]. And the activation of NF-κB were related with atherosclerosis[17].

However, to date, the role of Plin2 in cellular uptake of oxLDL and the molecular mechanisms are poorly understood. Given the association among the expression of Plin2, LOX1, NF-κB and the pathophysiology of atherosclerosis, we hypothesized that Plin2 can promote the intracellular lipids accumulation and participate in pathophysiological process of atherosclerosis by increasing the expression of LOX1, which through the activation of NF-κB pathway. We hereby provide an aim to highlighting the potential of Plin2 as a target for cardiovascular disease prevention and treatment.

# 1 Methods

## 1.1 Cell culture

293FT cells and RAW264.7 cells, obtained from Cell Bank in Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, were maintained in Dulbecco's modified Eagle medium (DMEM) (GE Healthcare Life Sciences, Logan, UT, USA) containing 10% (*v*/*v*) fetal bovine serum (Gibco, Thermo Fisher Scientific, Inc. Waltham Mass, MA, USA) at 37℃ in a humidified atmosphere of 5% $CO_2$. When the RAW264.7 cells presented monolayer and fused up to 70%, treated with 50 mg/L of oxLDL (Peking Union-Biololgy Co. Ltd, Beijing, China) for 0 h or 24 h, or pretreated with 30 μmol/L JSH-23 (Abcam, Cambridge, UK) for 1 h following incubation with 50 mg/L of oxLDL for 24 h. Cells were harvested and splited for analysis of Plin2, LOX1 and p-p65 proteins. The 293FT cells, presented monolayer cell and fused up to 70%, were transfected with vectors.

## 1.2 Western blot analysis

Cell lysates were prepared by incubating for 30 min in an ice-cold RIPA buffer (GBCBIO Technologies Inc. Guangzhou, China) containing 50 mmol/L Tris, 150 mmol/L NaCl, 1.0% Triton X-100, 0.5% sodium dexycholate, 0.1% SDS and protease/phosphatase inhibitors (Sigma-Aldrich, St. Louis, MO, USA) followed by centrifuging at 10 000*g* for 10 min at 4℃ . The protein concentration was measured using the bicinchoninic acid protein assay kit (Thermo Scientific, Waltham Mass, MA, USA). Proteins were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred electrophoretically onto a polyvinylidene fluoride (PVDF) membranes (Merck Millipore, Billica, MA, USA). Then the membranes were blocked using 5% nonfat milk (Nestle, Vevey, Switzerland) in tris-buffered saline containing 0.1% Tween 20 (TBST) for 1 h at room temperature. The membrane was then incubated overnight at 4℃ with the primary antibodies including rabbit polyclonal anti-Plin2 (Thermo Scientific, Waltham Mass, MA, USA), mouse monoclonal and rat monoclonal anti-LOX1 (Santa Cruz Biotechnology, Delaware Avenue, Santa Cruz, CA, USA), mouse monoclonal anti-flag (Sigma-Aldrich, St. Louis, MO, USA), rabbit monoclonal anti-NF-κB p65 (Cell Signaling Technology, Beverly, MA, USA), rabbit monoclonal anti-phospho-NF-κB p65 (Ser536) (Cell Signaling Technology, Beverly, MA, USA), and rabbit polyclonal anti-β-actin (Sangon Biotech (Shanghai) Co., Ltd., Shanghai, China). Following primary antibodies incubation, the membranes were washed three times with TBST and then were incubated at room temperature for 1 h with horseradish peroxidase-conjugated secondary antibodies (1∶5 000 dilution; Cell Signaling Technology, Beverly, MA, USA). The membranes were washed three times with TBST and the protein bands were visualized using enhanced chemiluminescence (Sangon Biotech (Shanghai) Co., Ltd., Shanghai, China) and analyzed by densitometry using Image J software.

## 1.3 Reverse transcription−quantitative real−time PCR （RT−qPCR）

After treatment, isolation of total RNA from macrophages was performed using NucleoZol reagent (Gene Company Limited, Hong Kong, China) according to the manufacturer's instructions. Subsequently, RNA from each sample was reverse transcribed using the MightyScript First Strand cDNA Synthesis Master Mix kit (Sangon Biotech (Shanghai) Co., Ltd., Shanghai, China) in compliance with the manufacturer's instructions. The temperature protocol for this step was as follows: 25℃ for 5 min, 42℃ for 30 min and 85℃ for 5 min. Then a 2× SG Fast qPCR Master Mix kit (Sangon Biotech (Shanghai) Co., Ltd., Shanghai, China) with SYBR Green I fluorescent dye was used for PCR to quantify the gene expression. A comparative threshold cycle (Cq) method was used to calculate relative changes in gene expression determined from RT-qPCR experiments. The relative gene expression was calculated using the $2^{-\Delta\Delta Cq}$ method using GAPDH as an internal control. The following thermocycling conditions were used: initial denaturation at 95℃ for 3 min, and 45 cycles of 95℃ for 10 s and 55℃ for 30 s, and a final extension at 72℃ for 30 s. The primer sequences were presented as follows: GAPDH forward, 5'-CAAATTCAACGG-CACAGTCA-3' and reverse, 5'-CCCCATTTGATGT-TAGCGGG-3'; Plin2 forward, 5'-AACTTGGTAGA-TGGCTTTA-3' and reverse, 5'-CACCAGCCAGGT-AAGAG-3'.

## 1.4 BODIPY 493/503 staining

Lipid accumulation was measured by the fluorescent neutral lipid dye BODIPY 493/503

(4, 4-difluoro-1, 3, 5, 7, 8-pentamethyl-4-bora-3a, 4a-diaza-s-indacene; Sigma-Aldrich, St. Louis, MO, USA). The 4, 6-diamidino-2-phenylindole (DAPI; Shanghai Macklin Biochemical Co., Ltd, Shanghai, China) was used to identify nuclei of RAW264.7 cells. The cells were placed into 6-well plates with slides at a density of $4\times10^5$ cells/cm$^2$ and cultured for 24 h, then treated with 50 mg/L oxLDL for 0 h or 24 h, or pretreated with JSH-23 (30 μmol/L) for 1 h following treated with 50 mg/L of oxLDL for 24 h, and were rinsed twice with phosphate-buffered saline (PBS), then incubated in 2 μmol/L BODIPY staining solution in the dark for 30 min at 37℃. The cells were washed twice in 3 ml PBS and were fixed in 3 ml 4% paraformaldehyde (PFA) (Guangzhou Jetway Biotech Co., Ltd, Guangzhou, China) for 30 min at room temperature. Removed 4% PFA and washed samples three times with PBS. Use forceps to mount cover slips onto glass slides and imaged immediately with a Zeiss Axio Observer Inverted fluorescence microscope.

### 1.5 Data sets analysis

Transcriptome data was obtained from the GEO database (https://www.ncbi.nlm.nih.gov/gds). In order to illuminate the expression change of Plin2 and LOX1 in atherosclerosis, we downloaded the data of carotid artery atheroma (GSE43292) for gene expression (Affymetrix Human Gene 1.0 ST Array) ($n$=64) [18]. The data included 32 patients who experienced endarterectomy. So there were 32 carotid atheroma plaque samples and 32 macroscopically intact carotid tissues adjacent to the atheroma plaque samples respectively. The expression of Plin2 and LOX1 in the two kinds of samples were compared. To explore the mechanism of accumulation of lipids mediated by Plin2, the relationship between Plin2, LOX1 expression level and the activation of NF-κB signaling pathway was next analyzed by Gene Set Enrichment Analysis (GSEA) in a publicly available GEO database GSE43292.

### 1.6 Vector transfection and stable cell line construction

A length of 1 322 bp including flag label and mouse Plin2 coding sequence was synthesized by Guangzhou IGE Biotechnology Co. LTD (Guangzhou, China). Then the sequence was subcloned into the *Xba*I and *Not*I sites of pCDH-CMV-MCS-EF1-mcherry-T2A-puro empty vector. A targeted interfering sequence of mouse Plin2 coding sequence was synthesized by HonorGene (Changsha, China) and was subcloned into the *Bam*HI and *Eco*RI sites of pHG-LVsh empty vector. The Plin2 siRNA primer sequences were as follows: forward, ATGCA-CAGTGCCAACCAGA and reverse, TCTGGTTGG-CACTGTGCAT. The two recombinant vectors were validated by sequencing. To produce viral particles, *in vitro* 293FT cells were co-transfected with the vector pCDH-CMV-MCS-EF1-mcherry-T2A-Plin2 or pHG-musPlin2-sh and the helper plasmids psPAX2 and pMD2.G using Lipofectamine® 3000 reagent (Thermo Scientific, Waltham Mass, MA, USA) according to the manufacturer's instruction. Harvested supernatant was filtered through a 0.45 μm filter and used as rich viral source for transduction. The RAW264.7 cells were seeded in 6-well plates until they reach a cell density of approximately 70%−80% and then the filtrated virus mixed with final concentration of 8 mg/L polybrene was added to the plates and incubated for 24 h. Next, the culture medium was replaced by fresh DMEM containing 10% fetal bovine serum. Afterward, 48 h after infection with the viruses, puromycin were added to 6 mg/L, then maintained the concentration of puromycin as 3 mg/L. After 4 weeks of selection, individual colony was isolated and expanded. The cells were harvested and splited for analysis of Plin2 and LOX1 proteins.

### 1.7 Statistical analysis

Data were expressed as mean±standard deviation (SD). Statistical analysis were performed using GraphPad Prism software. All experiments were performed as three separate replicates. All data were analyzed using unpaired Student's $t$-test and one-way analysis of variance (ANOVA). $P<0.05$ were considered to indicate statistically significant differences.

## 2 Results

### 2.1 The expressions of Plin2 and LOX1 in atheroma plaque and in oxLDL incubated RAW264.7 cells

In order to illuminate the expression change of Plin2 and LOX1 in atherosclerosis, we downloaded the gene expression data from the GEO database of carotid artery atheroma (GSE43292). The results showed that the expressions of Plin2 and LOX1 in

carotid atheroma plaque samples (stage IV and over of the Stary classfication) containing core and shoulders of the plaque were significantly higher than that in the adjacent to the atheroma plaque samples (stages I and II) (Figure 1a, b).

Since the above analysis indicated the relationship between carotid atheroma plaque and Plin2, LOX1, we next examined the effects of oxLDL on Plin2 and LOX1 expressions in RAW264.7 cells and found that, oxLDL increased Plin2 and LOX1 expressions after oxLDL incubation for 24 h (Figure 1c−e).

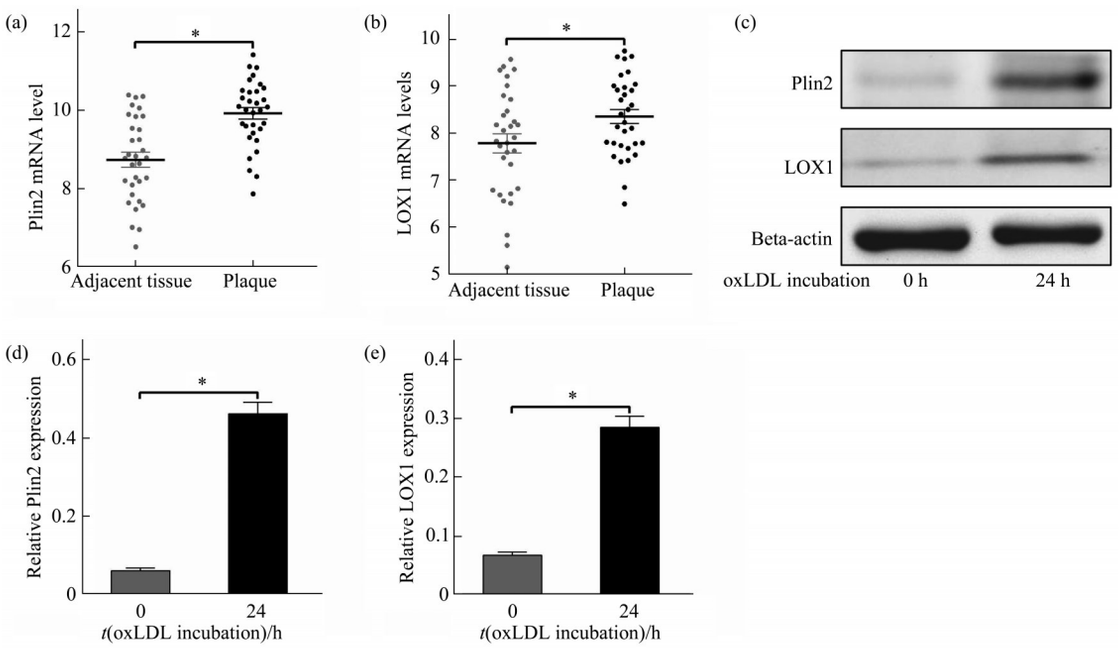


**Fig. 1 The expressions of Plin2 and LOX1 in atheroma plaque (*n*=32) and in oxLDL incubated RAW264.7 cells (*n*=3)**

(a, b) The expressions of Plin2 (a) and LOX1 (b) in the adjacent to the atheroma plaque samples were compared with carotid atheroma plaque samples. (c) The expressions of Plin2 and LOX1 protein immunoblotted with anti-Plin2 or anti-LOX1 antibodies after oxLDL incubation. (d, e) The results from densitometric analysis of Plin2 (d) and LOX1 (e) protein levels relative to beta-actin from 3 independent experiments were shown. Results were shown as *mean*±*SD*; **P*＜0.05.

## 2.2 The effect of Plin2 on the expression of LOX1

As we known that the expression of Plin2 increased in atherosclerosis, which participated in the process of cardiovascular diseases. And the above studies showed that the expression of LOX1 also elevated in atheroma plaque and in oxLDL incubated RAW264.7 cells. So we further analyzed whether the expression of LOX1 could be regulated by Plin2. Western blot analysis was used to detect the expression of LOX1 after Plin2 knockdown or overexpression in RAW264.7 cells. The Plin2 knockdown and overexpression were effective in cells (Figure 2a−c, e−g). And the overexpression Plin2 was accompanied by a significant increasing of LOX1 protein (Figure 2f, h). But Plin2 maked no difference on LOX1 when it was interfered (Figure 2b, d).

## 2.3 The possible signaling pathway was related to Plin2 expression

To explore the mechanism of Plin2 expression in the accumulation of intracellular lipid droplets, we next studied the relationship between Plin2/LOX1 expression level and the NF-κB signaling pathway using GSEA of GEO datasets. GSEA results showed that Plin2/LOX1 expression was positively correlated with the activation of NF-κB in a published available Genome-wide expression study of human carotid atheroma (GSE43292) (Figure 3), indicating that NF-κB may be related to the Plin2 expression.

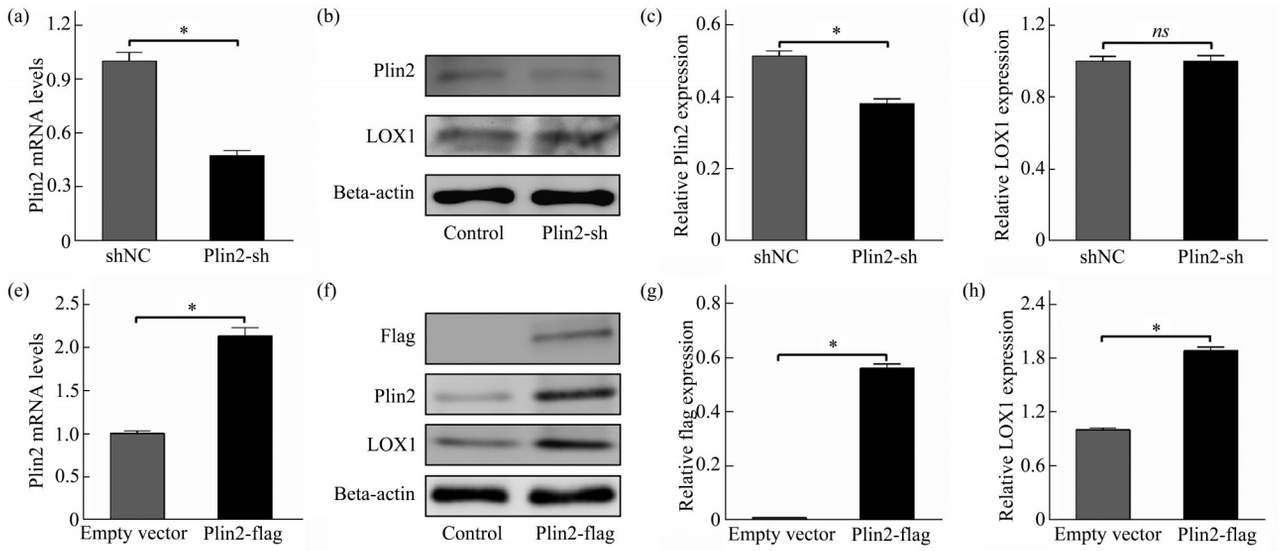


**Fig. 2　The effect of Plin2 on the expression of LOX1 (*n*=3)**

The mRNA and protein expressions of Plin2 were determined by RT-qPCR and Western blot after Plin2 knockdown (a, b) and overexpression (e, f). The protein expression of LOX1 was determined by Western blot after Plin2 knockdown (b) and overexpression (f). The results from densitometric analysis of Plin2 (c) , flag (g) and LOX1 (d, h) protein levels relative to beta-actin from 3 independent experiments were shown. Results were shown as *mean*±*SD*; *ns*, no significance; **P*＜0.05.

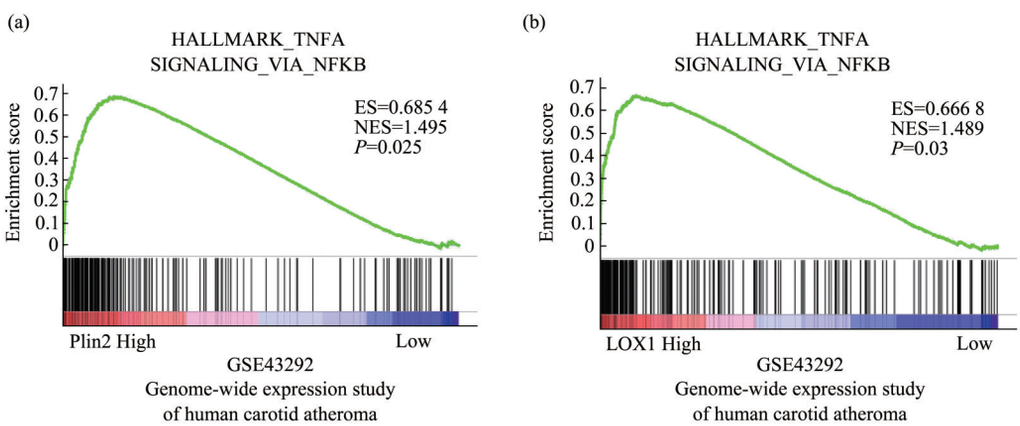


**Fig. 3　The correlation of Plin2 and LOX1 expression with the activation of NF−κB (*n*=64)**

GSEA plot showed that Plin2 (a), LOX1 (b) expression was positively correlated with the activation of NF-κB in a published available genome-wide expression study of human carotid atheroma (GSE43292). ES: enrichment score; NES: normalized enrichment score.

### 2.4　The effect of NF−κB inhibitor on Plin2 and LOX1 expression in oxLDL−induced RAW264.7 cells

To further uncover the NF-κB signaling pathway participated in Plin2-induced LOX1 expression under oxLDL incubation, RAW264.7 cells were pretreated with 30 μmol/L NF-κB inhibitor JSH-23[19] for 1 h, and then incubated with 50 mg/L oxLDL for 24 h. It shows that NF-κB activity was significantly inhibited after being pretreated with JSH-23 (Figure 4a, d), indicating that the inhibitor treatment was effective. Meanwhile, Plin2 and LOX1 expressions were significantly inhibited in spite of oxLDL treatment (Figure 4a−c). The amounts of intracellular lipid droplets also decreased after NF-κB inhibitor incubation (Figure 4e).

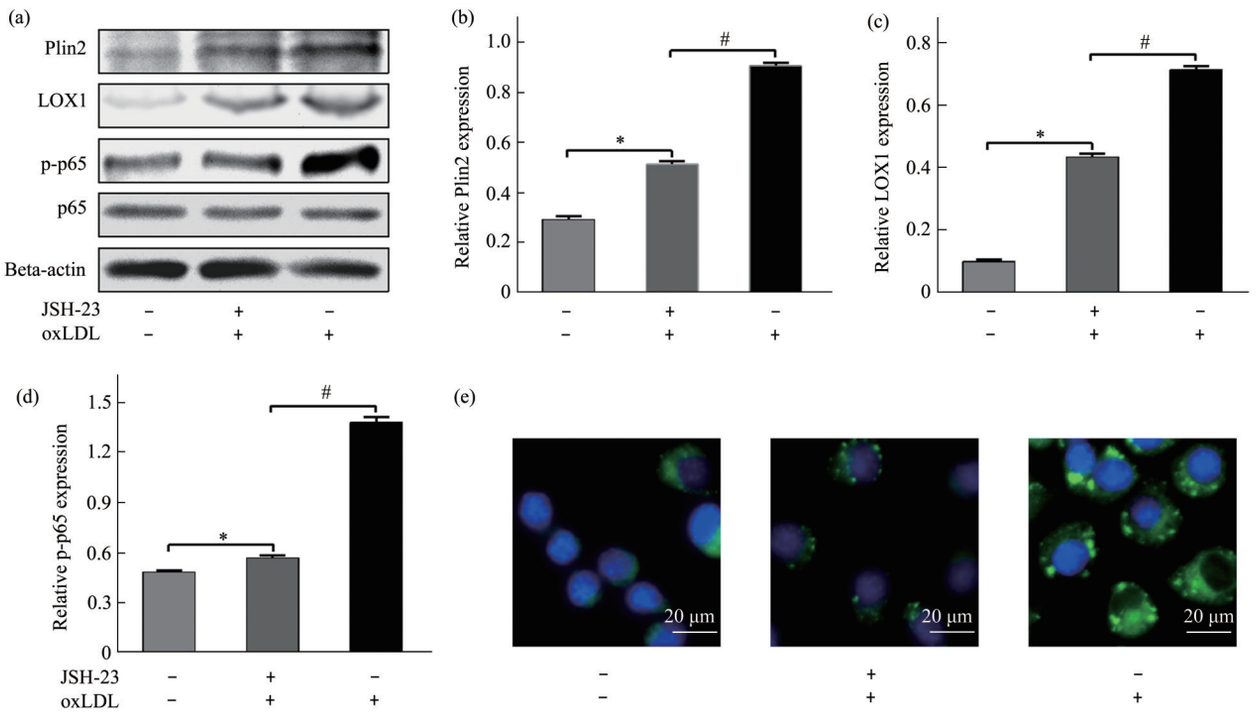


**Fig. 4　Plin2 and LOX1 expressions, p−p65 and intracellular lipid droplets change after NF−κB inhibitor JSH−23 pretreated (*n*=3)**

(a) The Western blot analysis for Plin2, LOX1, p-p65 and p65. (b−d) Results from densitometric analysis of Plin2, LOX1 and p-p65 protein levels relative to beta-actin from 3 independent experiments were shown as *mean*±*SD*; **P*＜0.05 versus the control group, #*P*＜0.05 versus the oxLDL incubation for 24 h. (e) The lipid droplets in cells after incubated with or without NF-κB inhibitor JSH-23 following with oxLDL for 0 h, 24 h. The cells were stained with BODIPY 493/503 staining.

### 2.5　The effect of NF−κB inhibitor on Plin2 and LOX1 expression in oxLDL−induced RAW264.7 cells after Plin2 overexpression or knockdown

To further confirm the effect of NF-κB signaling on Plin2 and LOX1 expression in oxLDL-induced intracellular lipid droplets accumulation. Firstly, we constructed the RAW264.7 cell lines of Plin2 overexpression or knockdown. Then the cells were pretreated with NF-κB inhibitor JSH-23 for 1 h and followed with oxLDL incubation for 24 h. The protein level of p-NF-κB, Plin2 and LOX1 were analyzed by Western blot, the amounts of intracellular lipid droplets were detected by BODIPY staining. The activity of NF-κB, the expressions of Plin2 and LOX1 were significantly inhibited after pretreatment with JSH-23 in spite of Plin2 overexpression plus oxLDL incubation (Figure 5a, c−e), indicating that inhibitor treatment was effectively inhibited the expressions of Plin2 and LOX1. Under the condition of oxLDL incubation, although the expressions of Plin2 were inhibited in Plin2 siRNA group after JSH-23 pretreatment compared with the Plin2 siRNA group without JSH-23 pretreatment, there was not statistically significant (Figure 5a, c). And under the condition of oxLDL incubation, the expressions of LOX1 and the protein level of p-NF-κB were inhibited in Plin2 siRNA group after JSH-23 pretreatment compared with the Plin2 siRNA group without JSH-23 pretreatment (Figure 5a, d, e). The lipid changes in cells was similar to the changes of Plin2 and LOX1 proteins (Figure 5b).

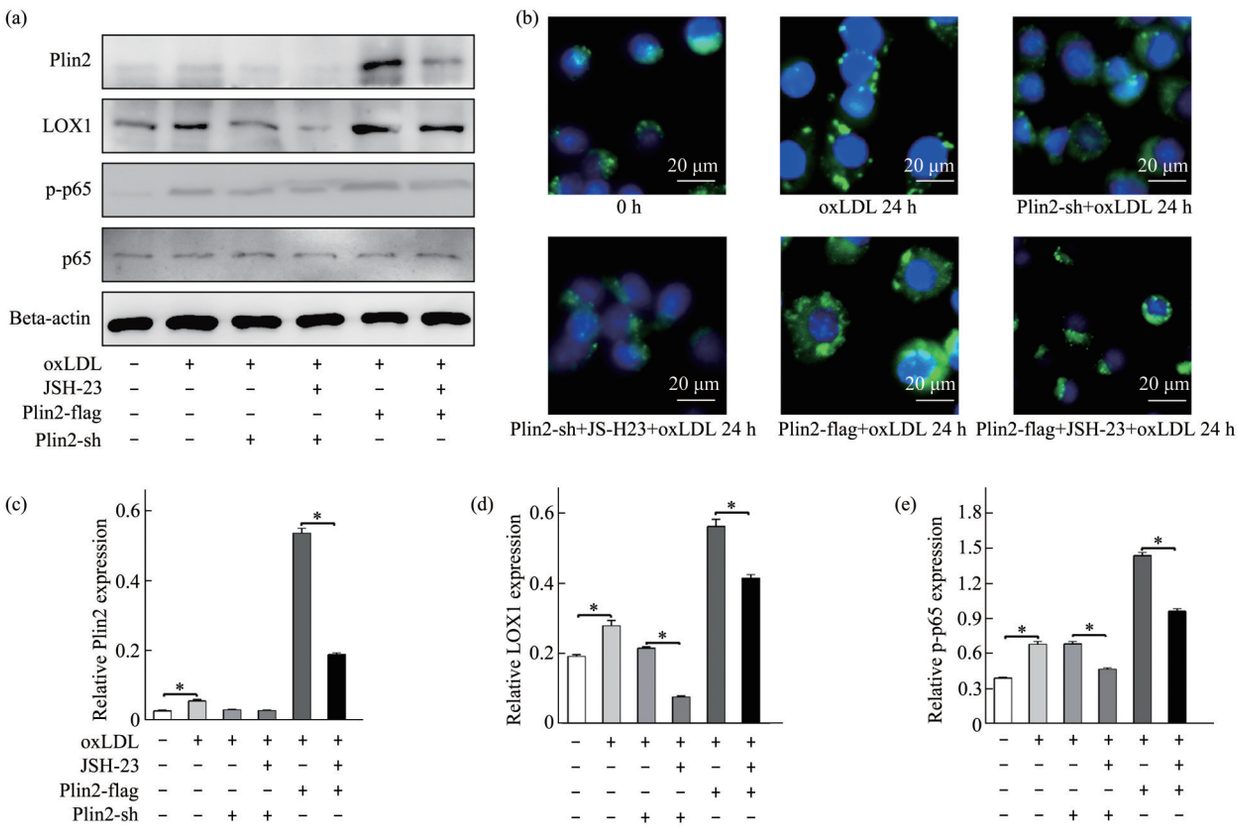


**Fig. 5　The expression of Plin2 and LOX1 and the accumulation of lipid droplets on NF−κB inhibitor in oxLDL−induced RAW264.7 cells after Plin2 overexpression or knockdown (*n*=3)**

(a) The level of LOX1, Plin2 and p-p65 proteins in Plin2 overexpression or knockdown cells with or without treatment of NF-κB inhibitor. (b) The cells were stained with BODIPY 493/503 staining. (c−e) Results from densitometric analysis of Plin2, LOX1 and p-p65 protein levels relative to beta-actin from 3 independent experiments were shown as *mean*±*SD*; **P*＜0.05.

## 3　Discussion

Atherosclerosis is a progressive disease of multifactorial origin, which occurs in response to endothelial injury. Plin2 was upregulated in atherosclerotic plaques and associated with the development of atherosclerosis[4]. Our previous studies suggested that oxLDL increased the expression of Plin2, which involved in the accumulation of lipid droplets and promoted the formation of foam cells[20]. In the study, we also found that the expression of Plin2 increased in atheroma plaque and was up-regulated by oxLDL in RAW264.7 cells. This was accompanied with lipid droplets accumulation. And there was a more obvious accumulation of lipid droplets in overexpression Plin2 plus oxLDL incubation cells than that in the only oxLDL incubation group. In contrast, the lipid droplets in Plin2 siRNA transfected cells significantly decreased compared with the control group after oxLDL incubation. Furthermore, other investigators have reported that the protein levels of Plin2 were significantly increased in patients with neoatherosclerosis after drug-eluting stent implantation[21]. The Pro251 variant of Plin2 increased autophagy activity, cholesterol efflux and a controlled inflammatory response, which exerted its beneficial effects on subclinical atherosclerosis[22]. Moreover, Plin2 overexpression was induced by proteasome impairment in monocytes of children with overweight/obesity and could contribute to the onset of arteropathy[23]. All of these results showed that Plin2 might play a pathogenetic role in the onset and progression of atherosclerosis. It even plays an important role in the clinical prognosis.

Owing to its central position in the pathogenetic mechanism, LOX1 is an attractive focus of

manipulation to regulate atherosclerosis. And it is one of the major contributors of oxLDL uptake in macrophages. Many studies have shown that LOX1 participated in the injuring of the endothelial cells and the following lipids, particularly oxLDL, accumulation in the subendothelial layer to promote the formation of atherosscleorsis[24-25]. And oxLDL-induced LOX1 activation may contribute to vascular dysfunction[26]. Moreover, atherosclerosis is associated with macrophage accumulation. LOX1 also has been shown to induce macrophage attachment. It is implicated in oxLDL induced oxidative stress of macrophages in atherosclerosis and *P. gingivalis*-induced monocyte migration and adhesion to endothelial cells[27]. And LOX1 targeting siRNA significantly reversed the alterations in oxidative stress parameters induced by oxLDL[28]. The deletion of LOX1 reduced atherosclerosis in LDL receptor KO mice fed a high cholesterol diet. And its deletion translates into reduction in macrophage trafficking in the aorta of LDL receptor KO mice[11]. Here we found that oxLDL increased the expression of LOX1 in cells. On the contrary, the augmented uptake of oxLDL was almost completely abrogated by treatment with an anti-LOX1 antibody[29]. These may provide many therapeutic ideas for targeting the role of LOX1 in atherosclerosis.

A lot of evidences showed that oxLDL could enhance the expression of Plin2 in RAW264.7 cells or peritoneal macrophages. Of note, similar to other studies, in this article we also found that oxLDL not only enhanced the expression of Plin2, but also induced of LOX1 expression[7,14,30] and NF-κB activation[31]. And All of them were related with the onset and progression of atherosclerosis. Furthermore, at the condition of inflammatory stimulation such as LPS could promote lipid accumulation *via* the upregulation of Plin2 expression and induced LOX1 expression, NF-κB nuclear translocation, oxLDL endocytosis and monocytes adhesion[32]. To elucidate the potential relationship of Plin2 and LOX1 in atherosclerosis, our present study identified that overexpressing Plin2 could increase the expression of LOX1, especially after oxLDL incubation. Surprisingly, Plin2 maked no difference on LOX1 when it was interfered. We speculated that there was other molecular regulated LOX1 such as miRNAs other than Plin2[33]. Taken together, these results showed that Plin2 could increase the expression of LOX1, and then promoted the development of atherosclerosis. On the basis of overexpression Plin2 further increasing the LOX1 expression, we speculated that Plin2 could participate in atherosclerosis progression by regulating the role of LOX1.

Atherosclerosis begins as an innate immune response to modified cholesterol-rich lipoproteins trapped within the subendothelial space of the vessel wall, recruiting inflammatory monocytes from the circulation[34]. These monocytes differentiate into macrophages that avidly engulf modified lipids such as oxLDL *via* scavenger receptors to become foam cells. These foam cells engaged the NF-κB pathway to transcriptionally activate the production of pro-inflammatory cytokines and chemokines to sustain the local inflammatory environment, resulting in additional monocyte recruitment[35]. In endothelial cells, knockdown RIPK1 prevented NF-κB translocation to the nucleus in response to TNFα, where accordingly there was a reduction in monocyte attachment[36]. And the potential impacts of saffron aqueous extract on atherosclerosis were related to both LOX1 and NF-κB[37]. LPS and oxLDL dramatically induced LOX1 expression *via* NF- κB pathway in vascular cells, and inhibiting of NF- κB resulted in a significant reduction of LOX1[29], which suggested that NF-κB was highly likely to be responsible for the upregulation of LOX1 expression[38-39]. In the study, GSEA results showed that Plin2, LOX1 expression was positively correlated with the activation of NF-κB in atherosclerosis, indicating that NF-κB may be related to the Plin2 and LOX1 expression. And we further found that the expressions of Plin2 and LOX1, the amounts of intracellular lipid droplets were reduced by NF-κB inhibitor JSH-23. These results suggested that Plin2 may regulate the expression of LOX1 by activating of NF-κB. Even though there were other signal pathways concerned with Plin2 regulation such as protein Kinase C (PKC) and acyl coenzyme A: cholesterol acyltransferase 1 (ACAT1).

Furthermore, unlike Plin2, many findings suggest that Plin1, another member of the PAT family, overexpression in macrophages protects against the progression of atheroma in *ApoE*-knockout mice, which partly because of its overexpression increased the size of lipid droplets in M1 macrophages. That is different members of the PAT family have different

roles in the progression of atherosclerotic lesions[40]. So we should study the roles of some PAT members in future for better understanding the pathogenesis of atherosclerosis.

## 4 Conclusion

This study demonstrated that oxLDL induced Plin2 promoted foam cell formation was at least partially mediated by NF-κB-Plin2-LOX1 pathway in a mouse macrophage-like cell line RAW264.7 cells (Figure 6). It suggested that a potential role of these genes product in the progression of atherosclerosis and their relationship that there was no report in previous literature. And for better prevention of atherosclerosis, we can use small molecule compounds for binding to LOX1 and NF-κB in combination with intervene of Plin2. If clinical samples and animal models of atherosclerosis such as mice can be obtained for further study, better evidence will be provided for our speculation. In conclusion, these unique signals in RAW264.7 cells induced by oxLDL may be one of the mechanisms in atherosclerosis progression, which may be a novel therapeutic approach for atherosclerosis about Plin2.

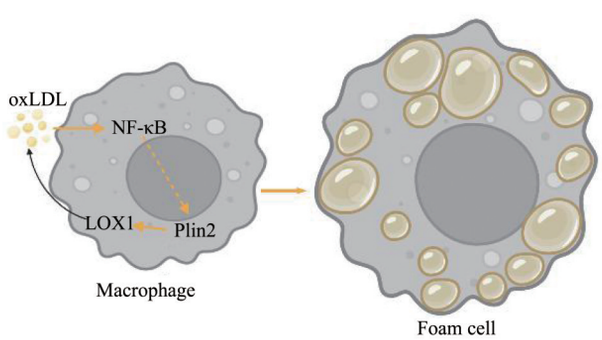


**Fig. 6 The mechanism of oxLDL induced Plin2 and foam cell formation in RAW264.7 macrophages cells**

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# Plin2通过NF-κB通路参与oxLDL诱导巨噬细胞LOX1的表达*

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**摘要　目的**　oxLDL可上调Plin2的表达，进而促进泡沫细胞的形成，LOX1是oxLDL的受体。本文探讨Plin2与LOX1在动脉粥样硬化发生发展过程中的关系。**方法**　从GEO数据库中下载GSE43292，分析Plin2、LOX1的表达及Plin2、LOX1与NF-κB信号通路的相关性。采用oxLDL处理的RAW264.7细胞作为动脉粥样硬化的细胞模型进行研究，蛋白质免疫印迹法检测细胞中Plin2、LOX1和p-p65的表达，荧光中性脂质染料BODIPY 493/503染色法检测细胞内脂滴。**结果**　通过分析GSE43292数据发现，Plin2、LOX1在颈动脉粥样硬化斑块中的表达显著高于颈动脉邻近组织。oxLDL处理RAW264.7细胞24 h后，Plin2与LOX1的表达、细胞内脂滴明显增加。过表达Plin2的细胞中LOX1表达升高；当用oxLDL孵育过表达Plin2的细胞后，LOX1的水平升高更为显著；但在没有oxLDL处理的情况下，敲减Plin2对细胞内LOX1的表达没有影响。基因集富集分析（gene set enrichment analysis，GSEA）结果显示，在动脉粥样硬化中，Plin2和LOX1的表达与NF-κB的活化呈正相关。此外，尽管采用oxLDL处理细胞，NF-κB抑制剂JSH-23预处理仍可显著降低Plin2与LOX1的表达、细胞内的脂质积聚，过表达Plin2后，JSH-23亦能显著抑制oxLDL孵育的细胞中Plin2和LOX1的表达。**结论**　Plin2可通过上调LOX1的表达促进细胞内脂质积聚，参与动脉粥样硬化，这一过程至少部分是通过激活NF-κB通路实现的。

**关键词**　Plin2，动脉粥样硬化，植物凝集素样氧化低密度脂蛋白受体1，核因子κB
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