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RESEARCH ARTICLE

Molecular mechanism of curcumin on the suppression of cholesterol accumulation in macrophage foam cells and atherosclerosis

Jing-Feng Zhao[1], Li-Chieh Ching[1*], Yu-Chu Huang[1*], Chien-Yu Chen[1], An-Na Chiang[2], Yu Ru Kou[1], Song-Kun Shyue[3] and Tzong-Shyuan Lee[1,4]*

[1] Department of Physiology, National Yang-Ming University, Taipei, Taiwan
[2] Institute of Biochemistry and Molecular Biology, National Yang-Ming University, Taipei, Taiwan
[3] Cardiovascular Division Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan
[4] Brain Research Center, National Yang-Ming University, Taipei, Taiwan

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Scope: Curcumin, a potent antioxidant extracted from *Curcuma longa*, confers protection against atherosclerosis, yet the detailed mechanisms are not fully understood. In this study, we examined the effect of curcumin on lipid accumulation and the underlying molecular mechanisms in macrophages and apolipoprotein E-deficient (apoE$^{-/-}$) mice.

Methods and results: Treatment with curcumin markedly ameliorated oxidized low-density lipoprotein (oxLDL)-induced cholesterol accumulation in macrophages, which was due to decreased oxLDL uptake and increased cholesterol efflux. In addition, curcumin decreased the protein expression of scavenger receptor class A (SR-A) but increased that of ATP-binding cassette transporter (ABC) A1 and had no effect on the protein expression of CD36, class B receptor type I (SR-BI), or ATP-binding cassette transporter G1 (ABCG1). The downregulation of SR-A by curcumin was via ubiquitin–proteasome–calpain-mediated proteolysis. Furthermore, the curcumin-induced upregulation of ABCA1 was mainly through calmodulin-liver X receptor α (LXRα)-dependent transcriptional regulation. Curcumin administration modulated the expression of SR-A, ABCA1, ABCG1, and SR-BI in aortas and retarded atherosclerosis in apoE$^{-/-}$ mice.

Conclusion: Our findings suggest that inhibition of SR-A-mediated oxLDL uptake and promotion of ABCA1-dependent cholesterol efflux are two crucial events in suppression of cholesterol accumulation by curcumin in the transformation of macrophage foam cells.

Keywords:
ABCA1 / Calpain / Curcumin / LXRα / SR-A

1 Introduction

Atherosclerosis, one of the leading causes of death from coronary artery disease in developing countries, is characterized by excessive cholesterol deposition and persistent inflammation within the artery wall resulting in myocardiac infarction and stroke [1, 2]. The migration of monocytes/macrophages into intima with subsequent cholesterol clearance and proinflammatory cytokine secretion is a crucial step in the initiation and progression of atherosclerosis [2–4]. Particularly, cholesterol accumulation in foam cells is mainly due to

Correspondence: Dr. Tzong-Shyuan Lee, Department of Physiology, School of Medicine, National Yang-Ming University, Taipei 11221, Taiwan
E-mail: tslee@ym.edu.tw
Fax: +886-2-2826-4049

Abbreviations: ABCA1, ABCG1, ATP-binding cassette transporter A1, G1; **ACAT-1**, acyl CoA: cholesterol acyltransferase-1; **ACC**, acetyl-CoA carboxylase; **apoAI**, apolipoprotein AI; **CHX**, cycloheximide; **FAS**, fatty acid synthase; **HMGCR**, 3-hydroxy-3- methylglutaryl coenzyme A reductase; **LDLR**, low-density lipoprotein receptor; **LOX**, lipoxygenase; **Luc**, luciferase; **LXR**α, liver X receptor α; **LXRE**, LXR responsive element; **MCP-1**, monocyte chemoattractant protein-1; **oxLDL**, oxidized LDL; **PPARs**, peroxisome proliferative-activated receptors; **RCTs**, reverse cholesterol transporters; **siRNA**, small interfering RNA; **SR-A**, scavenger receptor class A; **SR-BI**, class B scavenger receptor type I; **SREBPs**, sterol regulatory element binding proteins; **TNF-**α, tumor necrosis factor-α; **Ub**, ubiquitin; **VCP**, valosin-containing protein.

*These authors contributed equally to this work.

uncontrolled uptake of modified low-density lipoprotein (LDL) or impaired cholesterol efflux in macrophages [4–8]. Scavenger receptors (SRs), class A SR (SR-A), and CD36 are responsible for internalization of modified LDL [6, 7]. In contrast, the efflux of intracellular cholesterol to high-density lipoprotein (HDL) or apolipoprotein AI (apoAI) is mediated by reverse cholesterol transporters (RCTs), including class B scavenger receptor type I (SR-BI) and ATP-binding cassette transporter A1 and G1 (ABCA1 and ABCG1) [8–10]. Thus, the intracellular lipid content of foam cells is dynamically regulated by these SRs and RCTs. Growing evidence indicates that modulation of these SRs or RCTs by antioxidants or cytokines reduces the cholesterol accumulation of foam cells, thereby retarding the development of atherosclerosis [5, 9–11].

Curcumin, the main active polyphenol extracted from *Curcuma longa*, can induce apoptosis and inhibit proliferation in various types of cancer cells [12–15]. Furthermore, dietary supplementation with curcumin can retard tumorigenesis in human and experimental rodents [16–18]. In addition to its anticancer activity, curcumin has beneficial effects on cardiovascular diseases such as diabetes and atherosclerosis in experimental animal models [19–21]. The contribution of curcumin to anti-inflammation and antioxidation in treatment of atherosclerosis is well recognized [20, 21]. However, the effect and molecular mechanism by which curcumin regulates cholesterol metabolism in macrophage foam cells are not fully resolved.

We explored the interaction between curcumin effects and cholesterol homeostasis of macrophage foam cells. We aimed to investigate the effect of curcumin on oxidized LDL (oxLDL)-induced cholesterol accumulation in macrophages; to delineate the effect of curcumin on the expression of SR-A, CD36, SR-BI, ABCA1, and ABCG1; and explore the molecular mechanisms underlying the curcumin-mediated modulation in cholesterol accumulation. Curcumin effectively suppressed oxLDL-mediated lipid accumulation through calpain-dependent downregulation of SR-A and calmodulin-liver X receptor α (LXRα)-dependent upregulation of ABCA1 in macrophages.

2 Materials and methods

2.1 Reagents and kits

Curcumin, W7, apoAI, MG-132, chloroquine, cycloheximide (CHX), and mouse anti-α-tubulin antibody (Ab) were from Sigma (St. Louis, MO). LXRα small interfering RNA (siRNA), control siRNA, rabbit anti-CD36, goat anti-SR-A, rabbit anti-valosin-containing protein (anti-VCP), mouse anti-Histone H1, mouse anti-ubiquitin, goat anti-peroxisome proliferative-activated receptor α (PPARα), mouse anti-PPARγ, rabbit anti-acetyl-CoA carboxylase (ACC), rabbit anti-fatty acid synthase (FAS), rabbit anti-3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR), goat anti-LDL receptor (LDLR), goat anti-12-lipoxygenase (12-LOX), mouse anti-15-lipoxygenase (15-LOX), and Abs were from Santa Cruz Biotechnology (Santa Cruz, CA). Mouse anti-ABCA1, goat anti-acyl CoA: cholesterol acyltransferase-1 (ACAT-1) and rabbit anti-LXRα Abs were from Abcam (Cambridge, MA). Mouse anti-sterol regulatory element binding protein 1 (SREBP1) and SREBP2 Abs were purchased from BD Biosciences (San Jose, CA). Rabbit anti-SR-BI and rabbit anti-ABCG1 Abs were from Novus Biologicals (Littleton, CO). Rabbit anti-apoAI Ab was from Biodesign International (Saco, ME). The assay kit for calpain activity was from BioVision (Lyon, France). TurboFect reagent was from Fermentas (Glen Burnie, MD). 3-hexanoly-7-nitro-2,1,3-benzoxadial-4-yl (NBD) cholesterol and TO901317 were from Cayman (Ann Arbor, MI). Assay kits for detecting cholesterol and triglyceride were from RANDOX (Antrim, UK). Dil-oxLDL was from Biomedical Technologies (York, UK). ELISA kits for tumor necrosis factor α (TNF-α), interleukin-6 (IL-6), and monocyte chemotactic protein-1 (MCP-1) were from R&D Systems (Minneapolis, MN).

2.2 Cell culture

The mouse macrophage cell line J774.A1 (Bioresource Collection and Research Center; Hsinchu, Taiwan) was cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), penicillin (100 U/mL), and streptomycin (100 μg/mL).

2.3 Preparation of oxLDL

The oxLDL was prepared as described [8]. LDL was exposed to 5 μM $CuSO_4$ for 24 h at 37°C, and Cu^{2+} was removed by extensive dialysis. The extent of modification was determined by measuring thiobarbituric acid-reactive substances (TBARs). We used oxLDL containing approximately 30–60 nmol TBARs defined as malondialdehyde equivalent per milligram LDL protein.

2.4 Oil-red O staining

Cells were fixed with 4% paraformaldehyde and then stained with 0.5% Oil-red O. Hemotoxylin was used as counterstaining. The density of lipid content was evaluated by alcohol extraction after Oil-red O staining. The absorbance at 540 nm was measured by use of a microplate reader (BioTek Instrument, Winooski, VT).

2.5 Cholesterol measurement

Cellular cholesterol was extracted by use of hexane/isopropanol (3:2, v/v). After removing cellular debris, the supernatant was dried under a nitrogen flush. The level

of cholesterol was measured by use of cholesterol assay kits.

2.6 Cholesterol efflux assay

We have previously applied NBD-cholesterol to study the experiments of cholesterol efflux in macrophages [8]. NBD-cholesterol uptake is independent of SR-A in macrophages (Supporting Information Fig. 1). Macrophages were treated with concentrations of curcumin (0, 5, 10, 20, 40 μM) for 12 h, then underwent equilibration of NBD-cholesterol (1 μg/mL) for an additional 6 h in the presence of apoAI (10 μg/mL). NBD-cholesterol-labeled cells were washed with phosphate-buffered saline and incubated in RPMI 1640 medium for 6 h. The fluorescence-labeled cholesterol released from the cells into the medium was measured by use of a multilabel counter (PerkinElmer, Waltham, MA) with 485 nm excitation and 535 nm emission.

2.7 DiI-oxLDL binding assay

DiI-oxLDL uptake was performed as described [8]. Macrophages were treated with or without curcumin (0, 5, 10, 20, 40 μM) for 24 h, then 10 μg/mL DiI-oxLDL at 4°C for 4 h. Cells were washed and lysates were analyzed by fluorometry (Molecular Devices, Downingtown, PA) with 514 nm excitation and 550 nm emission.

2.8 Quantitative real-time PCR (qRT-PCR)

Total RNA was isolated from cells by Tri reagent and converted into cDNA by use of reverse transcriptase (Biolabs, Ipswich, New England) with oligo-dT primer. cDNAs were then used as templates for qRT-PCR. qRT-PCR involved the TaqMan® probe-based real-time quantification system (Foster, CA). mRNA level was calculated relative to GAPDH mRNA as the invariant control.

2.9 Western blot analysis

Cells were lysed with phosphate buffered saline containing 1% Triton X-100, 0.1% SDS, 0.5% sodium deoxycholate, 1 μg/mL-1 leupeptin, 10 μg/mL-1 aprotinin, and 1 mM PMSF on ice. After sonication, crude extracts underwent centrifugation at 12 000 × *g* for 5 min at 4°C. The supernatants were collected as cell lysates. All protein concentrations were determined by a protein assay. Aliquots (50 μg) of cell lysates were separated on 8% SDS-PAGE and then transblotted on an Immobilon™-P membrane (Millipore, Bedford, MA). After being blocked with 5% skim milk, blots were incubated with primary Abs, then secondary Abs. The protein bands were detected by use of an enhanced chemiluminescence kit (PerkinElmer, Boston, MA) and quantified by ImageQuant 5.2 software (Healthcare Bio-Sciences, PA).

2.10 Measurement of calpain activity

Calpain activity was measured by use of a kit (BioVision). Briefly, cellular lysates (100 μg) were mixed with reaction buffer and fluorogenic substrate Ac-LLY-AFC. The level of released AFC was measured over 1 h at 37°C by fluorometry with 400 nm excitation and 505 nm emission.

2.11 Transient transfection and luciferase reporter assay

Cells were transfected with the plasmids phABCA1 (-928)-Luc, a reporter plasmid for the human ABCA1 promoter; or phABCA1-DR4m-Luc, a reporter plasmid with a mutation in the LXRα responsive element (LXRE) or LXR ligand-binding domain system, CMX-GAL-hLXRα [22] (kindly provided by Dr. B. M. Forman, Gonda Diabetes Center, City of Hope National Medical Center, Duarte, CA, USA) by use of TurboFect™. The pGL3-renilla plasmid was cotransfected as a transfection control. After transfection for 24 h, cells were treated with curcumin (40 μM) or TO-901317 (10 μM) for another 24 h. Cells were then lysed for Luc and renilla activity assays.

2.12 siRNA transfection

Macrophages were transfected with scramble or LXRα siRNA with use of TurboFect for 24 h, treated with curcumin for another 24 h, then lysed for western blot analysis and qRT-PCR.

2.13 Animals

The investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996), and all animal experiments were approved by the Animal Care and Utilization Committee of National Yang-Ming University, Taiwan. Apolipoprotein E-deficient (apoE$^{-/-}$) and SR-A$^{-/-}$ mice were obtained from the Jackson Laboratory (Bar Harbor, ME). Mice were housed in barrier facilities on a 12-h light/12-h dark cycle and fed with regular chow diet that contained 4.5% fat by weight (0.02% cholesterol) (Newco Distributors, Redwood, CA). Four-month-old male mice received daily oral administration of curcumin (20 mg/kg body weight) or saline (vehicle control) by gastric gavages ($n = 10$, each group). After 4 weeks, mice were euthanized with CO_2. Serum, hearts, and aortas were collected for blood biochemistry, histology, and western blot analysis.

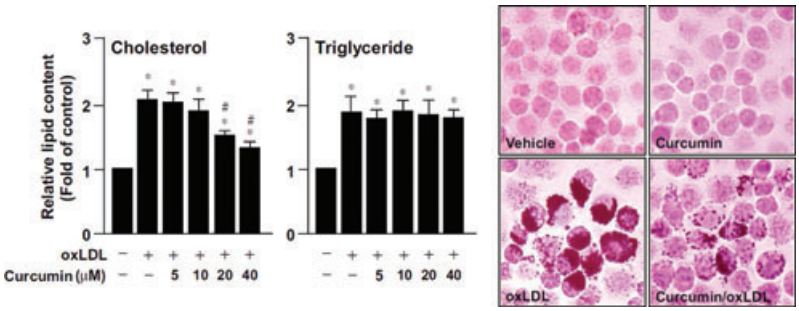

Figure 1. Curcumin attenuates oxidized low-density lipoprotein (oxLDL)-induced cholesterol accumulation in macrophages. Macrophages were co-treated with curcumin (0, 5, 10, 20, 40 μM) and oxLDL (50 μg/mL) for 24 h, then intracellular level of cholesterol and triglycerides was determined by an enzymatic method or cells were fixed and stained with Oil-red O. Cellular nuclei were stained with hematoxylin. The magnification of each panel is 400 × Data are mean ± SEM from five independent experiments. $^{*}p < 0.05$ versus control; $^{\#}p < 0.05$ versus oxLDL alone.

2.14 Quantification of atherosclerotic lesions

Harvested hearts were fixed with 4% paraformaldehyde, embedded in paraffin and serially sectioned at 8 μm. For the quantification of atherosclerotic lesions, 50 serial sections from the aortic sinus of each mouse were collected. A total of 10–12 sections sampled from every four consecutive sections were deparaffinized and subjected to hematoxylin and eosin staining. The photomicrographs of atherosclerotic lesions at aortic sinus were taken under a Motic TYPE 102M microscope (Motic Images Plus 2.0, China). The lesion size was then calculated from the average of the area quantified from the 10–12 sections by use of a computer imaging software (Motic Images Plus 2.0, China).

2.15 Serum lipid profile analysis

The serum levels of total cholesterol, HDL-cholesterol (HDL-c), non-HDL-c, and triglycerides were measured by use of blood biochemical analyzer (Spotchem EZ SP 4430, ARKRAY, Inc., Kyoto, Japan).

2.16 Measurement of inflammatory cytokines

The concentrations of TNF-α, IL-6, and MCP-1 in serum of mice were measured by ELISA kits according to the manufacturer's instructions.

2.17 Statistical analysis

All data represent the mean ± SEM values from at least five independent experiments. Mann–Whitney test was used to compare two independent groups. Kruskal–Wallis followed by the Bonferroni post hoc analyses was used for comparing multiple groups. Differences were considered statistically significant at $p < 0.05$.

3 Results

3.1 Curcumin reduces oxLDL-induced cholesterol accumulation in macrophages

Modified LDL such as oxLDL causes excess cholesterol deposition in macrophages and leads to the formation of foam cells that play a crucial role in the initiation and progression of atherosclerosis [2–4]. We thus explored the effect of curcumin on oxLDL-induced foam cell formation. Treatment with curcumin markedly decreased oxLDL-induced cholesterol accumulation in macrophages, as revealed by cellular cholesterol content and Oil-red O staining (Fig. 1). However, curcumin did not alter the intracellular triglyceride accumulation induced by oxLDL. Therefore, curcumin may regulate cholesterol metabolism of macrophage foam cells. Additionally, treatment with curcumin did not affect the protein expression of other lipid metabolism-related genes such as SREBP1, SREBP2, PPARα, PPARγ, ACC, FAS, HMGCR, LDLR, ACAT-1, 12-LOX, and 15-LOX (Supporting Information Fig. 2).

3.2 Effect of curcumin on oxLDL internalization and expression of SRs

To investigate the mechanisms underlying the suppression of foam cell formation by curcumin, we assessed the effect of curcumin on cholesterol uptake and expression of SRs, including SR-A and CD36. Curcumin treatment decreased DiI-oxLDL-induced cholesterol uptake (Fig. 2A) and the protein expression of SR-A but had no effect on the protein expression of CD36 (Fig. 2B and C). Importantly, curcumin did not affect the mRNA expression of SR-A (Fig. 2D). Transcription-independent downregulation of SR-A may be involved in curcumin-mediated reduction of oxLDL internalization in macrophages.

3.3 Curcumin promotes the turnover of SR-A protein via the proteasome pathway

We further delineated the molecular mechanisms underlying the effect of curcumin on the protein expression of SR-A

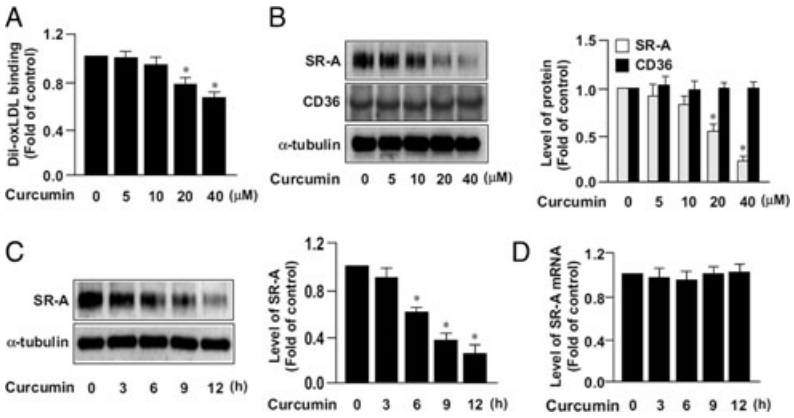

Figure 2. Effect of curcumin on oxLDL internalization and expression of scavenger receptors. (A) Analysis of Dil-oxLDL binding in macrophages treated with concentrations of curcumin for 12 h, then incubated with Dil-oxLDL (10 μg/mL) for an additional 4 h at 4°C. (B) Western blot analysis of protein levels of SR-A, CD36, and α-tubulin as a control in cells incubated with concentrations of curcumin for 12 h. (C) Western blot analysis of protein levels of SR-A and α-tubulin in cells treated for the indicated times with curcumin (40 μM). (D) Real-time PCR analysis of mRNA expression of SR-A and GAPDH as a control in macrophages treated with curcumin (40 μM) for indicated times. Data are mean ± SEM from five independent experiments. $^{*}p < 0.05$ versus control.

by examining the protein stability of SR-A with curcumin treatment. The degradation rate of SR-A protein was time-dependently promoted with curcumin treatment (Fig. 3A). We further defined the possible mechanism underlying the effect of curcumin on protein stability of SR-A by pretreatment with MG-132, a proteasome pathway inhibitor, which prevented the inhibitory effect of curcumin on protein expression of SR-A and Dil-oxLDL internalization. However, chloroquine, a lysosome pathway inhibitor, failed to produce such an effect (Fig. 3B and C).

Calpain, an important protease in proteasome pathway, participates in the pathogenesis of inflammatory diseases including atherosclerosis [23,24]. To determine whether calpain mediates the degradation of SR-A by curcumin, we examined the role of calpain in curcumin-induced downregulation of SR-A. Treatment with curcumin dose-dependently increased calpain activity (Fig. 3D). Moreover, calpeptin, a specific calpain inhibitor, diminished the inhibitory effect of curcumin on the protein expression of SR-A and Dil-oxLDL internalization (Fig. 3E and F), which suggests that calpain plays a key role in curcumin-provided protection against the formation of foam cells.

3.4 Curcumin increases the formation of the SR-A–ubiquitin–VCP complex

VCP, also known as p97, plays a mechanistic role in the delivery of ubiquitylated substrate to proteasomes for protein degradation [25, 26]. We, therefore, elucidated whether ubiquitin–VCP signaling is involved in the curcumin-induced degradation of SR-A. Co-immunoprecipitation assays revealed that curcumin time-dependently increased the ubiquitination of SR-A, as early as 1 h, with a peak at 2 h, then decreased (Fig. 4A). Interestingly, SR-A interacted with VCP under normal conditions, and this association was elevated with curcumin treatment (Fig. 4B). Therefore, the ubiquitin–VCP signaling pathway may play an important role in the curcumin-induced degradation of SR-A protein.

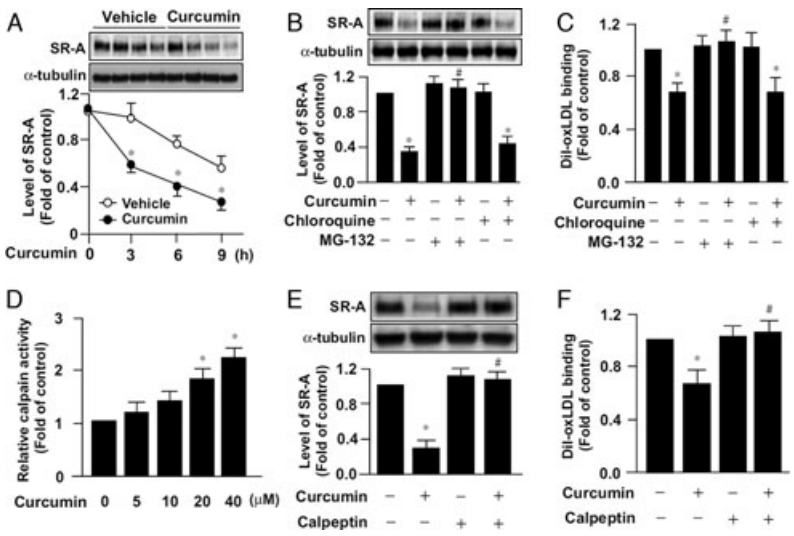

Figure 3. Curcumin promotes the turnover of SR-A protein through the proteasome pathway. (A) Macrophages were treated with or without curcumin (40 μM) in the presence of cycloheximide (CHX, 2 μg/mL) for the indicated times. (B) Western blot analysis of protein levels of SR-A and α-tubulin in macrophages pretreated with lysosome pathway inhibitor chloroquine (50 μM) or proteasome pathway inhibitor MG-132 (5 μM) for 2 h, then treated with curcumin for an additional 12 h, and (C) Dil-oxLDL binding analyzed by fluorometry with the previous treatment. (D) Calpain activity was evaluated by an assay kit. (E) Western blot analysis of protein levels of SR-A and α-tubulin in macrophages pretreated with calpain inhibitor calpeptin (10 μM) for 2 h, then treated with curcumin for an additional 12 h, and (F) Dil-oxLDL binding analyzed by fluorometry. Data are mean ± SEM from from independent experiments. $^{*}p < 0.05$ versus vehicle treatment; $^{\#}p < 0.05$ versus curcumin treatment.

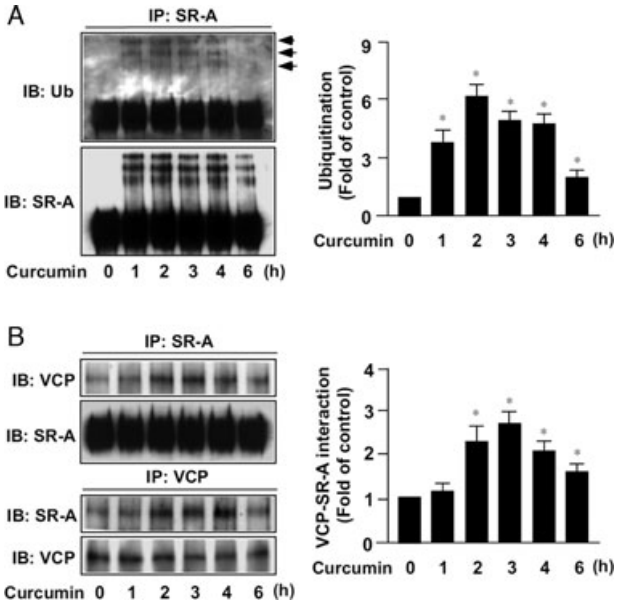

Figure 4. Curcumin increases the formation of SR-A–ubiquitin–VCP complex. Macrophages were treated with curcumin (40 μM) for the indicated times. Cellular lysates were immunoprecipitated (IP) with anti-SR-A antibody (Ab; A) or anti-VCP Ab (B) and then immunoblotting (IB) with anti-ubiquitin (Ub), anti-SR-A, or anti-VCP Ab and quantified (right). Ubiquitinated SR-A is indicated by arrows. Data are mean ± SEM from five independent experiments. $*p < 0.05$ versus vehicle.

3.5 Curcumin promotes apoAI-mediated cholesterol efflux and expression of ABCA1 in an LXRα-dependent manner

We next investigated the effect of curcumin on cholesterol efflux and the expression of the RCTs ABCA1, ABCG1, and SR-BI in macrophages. Curcumin dose-dependently increased apoAI-dependent cholesterol efflux (Fig. 5A). We also found that apoAI could be expressed by macrophages and secreted into the culture medium (Supporting Information Fig. 3). In addition, curcumin dose- or time-dependently increased the protein level of ABCA1 but did not affect the protein expression of ABCG1 and SR-BI (Fig. 5B–F). Furthermore, treatment with curcumin time-dependently enhanced the mRNA expression of ABCA1, as revealed by qRT-PCR (Fig. 6A), which suggests that curcumin upregulating the expression of ABCA1 may be through transcriptional regulation. Moreover, treatment with curcumin or TO901317 (an LXRα agonist) increased LXRα activity (Fig. 6B) and ABCA1 promoter activity by ~two-fold, which was abrogated with transfection of the ABCA1 promoter with a DR4 mutation (reporter plasmid with a mutation in the LXRE) (Fig. 6C), which indicates that transcriptional activation of LXRα is essential for the curcumin-induced upregulation of ABCA1 gene. To specifically confirm the role of LXRα in curcumin-induced suppression of foam cell formation, we found that inhibition of LXRα activation by LXRα siRNA significantly reduced LXRα protein (Supporting Information Fig. 4) and abolished the effect of curcumin and the increased mRNA and protein expression of ABCA1 (Fig. 6D and E). In addition, the promoted effect of curcumin on cholesterol efflux was prevented by LXRα siRNA treatment (Fig. 6F). Collectively, these results indicate the essential role of LXRα in curcumin-upregulated gene expression of ABCA1, which may contribute to the suppressive effect of curcumin on the formation of foam cells.

3.6 Calmodulin is required for the curcumin-mediated upregulation of ABCA1

Recently, calmodulin signaling was found to participate in curcumin-conferred protection in macrophages [27]. We, therefore, delineated the role of calmodulin in curcumin-induced upregulation of ABCA1. Inhibition of calmodulin activity by the pharmacological inhibitor W7 prevented the curcumin-induced increase in LXRα activity and ABCA1

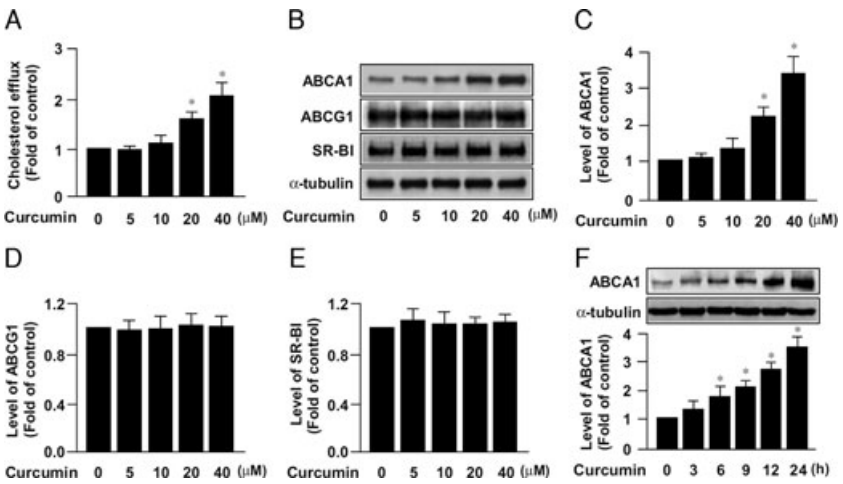

Figure 5. Curcumin promotes the cholesterol efflux and expression of ABCA1 in macrophages. (A) Macrophages were treated with indicated concentrations of curcumin for 12 h, then underwent equilibration of NBD-cholesterol (1 μg/mL) with apoAI (10 μg/mL) for an additional 6 h. Fold induction was defined as the level of cholesterol efflux relative to that in the vehicle-treated cells set as 1. (B–E) Western blot analysis of protein levels of ABCA1, ABCG1, SR-BI, and α-tubulin in macrophages with concentrations of curcumin and (F) ABCA1 level with curcumin (40 μM) for the indicated times. $*p < 0.05$ versus untreated group.

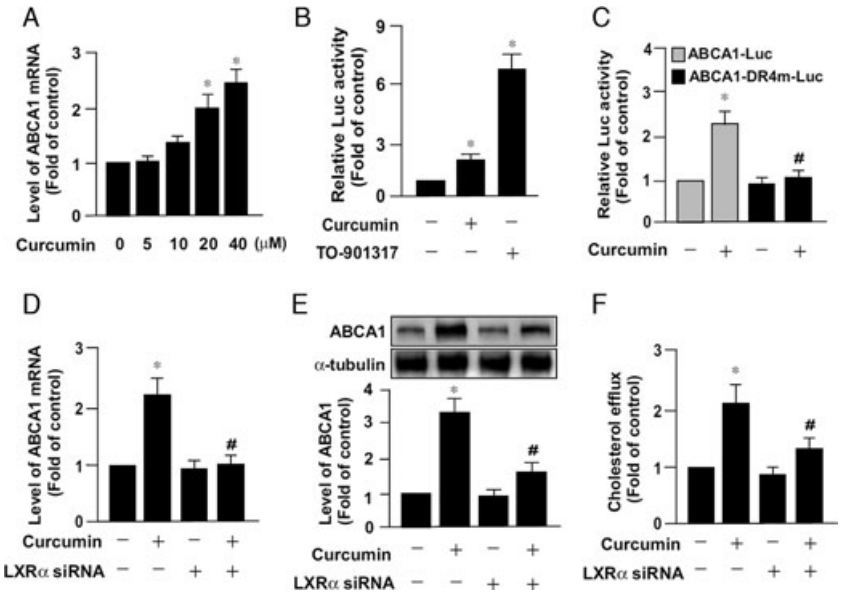

Figure 6. Liver X receptor α (LXRα) activation is essential for curcumin-induced upregulation of ABCA1 in macrophages. (A) Real-time PCR analysis of the mRNA expression of ABCA1 and GAPDH in macrophages incubated with concentrations of curcumin for 18 h. (B) Luciferase assay of cells transfected with the plasmid CMX-GAL-hLXRα with a GAL4-Luc reporter for 24 h, then treated with curcumin (40 μM) or TO-901317 (10 μM) for another 24 h. (C) Cells were transfected with the plasmid phABCA1-Luc or phABCA1-DR4m-Luc for 24 h, then treated with curcumin (40 μM) for 24 h. Renilla activity was an internal control. (D) Real-time PCR, (E) western blot, or (F) cholesterol efflux assay of macrophages transfected with control or LXRα siRNA for 24 h, then curcumin (40 μM) for an additional 18 h. Data are mean ± SEM from five independent experiments. $^{*}p < 0.05$ versus vehicle treatment; $^{\#}p < 0.05$ versus curcumin treatment.

promoter activity (Fig. 7A) and the mRNA and protein expression of ABCA1 (Fig. 7B and C). Moreover, pretreatment with W7 abolished cholesterol efflux elicited by curcumin (Fig. 7D), which suggests the crucial role of calmodulin in the curcumin-mediated suppression of cholesterol accumulation of foam cells.

3.7 Chronic treatment with curcumin retards the development of atherosclerosis in apoE$^{-/-}$ mice

It is known that apoE$^{-/-}$ mice fed with regular mouse chow diet or Western-type diet develop hypercholesterolemia and all phases of atherosclerotic lesions from fatty streaks to fibrous plaque, which are similar to those observed in humans [28, 29]. However, wild-type mice are resistant to the diet-induced hypercholesterolemia and atherosclerosis [29, 30]. By 4–5 months of age, multilayered foam cell lesions were developed within the aortic roots of the aortic apoE$^{-/-}$ mice fed with chow diet [28, 29]. We, therefore, used 4-month-old male apoE$^{-/-}$ mice to investigate the potential functional significance of cucumin in atherosclerosis. After oral administration of curcumin for 4 weeks, aortic roots showed smaller atherosclerotic lesions in apoE$^{-/-}$ mice than those observed in vehicle-treated apoE$^{-/-}$ mice (Fig. 8A). Furthermore, curcumin treatment increased the protein expression of ABCA1, ABCG1, and SR-BI but decreased that of SR-A without affecting the expression of CD36 in apoE$^{-/-}$ aortas (Fig. 8B and C). Moreover, serum levels of IL-6, TNF-α, and MCP-1 were lower in curcumin-treated than vehicle-treated apoE$^{-/-}$ mice (Fig. 8D). In addition, serum levels of total cholesterol and triglycerides and non-HDL-cholesterol were lower with curcumin and that of HDL cholesterol higher in curcumin-treated than vehicle-treated apoE$^{-/-}$ aortas (Fig. 8E).

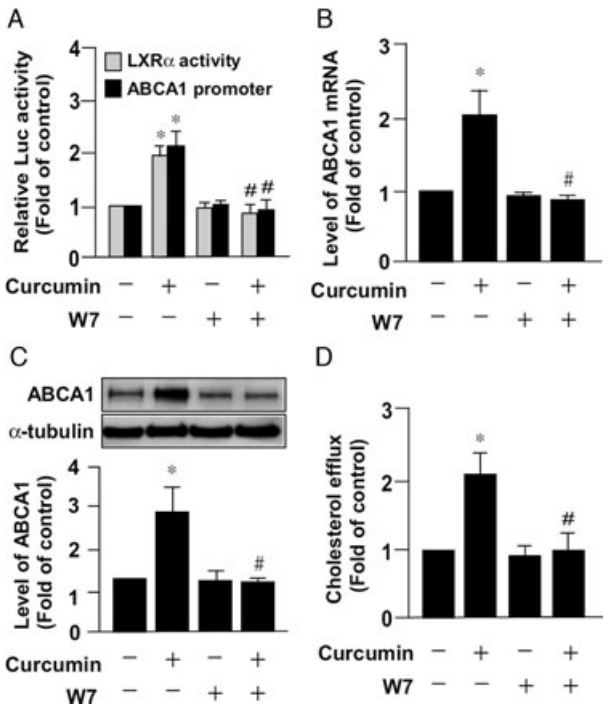

Figure 7. Calmodulin is required for the curcumin-induced increase in LXRα activity, ABCA1 expression, and cholesterol efflux in macrophages. (A) Luciferase assay of macrophages transfected with the plasmid CMX-GAL-hLXRα with a GAL4-Luc reporter or phABCA1-Luc for 24 h, pretreated with calmodulin inhibitor W7 (20 μM) for 2 h, then curcumin (40 μM) for an additional 24 h. Renilla activity was as an internal control. (B–D) Macrophages were preincubated with W7 for 2 h, and then treated with curcumin (40 μM). Real-time PCR or western blot analysis of the mRNA (B) or protein (C) expression, respectively, of ABCA1. (D) Cholesterol efflux analyzed by use of NBD-cholesterol. Data are mean ± SEM from five independent experiments. $^{*}p < 0.05$ versus vehicle treatment; $^{\#}p < 0.05$ versus curcumin treatment.

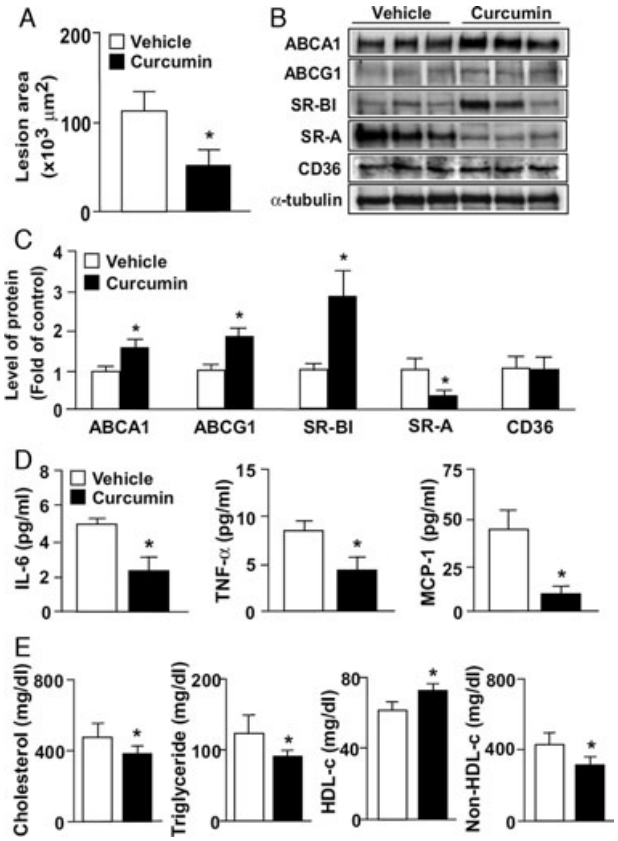

Figure 8. Curcumin retards the development of atherosclerosis in apoE$^{-/-}$ mice. Male apoE$^{-/-}$ mice at 4 months of age received daily oral administration of curcumin (20 mg/kg body weight) or vehicle control ($n = 10$, each group) by gastric gavages for 4 weeks. (A) Atherosclerotic lesions in aortic roots. (B and C) Western blot analysis of protein levels of ABCA1, ABCG1, SR-BI, SR-A, CD36, and α-tubulin in aortas from curcumin- or vehicle-treated apoE$^{-/-}$ mice. (D) ELISA of serum levels of IL-6, TNF-α, and MCP-1. (F) Serum levels of total cholesterol, triglyceride, HDL-cholesterol (HDL-c), and non-HDL-c. *$p < 0.05$ versus vehicle-treated apoE$^{-/-}$ mice.

4 Discussion

The protective effects of curcumin on atherosclerosis have been well established [19–21]. However, the efficacy of curcumin and the possible molecular mechanism involved in cholesterol metabolism of macrophage-derived foam cells remained elusive. We characterized the novel molecular mechanisms underlying the atheroprotection of curcumin in the formation of macrophage foam cells and in apoE$^{-/-}$ mouse aortas. In macrophages, incubation with curcumin attenuated oxLDL-induced cholesterol accumulation by reducing SR-A-dependent oxLDL uptake and promoting ABCA1-dependent cholesterol efflux. The decreased SR-A-mediated oxLDL internalization was due to increased protein degradation of SR-A. Our findings strongly suggest that curcumin has a novel effect in maintenance of cholesterol homeostasis during the transformation of foam cells in atherosclerosis and we delineated the underlying molecular mechanism.

SR-mediated oxLDL internalization and RCT-dependent cholesterol efflux are two key events in the regulation of intracellular cholesterol homeostasis of foam cells [4–10]. We may be the first to report that suppression of cholesterol accumulation by curcumin in macrophage foam cells is likely through decreased oxLDL uptake and increased cholesterol efflux. Additionally, curcumin markedly decreased the protein expression of SR-A by promoting proteolysis of the ubiquitin–proteasome system. SR-A plays an important role in oxLDL uptake in the formation of macrophage foam cells and the development of atherosclerosis [7]. Genetic deletion of SR-A in apoE$^{-/-}$ mice reduced cholesterol accumulation in foam cells and attenuated the progression of atherosclerosis [31]. Recent studies also suggest that transcriptional regulation of SR-A in macrophages can be downregulated by antiatherogenic antioxidants, which indicates its critical role in the pathogenesis of atherogenesis [31, 32]. Here, we report that the suppressive effect of curcumin on oxLDL internalization was through posttranscriptional regulation of SR-A expression, which could be caused by increased association of the ubiquitin–VCP–proteasome pathway and SR-A. Indeed, pharmacological inhibition of the proteasome pathway and calpain activation prevented the curcumin-downregulated SR-A. Our findings suggest that activation of the ubiquitin–VCP–proteasome pathway is implicated in the atheroprotection of curcumin in macrophages. In view of the function of SR-A, the increase in curcumin-induced protein degradation of SR-A likely contributes to reduced oxLDL uptake and subsequent attenuation of foam cell formation.

In addition to the inhibitory effect of curcumin on SR-A expression, curcumin promoted cholesterol efflux by upregulating ABCA1 expression. The curcumin-induced increase in ABCA1 protein expression appears to result from increased ABCA1 transcription. ABCA1 is the most important RCT responsible for cholesterol efflux from macrophage foam cells to apoAI and thus plays a crucial role in the maintenance of cholesterol homeostasis of foam cells [8, 10]. Loss or impaired RCT function in human or experimental animals causes hyperlipidemia, abnormal inflammatory response, and excessive cholesterol deposition in peripheral tissues, including aortas [8–10, 33, 34]. We further showed curcumin-induced upregulation of ABCA1 accompanied by increased LXRα activity. This notion was further supported by the curcumin-induced increase in promoter activity abrogated in macrophages transfected with the LXRE mutant. Moreover, the inhibition of LXRα activation by LXRα siRNA diminished the curcumin-mediated upregulation of ABCA1. The effect of curcumin promoting cholesterol efflux was abolished in functional analysis involving inhibited LXRα activation. Collectively, these results imply the essential role of LXRα-ABCA1-dependent cholesterol efflux in curcumin-induced suppression of the formation of foam cells.

We also found prolonged half life of ABCA1 protein with curcumin treatment (Supporting Information Fig. 5), which

implies that curcumin might stabilize ABCA1 protein. This result is consistent with previous findings that stabilization of ABCA1 protein enhances cholesterol efflux and leads to reduced lipid accumulation in foam cells [35, 36]. In contrast, destabilization of ABCA1 protein impairs lipid clearance and results in augmentated cholesterol accumulation [37, 38]. Calpain, an important protease of proteasome, can recognize the Pro-Glu-Ser-Thr sequence (PEST motif) within ABCA1 protein and subsequently promote the turnover of ABCA1 protein with proinflammatory stimuli [36, 39–41]. Intriguingly, we showed that curcumin increased calpain activity accompanied by increased ABCA1 stability. Our results may differ from previous studies because of calmodulin binding to ABCA1 and thus protecting it against calpain-mediated proteolysis. Calmodulin is a calcium-binding protein and orchestrates many signaling pathways by interacting with intracellular proteins [42]. In addition to the inhibitory effect of calmodulin on protein degradation of ABCA1, it can regulate ABCA1 transcription [43], which further supports our findings that calmodulin is required for curcumin-induced gene expression of ABCA1. In addition, we showed that calmodulin is the upstream signaling molecule for LXRα-ABCA-dependent cholesterol efflux by curcumin. On the other hand, several lines of evidence indicate that LXRα is also a key transcript factor for the gene expression of ABCG1 [8, 10]. In our study, we indeed found that treatment with curcumin caused an increase in mRNA expression of ABCG1 (data not shown) but failed to increase the protein expression of ABCG1. We thought that the possible explanation for the discrepancy between ABCA1 and ABCG1 protein expression by curcumin may be due to the difference in the calpain-dependent posttranscriptional regulation. Calmodulin is known to prevent protein degradation of ABCA1 by interacting with calmodulin-binding motif 1-5-8-14 (1244–1257 amino acids within ABCA1 protein) that is located near the PEST sequence (1283–1306 amino acids) [43]. However, database analysis showed that calmodulin-binding motif within ABCG1 protein (253 to 264 amino acids) is far from the PEST sequence (370–383 amino acids). This information suggests that curcumin-induced calmodulin activation may not prevent the calpain-dependent proteolysis of ABCG1, which may explain why curcumin has no effect on the protein expression of ABCG1. Taken together, our observations suggest that both transcriptional and posttranscriptional regulation may work in concert to modulate the expression of ABCA1 with curcumin treatment.

Recently, Kim and colleagues demonstrated that calmodulin signaling is critical for heme oxygenase-1 (HO-1)-mediated anti-inflammation effects of curcumin in macrophages [27]. HO-1 has beneficial effects against the development of atherosclerosis [44, 45]. Overexpression of HO-1 retards the progression of atherosclerosis in hyperlipidemic mice [44]. Deletion of HO-1 promotes the expression of SR-A, thus leading to accelerated foam cell formation and atherosclerosis [45]. Moreover, we previously reported that HO-1 contributes to the downregulation of SR-A and upregulation of ABCA1 by EGb761 in macrophages [23]. Curcumin is a potent HO-1 inducer [27]; however, whether HO-1 participates in the curcumin-mediated suppression of foam cell formation warrants further investigation.

Our findings of the suppressive effect of curcumin on foam cell formation are not limited to the cell culture system. Our *in vivo* experiments demonstrated that in apoE$^{-/-}$ mice, curcumin attenuated atherosclerosis progression, reduced systemic inflammation, decreased the serum levels of cholesterol and triglycerides, and increased that of HDL cholesterol, which are consistent with previous findings [46]. In fact, curcumin could inhibit tissue inflammation and hyperlipidemia in several animal models [46–48]. Curcumin can ameliorate oxLDL-induced cholesterol accumulation by inhibiting the SREBP1/caveolin-1 pathway in vascular smooth muscle cells [46]. Furthermore, curcumin prevented high fat diet-induced lipid accumulation in liver and adipose tissue, as well as obesity in rodents [47, 48]. Moreover, curcumin reduced cholesterol absorption in intestinal cells by downregulating the SREBP2-dependent expression of Niemann-Pick C1-like 1 [49]. Very recently, Shin *et al.* reported that administration with curcumin confers a protection against from the high fat diet-induced atherosclerosis by regulating hepatic cholesterol metabolism in LDL receptor-deficient mice [50]. Here, we further demonstrated increased protein levels of RCTs, including ABCA1, ABCG1, and SR-BI, and decreased protein expression of SR-A in curcumin-treated apoE$^{-/-}$ mice. Therefore, these therapeutic effects seem to be widespread, but the target cells for curcumin cannot be identified under this hyperlipidemic situation. Accordingly, curcumin may have a therapeutic function in various organs through dissimilar mechanisms.

In summary, we demonstrate new insights into the protective effect of curcumin in reducing cholesterol accumulation in the development of foam cells by downregulating SR-A and upregulating ABCA1 by a proteasome- and LXRα-dependent pathway, respectively. We provide new information for better understanding the molecular mechanisms of curcumin in inhibiting the formation of foam cells in atherosclerosis.

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5 References

[1] Glass, C. K., Witztum, J. L., Atherosclerosis. The road ahead. *Cell* 2001, *104*, 503–516.

[2] Lusis, A. J., Atherosclerosis. *Nature* 2000, *407*, 233–241.

[3] Berliner, J. A., Heinecke, J. W., The role of oxidized lipoproteins in atherogenesis. *Free Radic. Biol. Med.* 1996, *20*, 707–727.

[4] Kleemann, R., Zadelaar, S., Kooistra, T., Cytokines and atherosclerosis: a comprehensive review of studies in mice. *Cardiovasc. Res.* 2008, *79*, 360–376.

[5] Li, A. C., Glass, C. K., The macrophage foam cell as a target for therapeutic intervention. *Nat. Med.* 2002, *8*, 1235–1242.

[6] Collot-Teixeira, S., Martin, J., McDermott-Roe, C., Poston, R., McGregor, J. L. CD36 and macrophages in atherosclerosis. *Cardiovasc. Res.* 2007, *75*, 468–477.

[7] Kunjathoor, V. V., Febbraio, M., Podrez, E. A., Moore, K. J. et al., Scavenger receptors class A-I/II and CD36 are the principal receptors responsible for the uptake of modified low density lipoprotein leading to lipid loading in macrophages. *J. Biol. Chem.* 2002, *277*, 49982–49988.

[8] Cheng, L. C., Su, K. H., Kou, Y. R., Shyue, S. K. et al., α-Lipoic acid ameliorates foam cell formation via liver X receptor α-dependent upregulation of ATP-binding cassette transporters A1 and G1. *Free Radic. Biol. Med.* 2011, *50*, 47–54.

[9] Ji, A., Meyer, J. M., Cai, L., Akinmusire, A. et al., Scavenger receptor SR-BI in macrophage lipid metabolism. *Atherosclerosis* 2011, *217*, 106–112.

[10] Chen, C. Y., Shyue, S. K., Ching, L. C., Su, K. H. et al., Wogonin promotes cholesterol efflux by increasing protein phosphatase 2B-dependent dephosphorylation at ATP-binding cassette transporter-A1 in macrophages. *J. Nutr. Biochem.* 2011, *22*, 1015–1021.

[11] Lu, K. Y., Ching, L. C., Su, K. H., Yu, Y. B. et al., Erythropoietin suppresses the formation of macrophage foam cells: role of liver X receptor α. *Circulation* 2010, *121*, 1828–1837.

[12] Rao, C. V., Rivenson, A., Simi, B., Reddy, B. S., Chemoprevention of colon carcinogenesis by dietary curcumin, a naturally occurring plant phenolic compound. *Cancer Res.* 1995, *55*, 259–266.

[13] Wu, J. C., Lai, C. S., Badmaev, V., Nagabhushanam, K. et al., Tetrahydrocurcumin, a major metabolite of curcumin, induced autophagic cell death through coordinative modulation of PI3K/Akt-mTOR and MAPK signaling pathways in human leukemia HL-60 cells. *Mol. Nutr. Food Res.* 2011, *55*, 1646–1654.

[14] Chung, L. C., Tsui, K. H., Feng, T. H., Lee, S. L. et al., Curcumin provides potential protection against the activation of hypoxia and prolyl 4-hydroxylase inhibitors on prostate-specific antigen expression in human prostate carcinoma cells. *Mol. Nutr. Food Res.* 2011, *55*, 1666–1676.

[15] Villegas, I., Sánchez-Fidalgo, S., de la Lastra, C. A., Chemopreventive effect of dietary curcumin on inflammation-induced colorectal carcinogenesis in mice. *Mol. Nutr. Food Res.* 2011, *55*, 259–267.

[16] Conney, A. H., Lysz, T., Ferraro, T., Abidi, T. F. et al., Inhibitory effect of curcumin and some related dietary compounds on tumor promotion and arachidonic acid metabolism in mouse skin. *Adv. Enzyme Regul.* 1991, *31*, 385–396.

[17] Rao, C. V., Rivenson, A., Simi, B., Reddy, B. S., Chemoprevention of colon carcinogenesis by dietary curcumin, a naturally occurring plant phenolic compound. *Cancer Res.* 1995, *55*, 259–266.

[18] Strimpakos, A. S., Sharma, R. A., Curcumin: preventive and therapeutic properties in laboratory studies and clinical trials. *Antioxid. Redox. Signal.* 2008, *10*, 511–545.

[19] Wongcharoen, W., Phrommintikul, A., The protective role of curcumin in cardiovascular diseases. *Int. J. Cardiol.* 2009, *133*, 145–151.

[20] Seo, K. I., Choi, M. S., Jung, U. J., Kim, H. J. et al., Effect of curcumin supplementation on blood glucose, plasma insulin, and glucose homeostasis related enzyme activities in diabetic db/db mice. *Mol. Nutr. Food Res.* 2008, *52*, 995–1004.

[21] Olszanecki, R., Jawień, J., Gajda, M., Mateuszuk, L. et al., Effect of curcumin on atherosclerosis in apoE/LDLR-double knockout mice. *J. Physiol. Pharmacol.* 2005, *56*, 627–635.

[22] Forman, B. M., Ruan, B., Chen, J., Schroepfer, G. J. Jr. et al., The orphan nuclear receptor LXRα is positively and negatively regulated by distinct products of mevalonate metabolism. *Proc. Natl. Acad. Sci. USA* 1997, *94*, 10588–10593.

[23] Hori, N., Hayashi, H., Sugiyama, Y., Calpain-mediated cleavage negatively regulates the expression level of ABCG1. *Atherosclerosis* 2011, *215*, 383–391.

[24] Goodarzi, M. O., Taylor, K. D., Jones, M. R., Fang, B. et al., Replication of calpain-10 genetic association with carotid intima-media thickness. *Atherosclerosis* 2009, *205*, 503–505.

[25] Chapman, E., Fry, A. N., Kang, M., The complexities of p97 function in health and disease. *Mol. Biosyst.* 2011, *7*, 700–710.

[26] Tanaka, A., Cleland, M. M., Xu, S., Narendra, D. P. et al., Proteasome and p97 mediate mitophagy and degradation of mitofusins induced by Parkin. *J. Cell. Biol.* 2010, *191*, 1367–1380.

[27] Kim, A. N., Jeon, W. K., Lee, J. J, Kim, B. C., Up-regulation of heme oxygenase-1 expression through CaMKII-ERK1/2-Nrf2 signaling mediates the anti-inflammatory effect of bisdemethoxycurcumin in LPS-stimulated macrophages. *Free Radic. Biol. Med.* 2010, *49*, 323–331.

[28] Nakashima, Y., Plump, A. S., Raines, E. W., Breslow, J. L. et al., ApoE-deficient mice develop lesions of all phases of atherosclerosis throughout the arterial tree. *Arterioscler. Thromb.* 1994, *14*, 133–140.

[29] Reddick, R. L., Zhang, S. H., Maeda, N., Atherosclerosis in mice lacking apo E. Evaluation of lesional development and progression. *Arterioscler. Thromb.* 1994, *14*, 141–147.

[30] Shi, W., Wang, N. J., Shih, D. M., Sun, V. Z. et al., Determinants of atherosclerosis susceptibility in the C3H and C57BL/6 mouse model: evidence for involvement of endothelial cells but not blood cells or cholesterol metabolism. *Circ. Res.* 2000, *86*, 1078–1084.

[31] Suzuki, H., Kurihara, Y., Takeya, M., Kamada, N. et al., A role for macrophage scavenger receptors in atherosclerosis and susceptibility to infection. *Nature* 1997, *386*, 292–296.

[32] Tsai, J. Y., Su, K. H., Shyue, S. K., Kou, Y. R. et al., EGb761 ameliorates the formation of foam cells by regulating the expression of SR-A and ABCA1: role of haem oxygenase-1. *Cardiovasc. Res.* 2010, *88*, 415–423.

[33] Zhang, Y., Luo, Z., Ma, L., Xu, Q. et al., Resveratrol prevents the impairment of advanced glycosylation end products (AGE) on macrophage lipid homeostasis by suppressing the receptor for AGE via peroxisome proliferator-activated receptor gamma activation. *Int. J. Mol. Med.* 2010, *25*, 729–734.

[34] Brooks-Wilson, A., Marcil, M., Clee, S. M., Zhang, L. H. et al., Mutations in ABC1 in Tangier disease and familial high-density lipoprotein deficiency. *Nat. Genet.* 1999, *22*, 336–345.

[35] Joyce, C. W., Amar, M. J., Lambert, G., Vaisman, B. L. et al., The ATP binding cassette transporter A1 (ABCA1) modulates the development of aortic atherosclerosis in C57BL/6 and apoE-knockout mice. *Proc. Natl. Acad. Sci. USA* 2002, *99*, 407–412.

[36] Arakawa, R., Yokoyama, S., Helical apolipoproteins stabilize ATP-binding cassette transporter A1 by protecting it from thiol protease-mediated degradation. *J. Biol. Chem.* 2002, *277*, 22426–22429.

[37] Wang, N., Chen, W., Linsel-Nitschke, P., Martinez, L. O. et al., A PEST sequence in ABCA1 regulates degradation by calpain protease and stabilization of ABCA1 by apoA-I. *J. Clin. Invest.* 2003, *111*, 99–107.

[38] Lee, T. S., Lin, C. Y., Tsai, J. Y., Wu, Y. L. et al., Resistin increases lipid accumulation by affecting class A scavenger receptor, CD36 and ATP-binding cassette transporter-A1 in macrophages. *Life Sci.* 2009, *84*, 97–104.

[39] Wang, Y., Oram, J. F., Unsaturated fatty acids phosphorylate and destabilize ABCA1 through a phospholipase D2 pathway. *J. Biol. Chem.* 2005, *280*, 35896–36903.

[40] Martinez, L. O., Agerholm-Larsen, B., Wang, N., Chen, W. et al., Phosphorylation of a pest sequence in ABCA1 promotes calpain degradation and is reversed by ApoA-I. *J. Biol. Chem.* 2003, *278*, 37368–37374.

[41] Arakawa, R., Tsujita, M., Iwamoto, N., Ito-Ohsumi, C. et al., Pharmacological inhibition of ABCA1 degradation increases HDL biogenesis and exhibits antiatherogenesis. *J. Lipid Res.* 2009, *50*, 2299–2305.

[42] Choi, J., Husain, M., Calmodulin-mediated cell cycle regulation: new mechanisms for old observations. *Cell Cycle* 2006, *5*, 2183–2186.

[43] Iwamoto, N., Lu, R., Tanaka, N., Abe-Dohmae, S. et al., Calmodulin interacts with ATP binding cassette transporter A1 to protect from calpain-mediated degradation and upregulates high-density lipoprotein generation. *Arterioscler. Thromb. Vasc. Biol.* 2010, *30*, 1446–1452.

[44] Juan, S. H., Lee, T. S., Tseng, K. W., Liou, J. Y. et al., Adenovirus-mediated heme oxygenase-1 gene transfer inhibits the development of atherosclerosis in apolipoprotein E-deficient mice. *Circulation* 2001, *104*, 1519–1525.

[45] Orozco, L. D., Kapturczak, M. H., Barajas, B., Wang, X. et al., Heme oxygenase-1 expression in macrophages plays a beneficial role in atherosclerosis. *Circ. Res.* 2007, *100*, 1703–1711.

[46] Yuan, H. Y., Kuang, S. Y., Zheng, X., Ling, H. Y. et al., Curcumin inhibits cellular cholesterol accumulation by regulating SREBP-1/caveolin-1 signaling pathway in vascular smooth muscle cells. *Acta Pharmacol. Sin.* 2008, *29*, 555–563.

[47] Asai, A., Miyazawa, T., Dietary curcuminoids prevent high-fat diet-induced lipid accumulation in rat liver and epididymal adipose tissue. *J. Nutr.* 2001, *131*, 2932–2935.

[48] Weisberg, S. P., Leibel, R., Tortoriello, D. V., Dietary curcumin significantly improves obesity-associated inflammation and diabetes in mouse models of diabesity. *Endocrinology* 2008, *149*, 3549–3558.

[49] Kumar, P., Malhotra, P., Ma, K., Singla, A. et al., SREBP2 mediates the modulation of intestinal NPC1L1 expression by curcumin. *Am. J. Physiol. Gastrointest. Liver Physiol.* 2011, *301*, G148–G155.

[50] Shin, S. K., Ha, T. Y., McGregor, R. A., Choi, M. S., Long-term curcumin administration protects against atherosclerosis via hepatic regulation of lipoprotein cholesterol metabolism. *Mol. Nutr. Food Res.* 2011, *55*, 1829–1840.

