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Original Article

APE1 inhibits foam cell formation from macrophages via LOX1 suppression

Zhaohui Hu[1*], Bo Hui[2*], Xuwei Hou[3], Ruhui Liu[1], Sergiy Sukhanov[4], Xiaohong Liu[5]

[1]Department of Cardiovascular Diseases, Tongji Hospital of Tongji University, Shanghai 200065, P. R. China; [2]Department of Cardiovascular Diseases, Qingdao Municipal Hospital of Qingdao University, Qingdao, P. R. China; [3]Department of Cardiology, The University of Missouri School of Medicine, Columbia, MO 65201, USA; [4]Heart and Vascular Institute, Tulane University School of Medicine, New Orleans, LA 70112, USA; [5]Cardiovascular Department of Internal Medicine, Central Hospital of Karamay, Karamay 834000, Xinjiang Uyghur Autonomous Region, P. R. China. [*]Equal contributors.

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Abstract: Background: Macrophage activation and massive foam cell formation are key events in the development of Atherosclerosis (AS). Apurinic apyrimidinic endonuclease 1/Redox factor-1 (APE1) is an enzyme responsible for DNA repair and redox regulation. Recent studies indicate that APE1 is also involved in inflammatory response. We sought to explore its effect on oxidized low-density lipoprotein (oxLDL) induced macrophage activation and foam cell formation. Methods: Human macrophage cell line THP-1 cells were cultured and treated with oxLDL. The mRNA and protein levels of inflammatory markers for macrophage activation were measured. Foam cell formation was detected by Oil red O staining. Meanwhile the major cellular receptors responsible for oxLDL uptake and efflux were detected. Chromatin immunoprecipitation-quantitative real time PCR (ChIP-qPCR) and dual luciferase reporter assays were performed to identify the molecular mechanisms through which APE1 affects macrophage activation and foam cell formation. Results: Aberrant APE1 expression dramatically decreases the mRNA and protein of oxLDL-induced inflammatory molecules in THP-1 cells, accompanied by significantly inhibited foam cell formation. Western blot assay showed that down-regulation of LOX1, a receptor of oxLDL, is responsible for the inhibitory effect of APE1 on oxLDL induced macrophage inflammation. ChIP-qPCR assay showed that APE1 inhibits binding of the LOX1 promoter to its transcription factor Oct1, leading to suppression of LOX1. Conclusion: Our data confirm the anti-inflammatory properties of APE1 and for the first-time report that APE1 suppresses foam cell formation from macrophages via the oxLDL receptor LOX1. This finding indicates that APE1 can be a therapeutic target for AS prevention.

Keywords: Oxidized low-density lipoprotein, apurinic apyrimidinic endonuclease 1/redox factor-1, macrophage, atherosclerosis

Introduction

Atherosclerosis (AS) is the pathological basis of cardio- and cerebrovascular disease [1]. As a chronic inflammatory process, AS is characterized by oxidized low-density lipoprotein (oxLDL) induced macrophage activation and massive foam cell formation in the vascular wall [2].

Apurinic apyrimidinic endonuclease 1/Redox factor-1 (APE1/Ref-1, abbreviated hereafter as APE1) is an enzyme primarily responsible for DNA repair and redox regulation [3]. Recent studies have reported that APE1 regulates the activation of inflammation regulatory transcription factors, such as Nuclear Factor kappa-light-chain-enhancer of activated B (NF-κB) and hypoxia-inducible factor (HIF)-1α, etc., implying it may play a role in inflammation regulation [4]. Indeed, it has been shown that APE1 inhibits adhesive molecules expression and inhibits inflammatory cytokines binding to their receptors in stimulated endothelial cells. APE1 in endothelial cells mitigates TNF-α-induced monocyte adhesion and expression of vascular cell adhesion molecules via a NOS-dependent mechanism [5]. APE1 regulates inflammatory response in lypopolysaccharide (LPS)-stimulated macrophages [6] and also is a key modula-

tor of aluminum-induced neuroinflammation in the brain [7]. Collectively, these previous studies suggest the importance of APE1 in inflammation regulation.

Macrophage activation and foam cell formation is a hallmark of AS [2, 8]. However, whether APE1 affects foam cell formation from macrophages has not been reported. In this study, we found that APE1 over-expression blunts oxLDL induced macrophage activation and inhibits foam cell formation capacity, suggesting that APE1 may serve as a promising target for AS prevention.

Methods

Cell lines and treatment

The human monocyte THP-1 cell line (ATCC TIB-202, American Type Culture Collection [ATCC]) was cultured in RPMI-1640 medium (ATCC 30-2001, ATCC) supplemented with 2 mM glutamine, 10% FBS, and 1% penicillin and streptomycin. All cells were maintained at 37°C at 5% CO_2 levels. For cell stimulation, oxLDL (Invitrogen, USA) was applied at the desired concentrations.

Construction of vectors and cell line transfection

The cDNA encoding APE1 was amplified and subcloned into the EcoR1 and Xho1 sites of the pcDNA3 vector (Invitrogen, USA). THP1 cells were transfected using lipofectamine 3000 (Invitrogen, USA). The expression of APE1 were evaluated using real-time Polymerase chain reaction (PCR) and Western blot assays. The LOX1 expression plasmids were constructed with a similar approach.

Cellular cholesterol and cholesteryl ester measurements

Cellular total cholesterol, free cholesterol and cholesteryl ester in THP-1 were analyzed using a Cholesterol/CE Quantitation Kit II (Biovision, USA) in compliance with the manufacturer's instruction. The concentration of cellular-protein was calculated using a Pierce BCA Protein Assay Kit (Thermo Scientific). Three independent assays were performed.

Analysis of DiI-oxLDL uptake

The fluorescence-labelled oxidative LDL (DiI-oxLDL, Invitrogen, USA) was used for this study. Cells were co-incubated with DiI-oxLDL (50 μg/ml) at 37°C for 4 hr. Fluorescence intensity was detected using a fluorescence microscope and calculated using Image Pro Plus software. Three independent experiments were performed.

Cholesterol efflux analysis

Cholesterol efflux to HDL was analyzed after HDL (Invitrogen, USA) incubation in RPMI for 6 h (100 μg/mL). After incubation, both extracellular (in cell growth medium) and intracellular (in cells) total (TC) and free (FC) cholesterol were analyzed. The Amplex Red Cholesterol Assay Kit (Invitrogen, USA) was used according to the manufacturer's protocol. Cholesterol esters (CE) were calculated as the difference between TC and FC. The HDL-mediated net cholesterol efflux was calculated by subtraction of the cholesterol mass of the medium from that of the cells. The CE/FC ratio was calculated.

Measurements of inflammatory cytokines secretion

Concentrations of IL6, TNF-α and IL1β were measured in the supernatants obtained from the control and oxLDL stimulated THP1 cells in the presence or absence of APE1 over-expression by using ELISA Kits (all from BD Biosciences, USA) according to the manufacturer's instructions.

Foam cell formation assay

Macrophages were plated on coverslips in 6-well plates. After treatment with oxLDL at desired concentrations, cells were washed three times, fixed with 4% formaldehyde (in PBS) for 15 min and rinsed, and then these cells were stained for 30 min with a filtered Oil Red O (Sigma-Aldrich) working solution at room temperature and rinsed with 60% isopropanol three times. Cells were then stained with hematoxylin for 5 seconds and rinsed. Pictures were taken by optical microscope.

Western blot analysis

Cells were seeded onto 6 well plates and were lysed in HEPES buffer (20 mM HEPES PH 7.2,

50 mM NaCl, 0.5% TritonX-100, 1 mM NaF, 1 mM DTT, 5 mM EDTA) containing protease inhibitors on ice. The lysates were collected and boiled for 20 minutes and separated on 10% SDS-PAGE, then transferred to nitrocellulose membranes, blocked with skim milk, incubated with primary antibodies and specific secondary antibodies, and specific proteins were detected using chemiluminescence method. The bands' intensities were scanned and calculated by densitometry (NIH ImageJ software).

Relative amounts of each protein were normalized by calculation. The following antibodies were used: Anti-SR-B, ABCG1, Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and β-actin antibodies were purchased from Santa Cruz, Anti-CD36, SR-A, LOX-1 antibodies were purchased from R&D system, and anti-ABCA1 antibodies were obtained from Abcam.

Quantitative real-time PCR (qPCR) assay

Total RNA was extracted using the Trizol reagent (Sigma-Aldrich, USA), and then reverse transcribed into cDNA using the Rever Tra Ace qPCR RT Kit (Sigma-Aldrich, USA). Real-time quantitative PCR analysis was determined using KAPA SYBR FAST qPCR Kit Master Mix (KAPABIO SYSTEM). The forward primer of APE1 5'-TGGGCTACACTGGAGCACCAG-3, the reverse primer of APE1 GGGTGTCGCTGTTGAAGTCA-3; The forward primer of LOX1 5'-TGACCCTGCC-ATGCCATGCT-3, the reverse primer of Lox1 TG-GGGATGGTGGAGGCCCTG-3; The forward primer of GAPDH 5'-TGGGCTACACTGGAGCACCAG-3, the reverse primer of GAPDH GGGTGTCGCTG-TTGAAGTCA-3; The forward primer of IL1β 5'-CAC CTT CTT TTC CTT CAT CTT TG-3, the reverse primer GTC GTT GCT TGT CTC TCC TTG TA-3; The forward primer of IL6 5'-TGA TGG ATG CTT CCA AAC TG-3, the reverse primer of IL6 GAG CAT TGG AAG TTG GGG TA-3; The forward primer of TNF-α 5'-ACT GAA CTT CGG GGT GAT TG-3, the reverse primer of TNF-α GCT TGG TGG TTT GCT ACG AC-3; The threshold cycle number of each gene was determined, and β-actin was used as the internal control to quantify the relative expression.

Computer analysis of the LOX-1 promoter

We used the publicly available TRANSFAC database (MatInspector software) to search for the potential cis-regulatory elements within the mouse LOX-1 promoter. The threshold was set at 0.88.

Chromatin immunoprecipitation (CHIP)-qPCR assays

Chromatin immunoprecipitation (ChIP) was performed using a Chromatin Immunoprecipitation kit (Abcam, USA) according to the manufacturer's instructions. Briefly, cells were treated with 1% formaldehyde to cross-link the proteins and DNA. Then cells were lysated and sonicated to shear DNA to sizes of 300 to 1000 bp. Equal aliquots of chromatin supernatants, into which 1 μg of either anti-NF-κB, anti-Oct1 (Abcam, USA) or anti-IgG as a negative control was added, were incubated overnight at 4°C on rocking bed. After reverse cross-linking of the protein/DNA complexes to free the DNA, PCR was performed using specific primers to amplify the LOX1 promoter. The primer was determined as: forward 5'-GGGGTACCCACCTACATT-ATGCAGC-3' and reverse 5'-GAAGATCTGAGTG-AAGCAGTCACGAACTTC-3'.

Dual luciferase assay

The luciferase activity was measured with a Dual-Luciferase Report Assay system (Promega, USA), and the luminescence was read by a luminometer (TD-20/20) according to the manufacturer's instructions. This system allows the quantification of activities of both Firefly luciferase (encoded by LOX-1 promoter-pGL3 plasmid construct) and Renilla luciferase (encoded by pRL-SV40 plasmid). The relative values of Firefly luciferase activity were determined by normalizing with Renilla luciferase activity for transfection efficiency.

Statistical analysis

All results were expressed as means ± SD with the indicated number of independent experiments. A two-tailed Student's t test was used to calculate the significance differences between two groups. Comparison of more than two groups was made using a one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test. All statistical analyses were performed with GraphPad Prism 6 and SPSS 22.0 software.

Significance was accepted at the level of $P<0.05$.

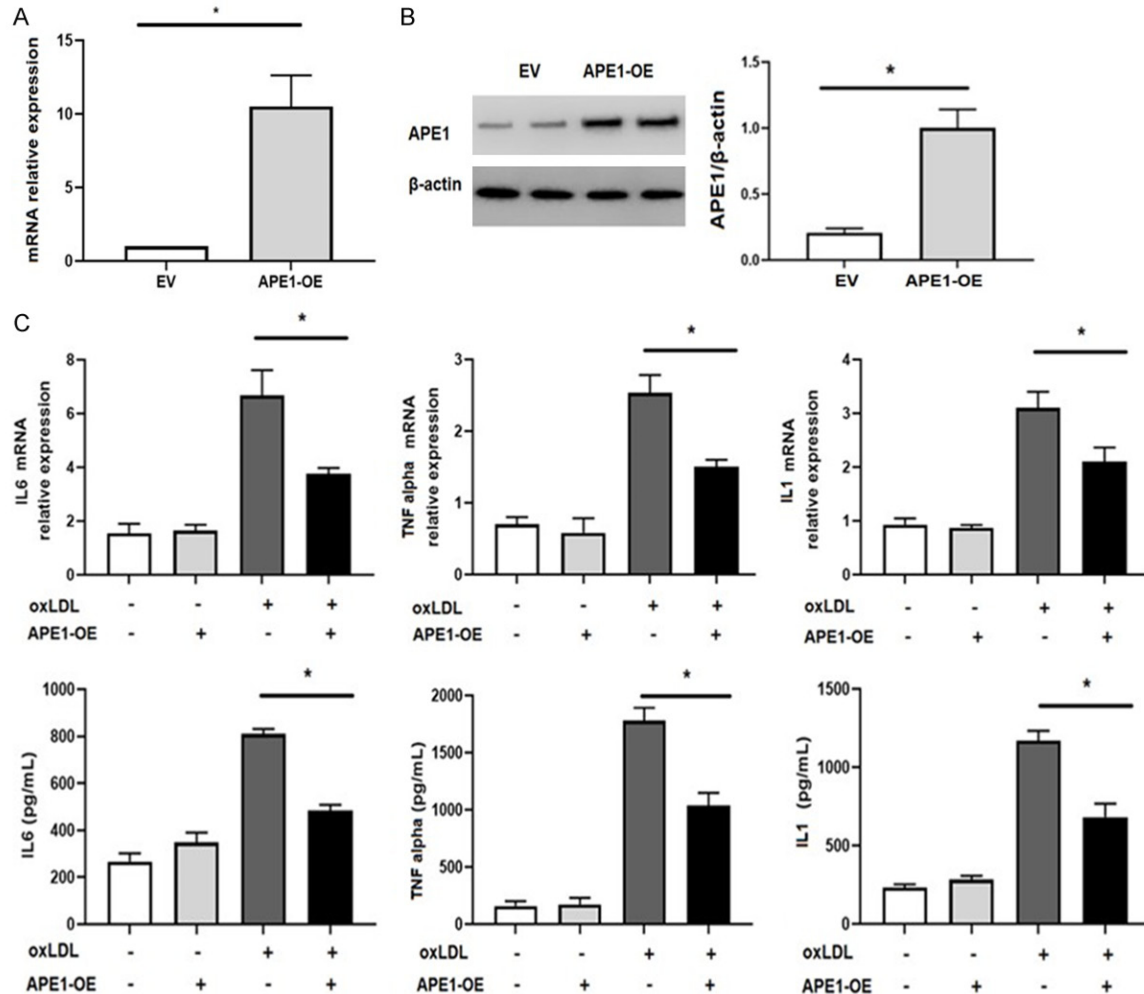

Figure 1. APE1 overexpression decreased oxLDL-induced upregulation of inflammatory molecules in THP-1 macrophages. A. mRNA expression of APE1 by qPCR 48 hours after plasmid transfection in EV and APE1-OE cells. B. Protein expression of APE1 by western blot assay 48 hours after plasmid transfection in EV and APE1-OE cells. C. mRNA and protein levels of IL6, TNF-α and IL1β by qPCR and ELISA assays in cultured macrophages and medium supernatants after oxLDL treatment (100 μg/mL for 12 hours). The ANOVA test was used for statistical analyses. EV, empty vector; APE1-OE, APE1 over-expression; *P<0.05.

Results

APE1 over-expression decreases oxLDL-induced inflammatory molecules in THP1 cells

We transfected THP1 cells with plasmids encoding APE1, followed by qPCR and western blot assays to determine the APE1 mRNA and protein expression levels, respectively. Our data show a 4~5 fold increase in APE1 mRNA (**Figure 1A**) and corresponding increase in APE1 protein expression (**Figure 1B**) in cells receiving APE1 encoding plasmids (APE1-OE) compared to those receiving empty vector (EV) transfection.

Macrophage activation is characterized by the secretion of pro-inflammatory cytokines, including Interleukin 6 (IL6), IL1β and Tumor necrosis factor alpha (TNF-α) [9].

We quantified expression levels of these inflammatory molecules by using ELISA assays in the conditioned media of cultured macrophages. As expected, we observed that oxLDL (100 μg/mL for 12 hours) dramatically increased the mRNA and protein expression of IL6, TNF-α and IL1β in EV transfected cells compared to the non-treated cells.

However, in APE1-OE cells the mRNA and protein levels of these molecules were significantly

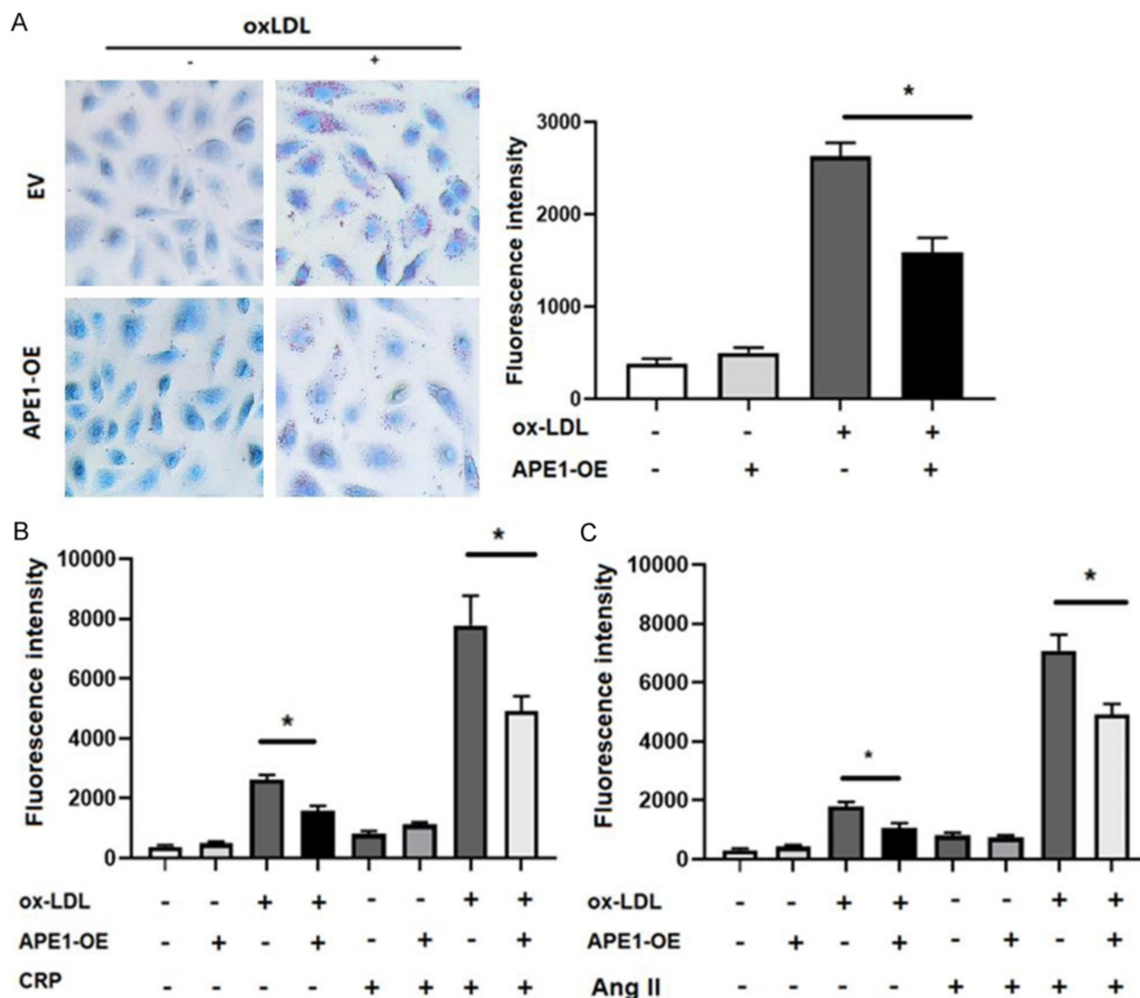

Figure 2. APE1 inhibits foam cell formation from THP-1 cells. A. Foam cell formation from macrophages after oxLDL treatment (100 μg/mL for 24 h) using Oil Red O staining. Left: Typical images for Oil Red O staining (60* times magnitude). Right: Quantification of fluorescence intensity in tested cells using ANOVA test. *P<0.05. B. APE1 inhibits foam cell formation from THP-1 cells under the co-treatment of oxLDL with CRP. C. APE1 inhibits foam cell formation from THP-1 cells under the co-treatment of oxLDL with Ang II. Quantification of fluorescence intensity was compared in tested cells using an ANOVA test. *P<0.05. Ang II, angiotensin II; CRP, C-Reactive Protein.

inhibited. These data suggest that APE1 suppresses oxLDL induced macrophage activation (**Figure 1C**).

APE1 inhibits foam cell formation

Whether APE1 affects foam cell formation from macrophages remains unknown. To address this question, we pretreated cells with oxLDL (100 μg/mL for 24 h), followed by Oil Red O staining. As anticipated, oxLDL induced a 3~4 fold increase in the foam cell formation capacity in EV THP1 cells compared to non-treated cells. Of note, in the APE1-OE cells, oxLD- induced accumulation of intracellular lipid droplets was significantly reduced, as shown in **Figure 2A**.

We next exposed THP1 cells to the other pro-atherogenic stimuli, such as Angiotensin II (Ang II, 10 μM for 24 h) and C-reactive protein (CRP, 20 μg/mL for 24 h), to further evaluate the effect of APE1 on foam cell formation from macrophages. We observed that co-incubation of Ang II (**Figure 2B**) and CRP (**Figure 2C**) with oxLDL further enhanced the fluorescence intensities, respectively, which were blunted by APE1 in APE1-OE cells (**Figure 2B**).

APE1 did not affect oxLDL efflux, but reduced Dil-oxLDL uptake

Formation of foam cells is the result of an imbalance between cholesterol influx and ef-

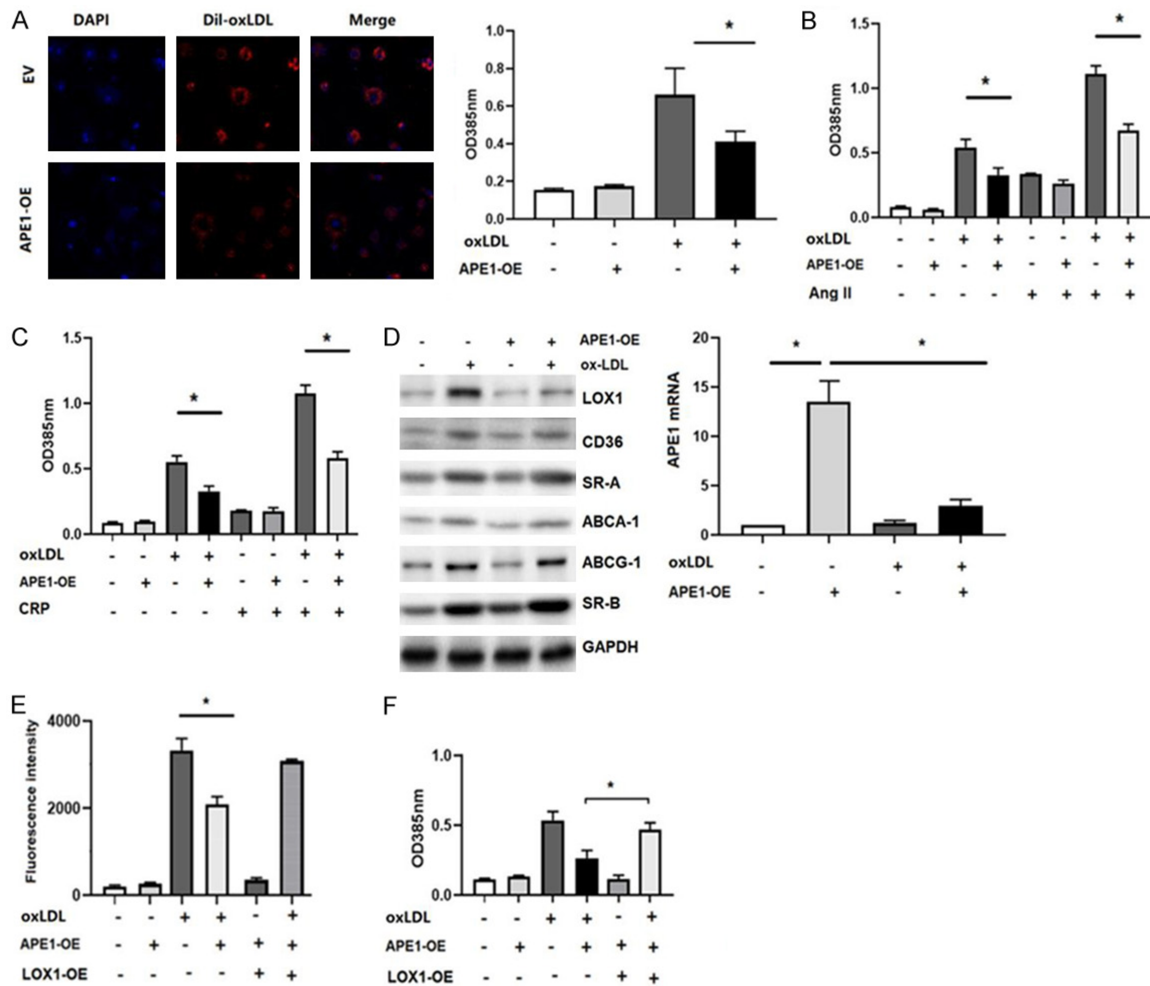

Figure 3. APE1 reduces Dil-oxLDL uptake in THP1 cells via suppression of LOX1. A. Typical images for the Dil-labeled oxLDL uptake assay under Dil-oxLDL incubation for 24 hours. B. Dil-oxLDL uptake by macrophages when THP1 cell was co-treated with Ang II. C. Dil-oxLDL uptake by macrophages when THP1 cell was co-treated with CRP. D. Western blot assay for scavenger receptors CD36, SR-A, LOX-1, ABCA1, ABCG1, and SR-B. APE1 suppressed oxLDL induced upregulation of LOX1 protein expression, while the others remained unchanged in APE1-OE cells. Right, APE1 suppressed oxLDL induced up-regulation of LOX1 mRNA. E. LOX1 expression blocked the inhibitory effect of APE1 on oxLDL uptake in THP1 cells receiving LOX1 and APE1 co-transfection. F. LOX1 expression blocked the inhibitory effect of APE1 on foam cell formation in THP1 cells receiving LOX1 and APE1 co-transfection. *P<0.05.

flux [10]. To identify the mechanism responsible for foam cell formation from macrophages, we measured the oxLDL uptake and efflux status, respectively.

Representative images for the Dil-labeled oxLDL uptake assay are shown in **Figure 3A**. To quantify the Dil-oxLDL uptake we measured the absorbance value at OD385nm levels in THP-1 cells. We found that Dil-oxLDL uptake was significantly inhibited in APE1-OE cells compared to EV cells (**Figure 3A**, right). This trend was evident when THP-1 cells were co-treated with oxLDL and Ang II or CRP, respectively (**Figure 3B** and **3C**). In our cholesterol efflux assay, however, we did not see APE1 changing the cholesterol efflux ability (data not shown). Collectively, these data suggest that APE1 inhibits foam cell formation by reducing oxLDL uptake capacity rather than changing the oxLDL efflux status.

To gain further insight into the mechanism governing the effect of APE1 on oxLDL uptake and foam cell formation, we quantified expression of major scavenger receptors responsible for oxLDL uptake, such as CD36, scavenger receptor A (SR-A) and lectin-like oxidized low density

lipoprotein receptor 1 (LOX-1), as well as molecules responsible for oxLDL efflux, including ATP-binding cassette transporter A1 (ABCA1), ATP-binding cassette transporter G1 (ABCG1), and scavenger receptor B (SR-B).

As shown in **Figure 3D**, we found that oxLDL significantly increased the protein expressions of these receptors in EV transfected cells; APE1 over-expression had no effect on the expression profile of these proteins in the absence of oxLDL treatment. However, in APE1-OE cells, we observed that the LOX1 mRNA and protein levels were significantly reduced. In contrast, the protein levels of the other scavenger receptors were not affected by APE1.

To further validate the role of LOX1 in mediating the inhibitory effect of APE1 on oxLDL uptake and foam cell formation, we co-transfected THP-1 cells with two types of plasmids encoding APE1 and LOX1 respectively, then exposed these cells to oxLDL. We found that LOX1 overexpression blocked the APE1-induced inhibition of oxLDL uptake (**Figure 3E**) and foam cell formation capacity (**Figure 3F**) as well as upregulation of macrophage activation markers (data not shown). These data confirm the role of LOX1 in mediating, at least in part, APE1-inihbited macrophage activation and macrophage transformation into foam cells.

APE1 inhibits LOX1 promoter binding to transcription factor Oct1, not NF-κB

It has been reported that LOX1 is regulated by Oct-1 and NF-κb at the transcriptional level in human endothelial cells [11, 12]. To determine whether APE1 induced LOX1 downregulation occurs at the transcription level, we performed a ChIP-qPCR assay to test if APE1 can modify the direct binding of the LOX1 promoter to NF-κB or Oct-1 in THP1 cells. The antibodies against NF-κB or Oct1 and the isotype-match IgG were used as controls. Next, the ChIP-derived DNA fragment complexes were amplified by qPCR using primers specific for the LOX1 full length promoter region. As shown in **Figure 4A**, in cells without oxLDL treatment, the amounts of LOX1 promoter DNA fragments were similar in the complexes immunoprecipitated by the anti-Oct1 antibody between EV and APE1-OE cells, suggesting that APE1 overexpression did not change the binding ability of the LOX1 promoter to Oct-1 at the baseline level. However, in the presence of oxLDL stimulation, the amount of LOX1 promoter qPCR products was significantly lower in APE1-OE cells compared to the EV cells, suggesting that APE1 affects the binding of LOX1 promoters to Oct1 under oxLDL stimulation. In contrast to this finding, the binding of LOX1 promoters to NF-κB was not affected by APE1, in the presence or absence of oxLDL treatment (**Figure 4B**).

To further confirm this finding from the CHIP-qPCR study, we performed a dual luciferase study. Our data showed that the oxLDL induced dramatic increase in the luciferase activities derived from LOX1 promoter and Oct-1 co-transfection in the EV cells. In APE1-OE cells, however, this enhancement was substantially suppressed (**Figure 4C** and **4D**). Consistent with our Chip-qPCR data, the luciferase activities for NF-κB were not affected by APE1 in APE-OE cells. Collectively, these data suggest that APE1 hinders LOX1 promoter binding to its transcription factor Oct1, and thus down-regulates LOX1 expression.

Discussion

In this study we demonstrated that APE1 suppresses oxLDL-induced macrophage activation and foam cell formation in THP-1 cells. Mechanistically, we determined that APE1 reduces LOX1 promoter binding to the transcription factor Oct1, causing downregulation of LOX1, which, in turn mediates, at least in part, the APE1-induced inhibitory effect on foam cell formation from macrophages. To the best of our knowledge, this is the first study to reveal the role of APE1 in macrophage activation and foam cell formation capacity.

Considering the importance of macrophages in the development in AS, our finding suggests that APE1 may serve as a promising target molecule for the prevention of AS.

So far, only a few studies have reported the role of APE1 in inflammation. APE1 is essential for the toll-like receptor 2 (TLR2)-dependent transcriptional activation of NF-κB and HIF-1α, leading to the expression of inflammatory cytokines including TNF-α, C-X-C Motif Chemokine Ligand 8, and IL-37 in human keratinocytes [13]. Furthermore, TLR2-dependent signaling

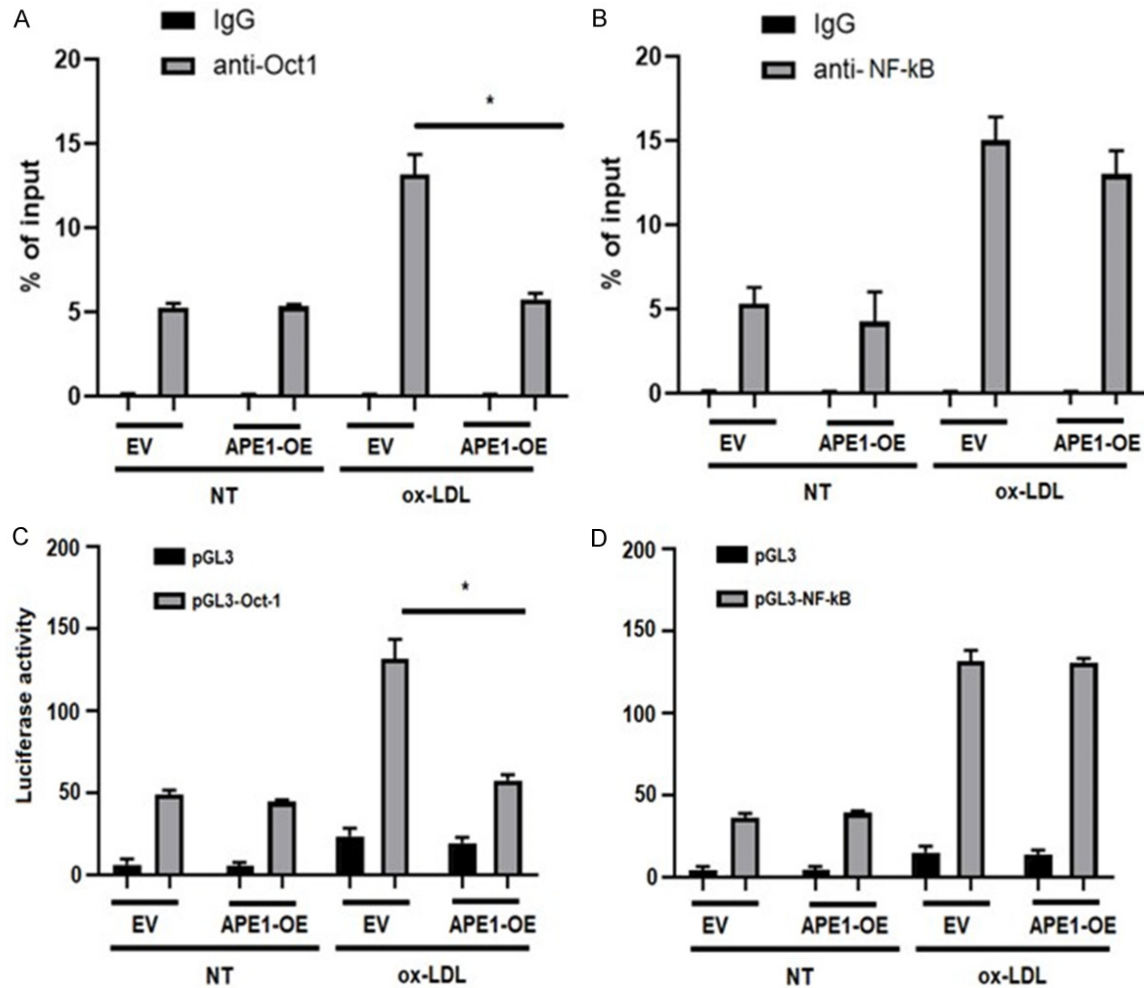

Figure 4. APE1 inhibits LOX1 promoter binding to transcription factor Oct1, but not to NF-κb. A and B. Show the CHIP-qPCR assays to test if APE1 modifies the binding ability of the LOX1 promoter to its transcript factors, NF-κB or Oct-1, in THP1 cells. The binding ability was indicated by the qPCR output from the complex pulled down by anti-Oct-1 or anti-NF-κB antibodies. A. At the baseline level LOX1 promoter binding to Oct-1 was similar between EV and APE1-OE cells. However, in the presence of oxLDL stimulation, the binding ability of the LOX1 promoter and Oct-1 was significantly lower in APE1-OE cells compared to the EV cells, suggesting that APE1 affects the binding of the LOX1 promoter to Oct-1 under oxLDL stimulation. B. The binding of the LOX1 promoter to NF-κB was not affected by APE1, in the presence or absence of oxLDL treatment. C and D. Show the dual luciferase study to confirm the results of CHIP-qPCR assay. C. oxLDL induced substantial increase in the luciferase activities derived from the LOX1 promoter and Oct-1 co-transfection in the EV cells. In APE1-OE cells, however, this enhancement was markedly suppressed by APE1. D. Consistent with our Chip-qPCR data, the luciferase activities for NF-κB was notaffected by APE1 in APE-OE cells in the presence or absence of oxLDL treatment. *$P<0.05$.

by ERK1/2, and Akt in keratinocytes is APE1 dependent [13]. Overexpression of APE1 suppresses VCAM-1 expression and inducible nitric oxide synthase (iNOS) activation under IL1β stimulation in endothelial cells, indicating an anti-inflammatory effect for APE1. It was postulated that this effect occurs by inhibiting inflammatory cytokine binding to the receptors in endothelial cells [5]. Consistent with other studies, we found that aberrant APE1 expression dramatically inhibits the expression of macrophage activation markers such as IL1, IL6 and TNF-α under baseline conditions and in response to other pro-artherogenic stimuli, such as CRP and AngII, further confirming the anti-inflammatory properties of APE1 under oxLDL stimulation. However, it should be noted that the pro-inflammatory effect of APE1 has been reported as well. In inflammatory pain rate model, APE1 expression is upregulated in neuronal cells and its inhibition helps to reduce the pain through suppressing inflammatory

cytokines [16]. Specifically, one previous study by Jedinak et al. reported a regulatory role of APE1 in an LPS-challenged RAW264.7 macrophage cell line [6]. They used E3330, a small-molecule inhibitor of APE1 redox signaling function to treat RAW264.7 cells and found that LPS-dependent production of IL6 and IL12 was markedly suppressed [6]. These findings seem contrary to our observation, which is APE1 overexpression leads to a low inflammatory profile in THP-1 cells. However, it should be noted that, Jedinak et al. studied the function of endogenous APE1, while we studied the function of exogenous APE1. The difference in the expression pattern may account for this inconsistency between our and Jedinak's groups. In addition, APE1 possess a multifunctional property that includes DNR damage repair, redox regulation and acetylation functions, all of which may also contribute to the discrepancy between Jedinak's and our data.

Whether APE1 affects the foam cell formation capacity of activated macrophages remains unknown so far. Our study provides evidence, for the first time, that APE1 inhibits cholesterol accumulation in macrophages. Scavenger receptors mediate lipoprotein internalization, which leads to foam-cell formation. We further identified that APE1 increased LOX1-dependent cholesterol uptake, but not cholesterol efflux, responsible for the inhibitory effect of APE1 on foam cell formation capacity. Vessel smooth muscle cell (VSMC) also plays an important role in the development of AS. Our previous study reveals that APE1 can form nuclear complex with glyceraldehyde-3-phosphate dehydrogenase and protect VSMC from H_2O_2 induced apoptosis [17]. Taken together, our findings strongly suggest a therapeutic significance for APE1 as a molecular target to alleviate AS progression.

We next explored the mechanism under which APE1 regulates LOX1 expression. Using ChIP-qPCR and dual luciferase assays, we found that APE1 markedly suppresses the binding ability of the LOX1 promoter with a key transcription factor, Oct1, to inhibit LOX1 protein expression. It is well known that APE1 is a multi-functional protein that performs the base-excision DNA repair activity and redox control of multiple transcription factors. Here we identified that APE1 also serves as a molecular regulator affecting the promoter binding of a target gene to a related transcription factor. Similar to our study, a previous study reported that APE1 can process miR-92b formation, thereby regulating expression of the tumor suppressor low-density lipoprotein receptor in cervical cancer cells [14]. Likewise, APE1 was also identified as a direct trans-acting factor for repressing human parathyroid hormone and renin genes by binding to the negative calcium-response element in their promoters [15].

Several limitations should be recognized for this study. First, this is an in vitro study; no *in vivo* data are available so far. Second, our findings were obtained using only THP1 cells; more data from other cell types are warranted. Third, although we discovered that APE1 inhibited LOX1 promoter binding to transcription factor Oct1, a further domain specific study is needed to better understand the effect of APE1.

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Disclosure of conflict of interest

None.

Address correspondence to: Xiaohong Liu, Cardiovascular Department of Internal Medicine, Central Hospital of Karamay, Karamay 834000, Xinjiang Uyghur Autonomous Region, P. R. China. E-mail: Lxh61666@sohu.com; Xuwei Hou, Department of Cardiology, The University of Missouri School of Medicine, Columbia, MO 65201, USA. E-mail: Houx@health.missouri.edu

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