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Daxx Mediates Oxidized Low-density Lipoprotein-Induced Cholesterol Accumulation and Apoptosis in Macrophages by Upregulating Caveolin-1 Expression*

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Abstract To explore whether Daxx mediates oxidized low-density lipoprotein (Ox-LDL)-induced cholesterol accumulation and apopotosis in macrophage and the underlying molecular mechanisms, intracellular lipid droplets and lipid levels were assayed by oil red O staining and high performance liquid chromatography (HPLC), respectively, the apoptotic effect of RAW264.7 cells induced by Ox-LDL was analyzed by flow cytometric analysis and acridine orange/ethidium bromide (AO/EB) staining, the mRNA expressions of Daxx was quantified by Real time RT- PCR, the protein expression of caveolin-1 was detected by Western-blotting, Daxx-specific small interfering RNA(Daxx siRNA) was transfected to RAW264.7 cell by lipofectamin. Ox-LDL up-regulated the expression of Daxx mRNA, increased the accumulation of intercellular cholesterol in RAW264.7 macrophages, and induced the apoptosis of RAW264.7 macrophages. However, Ox-LDL-induced intercellular cholesterol accumulation and apoptosis in RAW264.7 cells was prevented by Daxx siRNA. Ox-LDL also induced caveolin-1 expression and this effect is significantly suppressed by Daxx siRNA. It can be concluded that Daxx mediates Ox-LDL-induced cholesterol accumulation and apoptosis in macrophages by up-regulating caveolin-1 expression. These findings provide an important demonstration that Daxx might be associated with the development of atherosclerosis.

Key words Daxx, Ox-LDL, macrophage, apoptosis, caveolin-1
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Increasing evidence demonstrated that macrophages are intimately involved in atherosclerotic plaque formation and destabilization and arterial lesion formation [1-4]. It is now believed that apoptosis of lipid-containing macrophages contributes initially to lipid core formation and later to plaque instability [3]. Indeed, macrophage apoptosis occurs throughout all stages of atherosclerosis. Macrophage apoptosis in early lesions, with phagocytic clearance of apoptotic cells appears to be efficient, decreases macrophage burden, limits lesion cellularity and suppresses plaque progression [2, 4-5]. However, in advanced lesions, where phagocytic clearance of apoptotic macrophages is defective, macrophage apoptosis promotes the development of the necrotic core, which is thought to promote plaques disruption and acute thrombosis [2, 4]. Thus, further understanding of the mechanism involved macrophage apoptosis in advanced lesions

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will be critical for the formation of novel therapeutic approaches to combat atherosclerotic lesion progression[4].

We have recently demonstrated that the death-associated protein, Daxx plays an important role in cholesterol metabolism in hepatic cells, indicating that Daxx could be involved in the development of atherosclerosis [6]. Daxx, ubiquitously expressed and highly conserved in mammals, is a multifunctional protein that regulates a variety of cellular processes, including transcription and apoptosis [7]. Daxx plays a crucial role in the cellular apoptotic response induced by UV, oxidative stress, and glucose deprivation, in addition to its function during Fas mediated apoptosis[7].

Oxidized low-density lipoprotein (Ox-LDL) and intercellular cholesterol accumulation can induce apoptosis in cultured macrophages, and there is increasing evidence that both of these factors are the important inducer of macrophage apoptosis in atherosclerotic advanced lesions [1, 4, 8–10]. Ox-LDL also has been suggested as a major mechanism leading to macrophage cholesterol accumulation[11]. Therefore, we were interested in understanding whether Daxx is involved in Ox-LDL-induced cellular cholesterol accumulation and apoptosis in macrophage. In addition, caveolin-1 is increasingly believed to play a critical role in regulating the apoptosis and cellular cholesterol homeostasis in macrophage [12–13]. We also investigated the correlations between Daxx and caveolin-1 during regulation of cellular cholesterol accumulation and macrophages apoptosis.

1 Materials and methods

1.1 Cell and reagents

RAW264.7 murine macrophage-like cells, obtained from the Chinese Type Culture Collection (Beijing, China), were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) at 37℃ in humidified 5% CO_2 and 95% O_2. DMEM and FBS were purchased from Invitrogen/BRL (Grand Island, USA). Daxx siRNA and anti-caveolin-1 antibody were purchased from Santa Cruz Biotechnology (CA, USA). Ox-LDL was purchased from the Peking Union Institute of Biochemistry (Beijing, China).

1.2 Daxx small interfering RNA (siRNA) transfections

RAW264.7 cells (5×10^6) were seeded on six-well plates in the growth medium without antibiotics and allowed to reach 30%～50% confluence. Gene-specific siRNA oligomers (200 mmol/L) were diluted in 500 ml of Opti-MEM Ⅰ reduced serum medium (Opti-MEM; Invitrogen, CA, USA) and mixed with 5 ml of each Lipofectamin 2000 (Invitrogen) pre-diluted in 500 ml of Opti-MEM. After 20 min incubation at room temperature, the complexes were added to the cells in a final volume of 1 ml of medium. The Daxx siRNAs were 5′ GGAGUUGGACCUGUCAGAGCdTdT 3′ (sense) and 5′ GCUCUGACAGGUCCAACUCCdTdT 3′ (antisense). Transfected cells were then incubated for 48 h.

1.3 Apoptotic cells measured by flow cytometry (FCM) analysis

Treated RAW264.7 cells were collected after digestion with 0.25% trypsogen and washed twice with phosphate-buffered saline (PBS). Cells were then fixed with cold 70% ethanol at 4℃ overnight and then centrifuged at 250 *g* for 5 min, washed in PBS twice and resuspended in PBS at a concentration of 1×10^6 cells/ml. Cells were incubated with RNaseA for 45 min and then stained with 50 g/L propidium iodide (PI) in the dark at 4℃ for 60 min. The suspension was analyzed with a FACScan flow cytometer (EPICS-XL, Beckman Coulter, Fullerton, USA). The apoptotic rate was analyzed by FACScan software programs.

1.4 Apoptosis detected by acridine orange/ethidium bromide (AO/EB) staining

Morphological evidence of apoptosis was detected by AO/EB staining. Briefly, cells were incubated in different conditions and then harvested. After being fixed with 4% paraformaldehyde for 30 min at room temperature, the cells were washed 3 times with cold PBS and then stained with AO/EB (8 μl of a stock solution containing 100 mg/L AO and EB was added in 192 μl cells suspension) for 10 min. Stained cells were observed under a fluorescence microscope. After AO/EB staining the normal cells exhibited green nucleus and the apoptotic cells appeared orange nucleus.

1.5 Caveolin-1 expression analyzed by Western blot

SDS-polyacrylamide gel electrophoresis (PAGE) was carried out on 5% stacking and 12% resolving gel with low range molecular mass standards (Solarbio, China). Equal amounts of protein were loaded in each lane with loading buffer (Beyotime, China)

containing 0.1 mol/L Tris (pH6.8), 20% glycerol, 10% mercaptoethanol, 4% SDS and 0.2% Bromophenol Blue. Samples were heated at 100℃ for 5 min before gel loading. Following electrophoresis, the proteins were transferred to a PVDF transfer membrane (Solarbio, China). After this, the membranes were blocked with TBST (50 mmol/L Tris-HCl pH 7.4, 0.15 mol/L NaCl, 0.1% Tween-20) containing 5% BSA (Sigma, USA) for 2 h. Following this, the membranes were incubated with rabbit monoclonal anti-caveolin-1 primary antibodies (Santa Cruz Biotechnology, CA, USA) diluted 1∶1 000 at 4℃ over night. After washing with TBST, the membranes were incubated with anti-rabbit IgG labeled with horseradish peroxidase (Zsbio, China) diluted at 1∶1 000 at room temperature for 2 h. The membranes were washed again and developed with an enhanced chemiluminescence system (New England Biolabs, Beverly, MA, USA) followed by apposition of the membranes with autoradiographic films (Kodak, China). Anti-β-actin antibodies (1∶10 000; Santa Cruz) were used to quantify β-actin, used as the internal control. The integrated optical density for the protein band was calculated by Image-J software.

1.6 Daxx mRNA determined by quantitative Real-time PCR

Total RNA was extracted using RNA extraction Trizol reagent (Invitrogen). After contaminated genomic DNA was digested with DNase Ⅰ (Roche Diagnostics), first strand cDNA was synthesized using a SuperScript™ preamplification system (Invitrogen) from 2 μg of total RNA. PCR was performed using primers (sense and antisense) for cDNA 5′ CGT GGG CGT CAG GTT ACA G 3′ and 5′ ACC AGA TTC CGA GGA GGC TT 3′ (for Daxx) and 5′ CCT CTA TGC CAA CAC AGT GC 3′ and 5′ GTA CTC CTG CTT GCT GAT CC 3′ (for β-actin) (synthesized by Shanghai Kangchen Bio-technique Co., Ltd.). Primers were validated by analysis of template titration and dissociation curves. Each reaction (50 μl) contained 0.3 mmol/L primers, 25 μl of 2×SYBR Green PCR master mix reagent, and 2 μl of template and was amplified by 35 cycles of denaturation (95℃, 10 s), annealing (60℃, 15 s), and extension (72℃, 20 s). The quantification of Daxx and β-actin mRNA was achieved in an ABI PRISM 7700 sequence detection system (Applied Biosystems) and analyzed using ABI PRISM sequence detector software (version 1.6.3; Applied Biosystems). Transcript levels were normalized to the amount of β-actin transcript.

1.7 Intercellular cholesterol measured by high performance liquid chromatography (HPLC) analysis

Cells were scraped from the culture flasks into 0.9% NaCl (1 ml per 50 cm^2 flask) and homogenized on ice by sonication for 10 s. An equal volume of freshly prepared cold (–20℃) KOH in ethanol (150 g/L) was added to cell lysates, and the mixture was repeatedly vortexed until clear. An equal volume of 3∶2 hexaneisopropanol (v/v) was then added. The mixture was centrifugated at 800 g (15℃) for 5 min. The extraction procedure was repeated twice. The combined organic phase was transferred to clean tapered glass tubes and thoroughly dried under nitrogen at 40℃. 100 μl isopropanol-acetonitrile (20∶80, v/v) was added. After solubilization and centrifugation, the samples were introduced into HPLC device (Agilent 1100, Agilent Technologies, Inc, Palo Alto, USA). Cholesterol was eluted with 1 ml/min of eluent consisting of 20∶80 isopropanol-acetonitrile (v/v) and detected by ultraviolet absorption at 206 nm.

1.8 Lipid droplet observed by using oil red O staining

Cells were washed 3 times with phosphate-buffered saline (PBS) and were then fixed with 10% formalin at room temperature for 10 min. After being fixed, the cells were stained with oil red O solution (stock solution: 3 g/L oil red O dissolved in isopropanol, working solution: 60% oil red O stock solution and 40% distilled water) at 60℃ for 10 min and then counterstained with haematoxylin for 1 min. Microscopic images were obtained under an Olympus microscope (Tokyo, Japan).

1.9 Statistical analysis

A two-tailed Student's t-test was used for statistical comparisons between any two specific experimental groups as indicated in the text and figure legends. The results were expressed as $\bar{x} \pm s$. $P < 0.05$ was considered statistically significant.

2 Results

2.1 Ox-LDL up-regulates *Daxx* mRNA expression in RAW264.7 cells

To test the specific role of Daxx during Ox-LDL-induced macrophage apoptosis, we first analyzed the effects of Ox-LDL on Daxx expression in RAW264.7

macrophages. After 48 h incubation with 12.5, 25, 50, or 75 mg/L of Ox-LDL, Daxx expression in the total cell lysate was, respectively, 1.05-, 3.24-, 4.24- or 2.64-fold higher than that in control cells (Figure 1a). When macrophages were incubated for 12, 24, 48 or 72 h with 50 mg/L of Ox-LDL, Daxx expression was 0.94-, 2.94-, 4.24-, or 3.55-fold higher, respectively, than that in control cells (Figure 1b). The results indicated that Ox-LDL up-regulate the expression of Daxx in RAW264.7 macrophages.

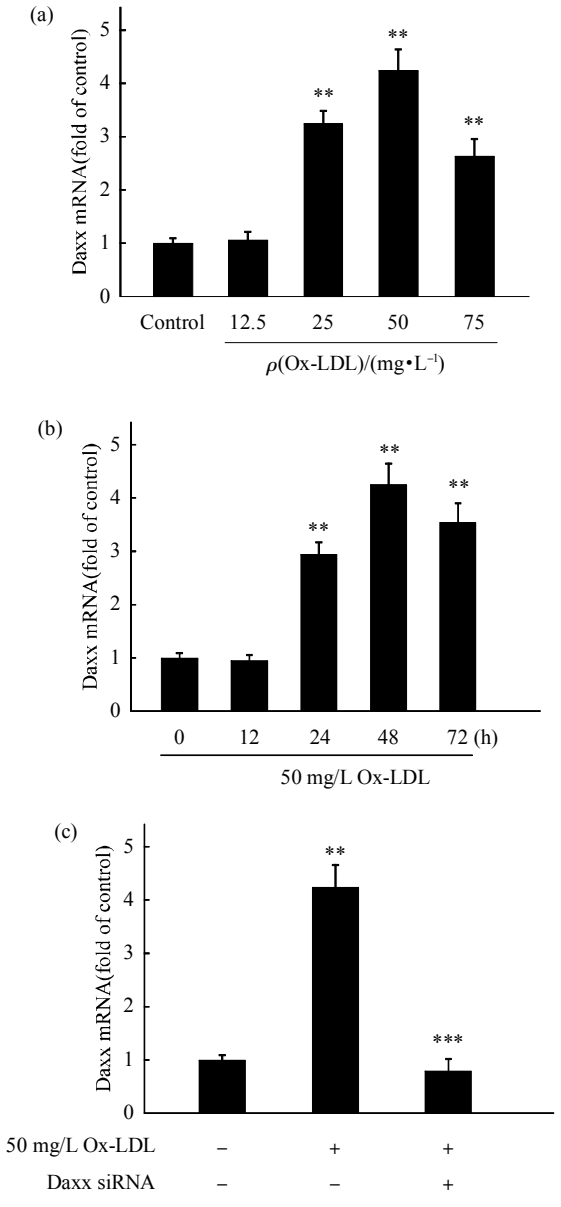

Fig. 1 The effect of Ox-LDL on Daxx mRNA expression in RAW264.7 cells

RAW264.7 cells were incubated with 0～75 mg/L of Ox-LDL for 48 h (a), with 50 mg/L of Ox-LDL for 12, 24, 48 or 72 h (b) or transfected with Daxx siRNA for 48 h, and then treated with 50 mg/L of Ox-LDL for 48 h (c), then Daxx mRNA expression was analyzed by quantitative Real-time PCR after normalization to β-actin mRNA. The data are expressed as a fold level of the control value and are the $\bar{x} \pm s$ for three independent experiments. $^{**}P < 0.01$, compared to control (untreated cells); $^{***}P < 0.01$, compared to 50 mg/L of Ox-LDL- treated cells.

In the present study, RNA interfering was used to examine the specific role of Daxx in Ox-LDL-induced macrophage apoptosis. Knockdown of Daxx was performed by transfection with Daxx-specific small interfering RNA (Daxx siRNA). The effectiveness of the siRNA treatment was validated by the result showing that Daxx siRNA completely prevented the up-regulation of Daxx expression induced by Ox-LDL (50 mg/L, Figure 1c).

2.2 Daxx siRNA attenuates apoptosis of RAW264.7 cells induced by Ox-LDL

Next, we investigated whether Ox-LDL induced RAW264.7 cells apoptosis and whether Daxx could be involved in the effect of Ox-LDL on RAW264.7 cells apoptosis. The concentration and time dependency effect of Ox-LDL on apoptosis of RAW264.7 cells were examined by FCM. Ox-LDL, at the concentrations of 12.5, 25, 50, or 75 mg/L for 48 h, increased the apoptotic rate of RAW264.7 cells in a dose-dependent manner (Figure 2a). When macrophages were incubated for 12, 24, 48 or 72 h with 50 mg/L Ox-LDL, the apoptotic rate of RAW264.7 cells were increased in a time-dependent manner (Figure 2b). However, the increase in apoptosis of RAW264.7 cells in response to Ox-LDL treatment was markedly inhibited by transfected with Daxx siRNA (Figure 2c). These data indicate that Daxx mediates Ox-LDL-induced apoptosis in macrophage.

Similarly, the nuclear staining assay by AO/EB staining for apoptosis also indicated that Daxx mediates Ox-LDL-induced apoptosis in macrophage. As shown in Figure 2d, exposed to 50 mg/L Ox-LDL for 48 h, RAW264.7 cells showed typical apoptotic morphological changes. However, in the cells which transfected with Daxx siRNA, the number of apoptotic cell induced by Ox-LDL was significantly reduced.

2.3 Daxx siRNA prevents Ox-LDL-induced cholesterol accumulation in RAW264.7 cells

Since intracellular cholesterol accumulation is a potent inducer of apoptosis in macrophage[1], we tested whether Ox-LDL induced cholesterol accumulation and whether Daxx could mediate the cholesterol accumulation induced by Ox-LDL. As shown in Figure 3a～c, the intracellular cholesterol was examined

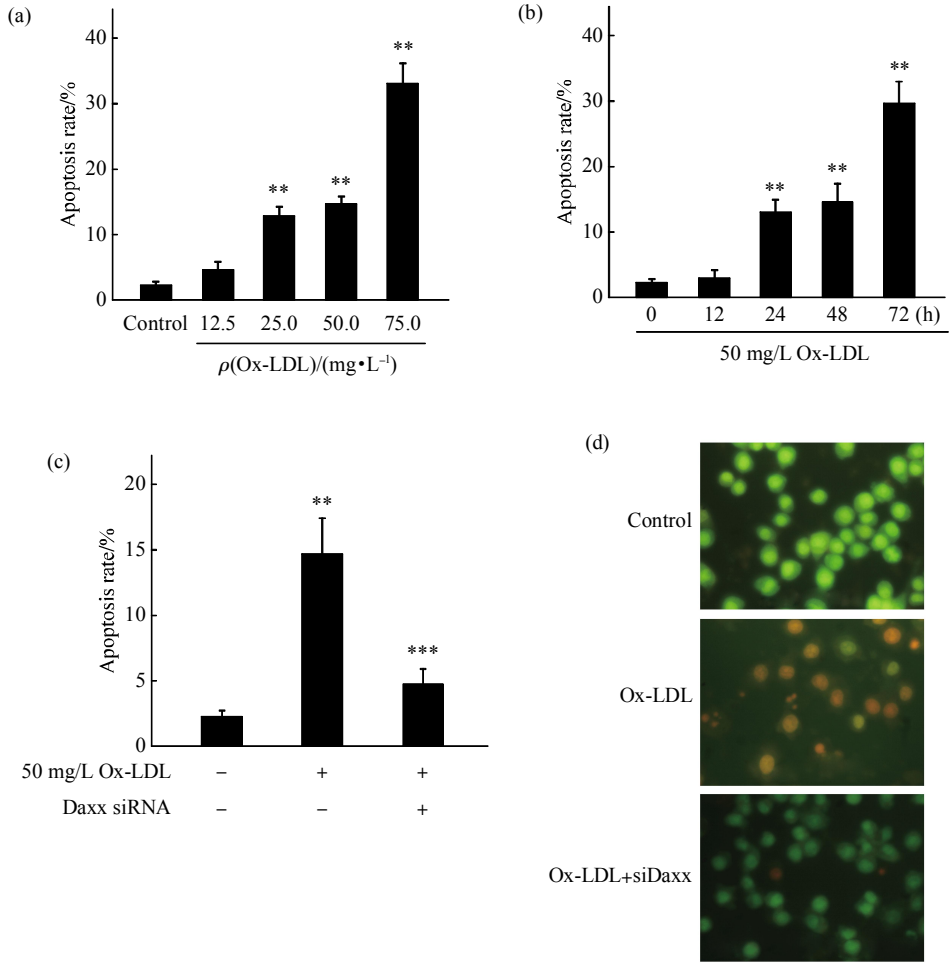

Fig. 2　Effect of Ox-LDL on apoptosis of RAW264.7 cells

(a)～(c) Cell apoptosis was detected by flow cytometry in RAW264.7 cells incubated with 0～75 mg/L of Ox-LDL for 48 h (a), with 50 mg/L of Ox-LDL for 12, 24, 48 or 72 h (b) or transfected with Daxx siRNA for 48 h, and then exposed to 50 mg/L of Ox-LDL for 48 h (c). The data are expressed by $\bar{x} \pm s$ for three independent experiments. $^{**}P < 0.01$, compared to control (untreated cells); $^{***}P < 0.01$, compared to 50 mg/L of Ox-LDL-treated cells. (d) Analysis of apoptosis by AO/EB staining in untreated RAW264.7 cells, RAW264.7 cells treated with 50 mg/L of Ox-LDL for 48 h, or Daxx siRNA-transfected RAW264.7 cells exposed to 50 mg/L of Ox-LDL for 48 h. Apoptotic cells were visualized under a fluorescent microscope (magnification × 400). The normal cells exhibited green nucleus and cell with orange nucleus is apoptotic.

by HPLC. Ox-LDL, at the concentrations of 12.5, 25, 50, or 75 mg/L for 48 h, increased the level of intracellular cholesterol in RAW264.7 cells in a dose-dependent manner (Figure 3a). When macrophages were incubated for 12, 24, 48 or 72 h with 50 mg/L of Ox-LDL, the level of intracellular cholesterol in RAW264.7 cells were increased in a time-dependent manner (Figure 3b). However, the increase in intracellular cholesterol in RAW264.7 cells in response to Ox-LDL treatment was markedly prevented by transfected with Daxx siRNA (Figure 3c). These data indicated that Daxx promoted Ox-LDL-induced cholesterol accumulation in macrophage.

Consistently with HPLC data, the oil red O staining for analysis of lipid droplets also indicated that Ox-LDL promotes intracellular cholesterol accumulation and Daxx mediates Ox-LDL-induced cholesterol accumulation in macrophage. Many lipid droplets were visualized in RAW264.7 cells treated with Ox-LDL(50 mg/L) for 48 h; however, the increase in lipid droplet caused by Ox-LDL was attenuated by transfected with Daxx siRNA (Figure 3d).

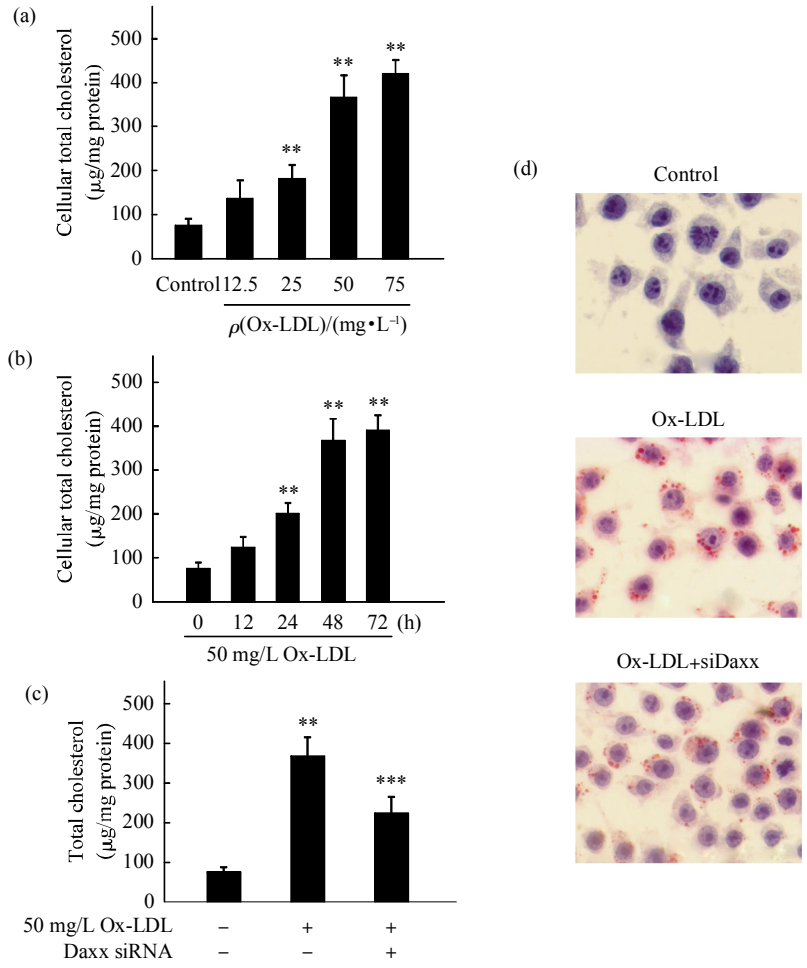

Fig. 3　Effect of Ox-LDL on cholesterol accumulation in RAW264.7 cells

(a)～(c) The levels of cellular cholesterol were detected by HPLC in RAW264.7 cells incubated with 0～75 mg/L of Ox-LDL for 48 h (a), with 50 mg/L of Ox-LDL for 12, 24, 48 or 72 h (b) or transfected with Daxx siRNA for 48 h, and then treated with 50 mg/L of Ox-LDL for 48 h (c). The data are expressed by $\bar{x} \pm s$ for three independent experiments. $^{**}P < 0.01$, compared to control (untreated cells); $^{***}P < 0.01$, compared to 50 mg/L of Ox-LDL-treated cells. (d) Analysis of lipid droplet by oil red O staining in untreated RAW264.7 cells, RAW264.7 cells treated with 50 mg/L of Ox-LDL for 48 h, or Daxx siRNA-transfected RAW264.7 cells exposed to 50 mg/L of Ox-LDL for 48 h. Lipid droplet after oil red O staining was visualized under a microscope (magnification × 400).

2.4　Daxx siRNA inhibits Ox-LDL-induced up-regulation of caveolin-1 in RAW264.7 cells

Caveolin-1 plays an important role in regulating the apoptosis and cellular cholesterol homeostasis in macrophage [12–13]. We tested whether Ox-LDL up-regulated caveolin-1 expression and whether Daxx could be involved in the up-regulation of caveolin-1 expression caused by Ox-LDL. Western blotting with antibodies to caveolin-1 revealed that the expression of caveolin-1 was at a higher level in Ox-LDL-treated cells compared to normal cells. As shown in Figure 4a, when RAW264.7 cells were exposed to 50 mg/L Ox-LDL for different hours, the expression of caveolin-1 protein increased in a time-dependent manner. However, Daxx siRNA suppressed significantly the up-regulation of caveolin-1 expression induced by 50 mg/L Ox-LDL (Figure 4b).

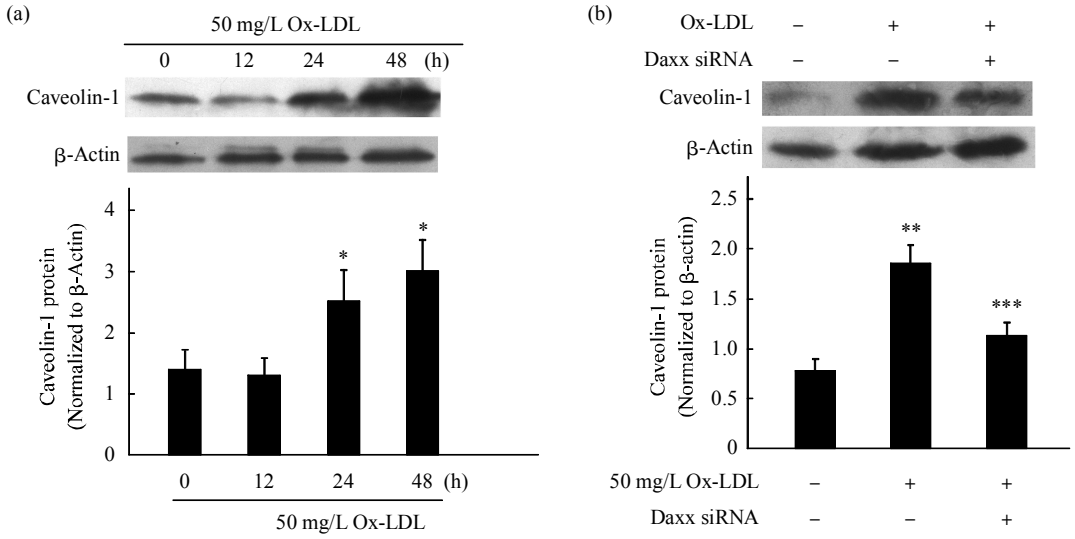

Fig. 4　Effect of Ox-LDL on caveolin-1 expression in RAW264.7 cells

(a) The levels of caveolin-1 expression were detected by Western blot in RAW264.7 cells incubated with 50 mg/L of Ox-LDL for 12, 24, or 48 h (a) or transfected with Daxx siRNA for 48 h, and then treated with 50 mg/L of Ox-LDL for 48 h (b). The data are expressed by $\bar{x} \pm s$ for three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, compared to control (untreated cells); $^{***}P < 0.01$, compared to 50 mg/L of Ox-LDL-treated cells.

3　Discussion

Apoptotic macrophage-foam cells represent a prominent feature of advanced atherosclerotic lesions[1–2, 4]. In advanced lesions, macrophage apoptosis causes lesional necrotic core formation, an event that is strongly associated and almost certainly promotes plaque rupture [2, 4]. Plaque rupture, in turn, leads to acute atherothrombotic vascular occlusion and tissue infarction [14]. Thus, elucidating the mechanisms of macrophage death in atherosclerotic advanced lesions is an important step for understanding the most critical stage of atherosclerotic vascular disease.

Ox-LDL plays a central role in the development and progression of atherosclerotic lesions [15]. The hypothesis that Ox-LDL mediate atherogenesis by inducing macrophage apoptosis has received widespread attention [4, 16–17]. In the present study, we found that Ox-LDL induced apoptosis of RAW264.7 cells in a time- and concentration-dependent manner. Our observation is consistent with the notion that Ox-LDL works as an important inducer of macrophage apoptosis [4]. The accumulation of intracellular free cholesterol has been identified as a unique inducer of macrophage death in advanced atherosclerotic lesions[1, 4]. It has been reported that cholesterol accumulation can induce apoptosis in cultured macrophages[10, 18]. In the present work, our data demonstrated that Ox-LDL could promote the accumulation of cholesterol in RAW264.7 macrophages. Ox-LDL has been suggested as a major mechanism leading to macrophage lipid accumulation[11]. Thus, our results suggest that Ox-LDL can directly induce macrophage apoptosis; on the other hand, it also can enhance cholesterol accumulation to lead macrophage apoptosis.

In this work, the most important finding is that Daxx mediates Ox-LDL-induced cholesterol accumulation and apoptosis in macrophages. Quantitative RT-PCR revealed that Ox-LDL increased the mRNA expression of Daxx mRNA in a dose- and time-dependent manner, which is companied with the increase in intercellular cholesterol accumulation and apoptotic development. Furthermore, Daxx specific siRNA prevents Ox-LDL-induced intercellular cholesterol accumulation and apoptosis in RAW264.7 macrophages. Therefore, we suggested that Daxx might mediate the cellular cholesterol accumulation and apoptosis induced by Ox-LDL in murine RAW264.7 macrophages. Several lines of evidence have indicated that Daxx plays a crucial role in the

cellular apoptotic response induced by UV, oxidative stress, and glucose deprivation [7, 19–22]. Our data are consistent with these previous studies[7, 19–22]. Our recent study also demonstrated that up-regulation of Daxx expression mediates Ox-LDL-induced apoptosis of THP-1 macrophages[23].

Caveolin-1 has been suggested to play a critical role in the development of atherosclerosis[3, 24]. Caveolin-1 expression is increased in atherosclerotic lesions in hypercholesterolemic rabbits and apo-E-deficient mice[25]. It is believed that caveolin-1 plays a critical role in regulating the cellular cholesterol homeostasis and apoptosis in macrophage [12–13, 26–27]. Matveev, *et al*.[26] demonstrated that up-regulation of caveolin-1 is accompanied by an increase in selective cholesterol ether uptake in THP-1 macrophages. Gargalovic, *et al*.[13] identified that increased caveolin-1 expression is associated with cellular apoptosis in macrophage. Our previous study has suggested that Daxx could up-regulate caveolin-1 expression[6]. Thus, an important question that arises is whether Daxx mediate Ox-LDL-induced cholesterol accumulation and apoptosis in macrophages through regulating caveolin-1 expression. In the present study, our results demonstrated that treatment of RAW264.7 macrophages with Ox-LDL increased the level of caveolin-1 protein in a time-dependent manner, which was accompanied by an increase in Daxx expression. Moreover, the up-regulation of caveolin-1 expression induced by Ox-LDL was significantly suppressed by Daxx siRNA. Taken together, our results indicate that the role of Daxx in mediating Ox-LDL-induced cholesterol accumulation and apoptosis in macrophages is involved in up-regulation of caveolin-1 expression.

However, the involvement of caveolin-1 in atherosclerosis still remains controversial[3, 28]. In a recent finding, caveolin-1 was identified as a novel factor for preventing human atherosclerotic disease[29]. Indeed, caveolin-1 plays a rather complex, either positive or negative, role in the progression of atherosclerosis, depending on the cell types examined[28]. In endothelial cells, evidence has accumulated that caveolin-1 plays a pro-atherogenic role[24, 28]. In contrast, in vascular smooth muscle cells, the ability of caveolin-1 may be anti-atherogenic [30–31]. Finally, caveolin-1 in macrophages may have a pro-atherogenic effect [12–13, 25]. Our results supported the notion that caveolin-1 in macrophages plays a pro-atherogenic role.

Taken together, the present study showed that treatment with Ox-LDL could induce cholesterol accumulation and apoptosis in RAW264.7 cells, which is accompanied with an increased expression of Daxx and caveolin-1. Daxx siRNA could suppress the enhancement of cholesterol accumulation and apoptosis induced by Ox-LDL, and decrease caveolin-1 expression up-regulated by Ox-LDL in RAW264.7 cells. These data suggest that Daxx mediates Ox-LDL-induced cholesterol accumulation and apoptosis in macrophages by up-regulating caveolin-1 expression. Our findings provide an important demonstration that Daxx is associated with the development of atherosclerosis. Daxx may be considered as a novel target in the prevention of atherosclerosis.

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Daxx 通过上调 caveolin-1 的表达介导 Ox-LDL 诱导巨噬细胞胆固醇蓄积和凋亡 *

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摘要　为探讨 Daxx 对氧化型低密度脂蛋白(oxidized low-density lipoprotein，Ox-LDL)诱导巨噬细胞胆固醇蓄积和凋亡的介导作用及其可能的分子机制，用高效液相色谱法检测细胞内胆固醇含量，油红 O 染色观察细胞内脂滴的形成情况，流式细胞术和吖啶橙 / 溴化乙锭(AO/EB)染色法研究 Ox-LDL 对细胞凋亡的影响，Real time RT-PCR 检测细胞内 Daxx mRNA 的表达水平，Western blot 检测 caveolin-1 蛋白的表达，用特异性 siRNA 沉默 Daxx 在 RAW264.7 细胞中的表达．Ox-LDL 上调 Daxx mRNA 和 caveolin-1 的表达、增加细胞内胆固醇含量、促使 RAW264.7 细胞凋亡，用特异性 siRNA 干扰 Daxx 在 RAW264.7 细胞中的表达能降低 caveolin-1 的表达、减少细胞内胆固醇含量、以及抑制细胞凋亡．上述结果表明，Daxx 对 Ox-LDL 诱导 RAW264.7 巨噬细胞胆固醇蓄积和凋亡具有介导作用，这一作用可能与 Daxx 上调 caveolin -1 的表达有关．

关键词　Daxx，氧化型低密度脂蛋白，巨噬细胞，凋亡，caveolin-1
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