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Induction of DKK1 by ox-LDL negatively regulates intracellular lipid accumulation in macrophages

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ABSTRACT

Dickkopf1 (DKK1), a canonical Wnt/β-catenin pathway antagonist, is closely associated with cardiovascular disease and adipogenesis. We performed an in vitro study to determine whether oxidized low-density lipoprotein (ox-LDL) increased the expression of DKK1 in macrophages and whether β-catenin and liver X receptor α (LXRα) were involved in this regulation. Induction of DKK1 expression by ox-LDL decreased the level of lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) via a Wnt/β-catenin pathway and increased ATP-binding cassette transporter A/G1 (ABCA/G1) levels via a signal transducer and activator of transcription 3 (STAT3) pathway. Lower LOX-1 and higher ABCA/G1 levels inhibited cholesterol loading in macrophages. In conclusion, ox-LDL may induce DKK1 expression in macrophages to inhibit the accumulation of lipids through a mechanism that involves downregulation of LOX-1-mediated lipid uptake and upregulation of ABCA/G1-dependent cholesterol efflux.

1. Introduction

Oxidized LDL (ox-LDL) is associated with the pathogenesis of various human diseases, such as atherosclerosis, aortic valve stenosis [1] and age-related macular degeneration [2]. Monocytes in the blood roll and adhere to the arterial wall, migrate into the subendothelial space, and differentiate into macrophages, which internalize ox-LDL and other pathogenic debris [3] to reduce the toxicity of ox-LDL. Foam cells formed by macrophages play a key and complicated role in atherosclerosis. For instance, in the formation of early "fatty streaks", monocyte-derived macrophages loaded with lipids are transformed into foam cells. Successive accumulation of lipids in macrophages leads to apoptosis and necrosis.

The wingless (Wnt) signaling pathway is a conserved pathway involved in embryonic development and regulating cell differentiation, proliferation and migration [4]. Many diseases related to an abnormal Wnt pathway include degenerative diseases (Alzheimer's disease) [5], malignant diseases (multiple myeloma) [6], systemic diseases (rheumatoid arthritis) [7] and chronic diseases (atherosclerosis) [8]. The Wnt pathway as a modulator in disease is a current focus.

The Dickkopf-related protein family is secreted in antagonism to the Wnt pathway. Dickkopf1 (DKK1) has been widely reported. DKK1 cuts off the Wnt pathway and decreases the downstream target genes of β-catenin, such as cyclinD1, C-Myc, and peroxisome proliferator-activated receptor δ [8]. Previous studies showed DKK1 involved in cardiovascular diseases such as cardiogenesis [9], vascular calcification [10], and angiogenesis [11]; it also promotes the endothelial–mesenchymal transition in aortic endothelial cells [12]. DKK1 has an antifibrotic role in microvascular mural cells [13]. It is a mediator in platelet-mediated endothelial cell activation in atherosclerosis [14]. Our recent study showed a higher baseline plasma level of DKK1 in patients with ST-segment elevation myocardial infarction as compared with patients with non-ST-segment elevation acute coronary syndrome, and the rate of major adverse cardiac events was associated with increased DKK1 level [15].

Liver X receptor α (LXRα) is a nuclear receptor that plays an important role in maintaining cholesterol homeostasis. The activation of LXRα increases reverse cholesterol transport in macrophages by upregulating ATP-binding cassette transporter A/G1 (ABCA/G1) as its target gene [16]. Both ABCA1 and ABCG1 are members of the large superfamily of ABC transmembrane transporters, responsible for cellular cholesterol efflux. However, whether DKK1 has any effects on lipid accumulation by ABCA/G1 has not yet been studied.

Abbreviations: DKK1, Dickkopf1; LXRα, liver X receptor α; LOX-1, lectin-like oxidized low-density lipoprotein receptor-1; ABCA/G1, ATP-binding cassette transporter A/G1

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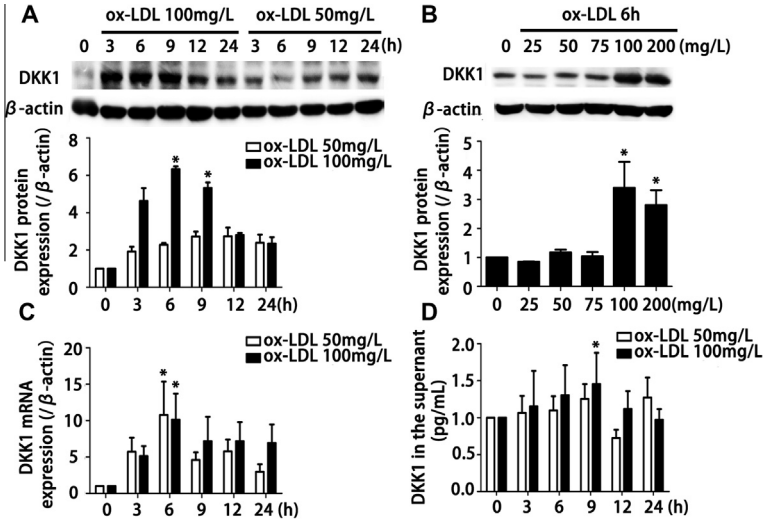

Fig. 1. Effect of ox-LDL oxidized low-density lipoprotein (ox-LDL) on DKK1 release in macrophages derived from THP-1 cells. (A–C) After stimulation with PMA (160 nM), THP-1 cells differentiated into macrophages were incubated with different concentrations of ox-LDL for various times. Shows western blot (A, B) and RT-PCR (C) analysis of protein and mRNA levels of DKK1 in macrophages. (D) ELISA of DKK1 secretion in cell culture medium. One-way ANOVA with LSD post hoc test, $n = 3$.

Whether and how DKK1 affects lipid metabolism in macrophages remain unclear. To determine the role and regulation of DKK1 in lipid metabolism, we examined the regulation of DKK1 by ox-LDL and its function in lipid accumulation and the underlying mechanisms.

2. Materials and methods

2.1. Materials

Recombinant human DKK1 (rDKK1) and human DKK1 ELISA kit were from R&D Systems (Minneapolis, MN, USA). Antibodies for phosophorylated-β-catenin (p-β-catenin), β-catenin, cyclinD1, p-signal transducer and activator of transcription 3 (p-STAT3), STAT3, β-actin and GAPDH were from Cell Signaling Technology (Danvers, MA, USA). Antibody for ABCG1 and protein A/G plus agarose were from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Antibodies for ABCA1, DKK1, LXRα and LXRβ were from Abcam (Cambridge, UK). Antibody for lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) was from Epitomics (Hangzhou, China). Geranylgeranyl pyrophosphate (GGPP), 22-(S)-hydroxycholesterol and 22-(R)-hydroxycholesterol were from Sigma (St. Louis, MO, USA). T0901317, GW3965 and Stattic were from Selleckchem (Shanghai). DiI-labeled ox-LDL (DiI-ox-LDL) and ox-LDL were from Yiyuan Biotechnologies (Guangzhou, China).

2.2. Cell culture

THP-1 cells obtained from the American Type Culture Collection (ATCC) were grown in RPMI 1640 medium with 10% fetal bovine serum and 1% penicillin/streptomycin in a 5% CO_2-humidified incubator at 37 °C. For THP-1 cell differentiation into macrophages, cells were seeded in culture plates at 2×10^6 cells/1 ml per well and allowed to adhere and differentiate overnight at 37 °C in the presence of 160 nM phorbol myristate acetate (PMA).

2.3. Real-time PCR

The total RNA was extracted from macrophages by use of TranZol reagent. cDNA was prepared from 2 μg RNA with PrimeScript RT reagent kit (Takara Bio Inc.; Otsu, Shiga, Japan). Real-time PCR involved use of the SYRB Premix Ex Taq kit (Takara Bio Inc.). The primer sequences were for DKK1, forward, 5′-GGGAATTACTGCA AAAATGGAATA-3′, reverse, 5′-ATGACCGGAGACAAACCAGAAC-3′; and β-actin, forward, 5′-CGTGCGTGACATTAAGGAGA-3′, reverse, 5′-CACCTTCACCGTTCCAGTTT-3′. The $2^{-\Delta\Delta Ct}$ method was used to assess the relative mRNA expression level normalized to that of β-actin.

2.4. Western blot analysis

Cells were lysed with RIPA lysis buffer including 1 mM PMSF. Equal amounts of extracted proteins were separated on 10% SDS–PAGE gel and transferred to PVDF membranes for incubation with primary antibodies overnight at 4 °C. The bands were visualized by enhanced chemiluminescence reagents and recorded by use of the LAS-4000 luminescent image analyzer (Fujifilm, Stamford, CT, USA).

2.5. Enzyme-linked immunosobent assay (ELISA)

THP-1 differentiated macrophages were stimulated with or without ox-LDL, and medium was collected and frozen at −80 °C. DKK1 in the supernatant was measured by ELISA assay according to the manufacturer's instruction.

2.6. RNA interference

Macrophages cultured with antibiotic-free medium were transfected with specific siRNA or negative control siRNA (GenePharma, shanghai) by using Lipofectamine 2000. After 6 h, complete culture medium was replaced. Gene silencing efficiency was determined by western blot analysis.

2.7. Foam cell formation and Dil-ox-LDL uptake assay

Differentiated THP-1 cells were cultured on slides and loaded with ox-LDL. After siRNA transfection or rDKK1 incubation, cells were fixed with 4% paraformaldehyde and stained with Oil Red O and counterstained with hematoxylin. Images were obtained by light microscopy to assess foam cell formation.

Dil-ox-LDL, labeled with red fluorescence, has been used to measure ox-LDL uptake by macrophages. Differentiated THP-1 cells were cultured on Lab-Tek II chamber slides and loaded with DiI-ox-LDL. After siRNA transfection or rDKK1 incubation, cells were fixed with 4% paraformaldehyde and counterstained with

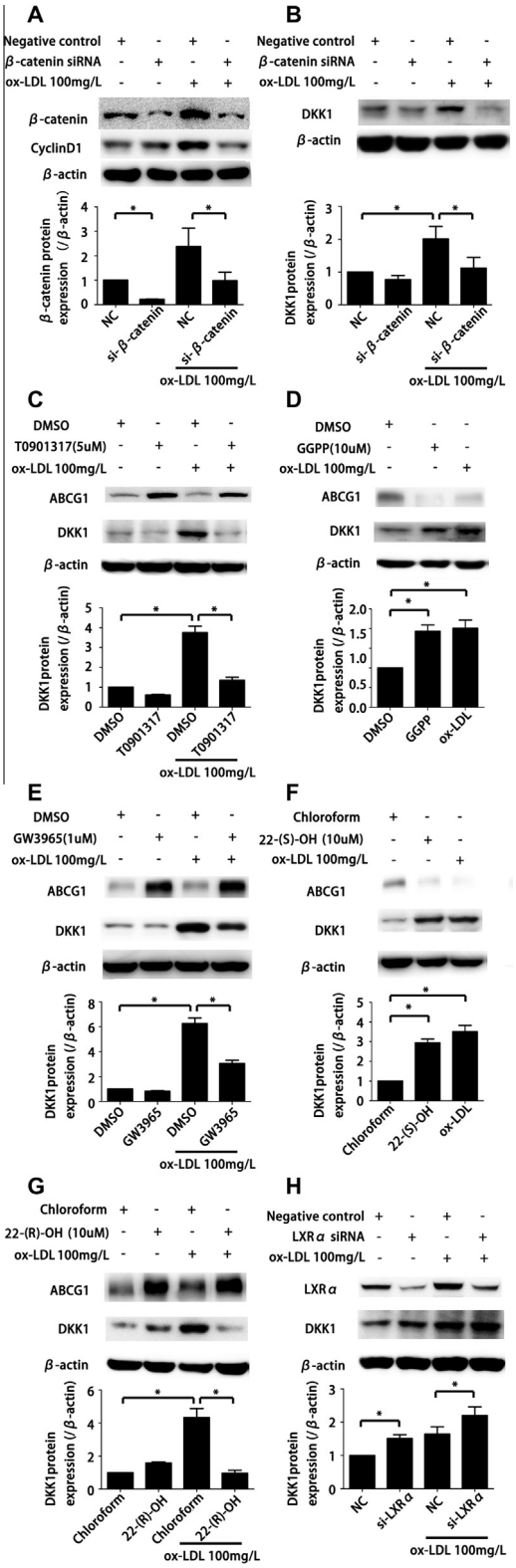

Fig. 2. The interaction of liver X receptor α (LXRα) and β-catenin is involved in ox-LDL regulation of DKK1. (A) Western blot analysis of siRNA knockdown of β-catenin and cyclinD1 in macrophages differentiated from THP-1 cells. (B) Level of DKK1 regulated by ox-LDL (100 mg/L) with or without β-catenin siRNA. (C, E, G) Cells were pretreated with DMSO or T0901317 (5 μM), GW3965 (1 μM), or 22-(R)-hydroxycholesterol (10 μM), then incubated with 100 mg/L ox-LDL for 6 h. (D, F) Cells were pretreated with GGPP (10 μM), 22-(S)-hydroxycholesterol (10 μM) or ox-LDL (100 mg/L) to mimic the inhibition of LXRα activity by ox-LDL. (H) Western blot analysis of DKK1 with knockdown of LXRα by siRNA. One-way ANOVA with LSD post hoc test, $n = 3–4$.

DAPI. Cells were examined by confocal microscopy at 549 nm excitation and 565 nm emission.

2.8. Immunoprecipitation

Differentiated THP-1 cells were incubated with or without ox-LDL, then lysed with lysis buffer for western blot analysis and immunoprecipitation. Cell lysates were precleared and incubated with cognate antibodies and 50% protein A/G agarose overnight at 4 °C with gentle rotation. Agarose-bound immunoprecipitates were rinsed and underwent SDS–PAGE (2×) before western blot analysis.

2.9. Statistical analysis

All experiments were repeated at least 3 times. Data are presented as mean ± S.E.M. Data analysis involved unpaired *t*-test and one-way ANOVA with LSD post hoc test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Ox-LDL upregulates the expression of DKK1 in macrophages

To investigate the effect of ox-LDL on DKK1 in macrophages, cells were stimulated with different concentrations of ox-LDL for different times. Stimulation with 100 mg/L ox-LDL significantly increased both the protein and mRNA expression of DKK1 at 6 h (Fig. 1A–C, $P < 0.05$) and significantly increased the secretion of DKK1 in culture medium at 9 h (Fig. 1D, $P < 0.05$). Therefore, ox-LDL at 100 mg/L for 6 h was used in subsequent experiments.

3.2. Interaction of LXRα and β-catenin is responsible for ox-LDL-upregulated DKK1

To further investigate the regulation of DKK1 by ox-LDL, we blocked the expression of β-catenin in macrophages by siRNA (Fig. 2A, $P < 0.05$). We also detected the level of cyclinD1 as the target gene of β-catenin to reflect the blockage of the canonical Wnt pathway. β-catenin siRNA knockdown reduced the ox-LDL-increased protein level of DKK1 in macrophages (Fig. 2B, $P < 0.05$). LXRα agonists can increase the expression of ABCG1 to accelerate cholesterol efflux [17]. Pretreatment with the LXRα agonists T0901317, GW3965 and 22-(R)-hydroxycholesterol abolished the ox-LDL-increased DKK1 expression (Fig. 2C, E, G, $P < 0.05$). The LXRα antagonists GGPP and 22-(S)-hydroxycholesterol increased the expression of DKK1, which resembled the upregulation of DKK1 by ox-LDL (Fig. 2D and F, $P < 0.05$). Knocking down LXRα level by siRNA increased the level of DKK1 as well (Fig. 2H, $P < 0.05$).

LXRα was reported to suppress the transactivation of β-catenin by directly binding with β-catenin [18]. To confirm the interaction of LXRα and β-catenin, immunoprecipitation assay revealed that ox-LDL stimulation decreased the interaction of LXRα and β-catenin (Supplementary Fig. S1A). We also explored the function of LXRβ and found that it had no significant effect on regulation of DKK1 by ox-LDL (Supplementary Fig. S1B and S1C, $P > 0.05$). These results suggested that ox-LDL upregulated the level of DKK1 by decreasing the repression of LXRα on β-catenin.

3.3. DKK1 inhibits lipid accumulation and foam cell formation in macrophages

We evaluated the effect of DKK1 on lipid accumulation in macrophages. After DKK1 knockdown with siRNA, cells were treated with 100 mg/L ox-LDL. Intracellular lipid droplet accumulation

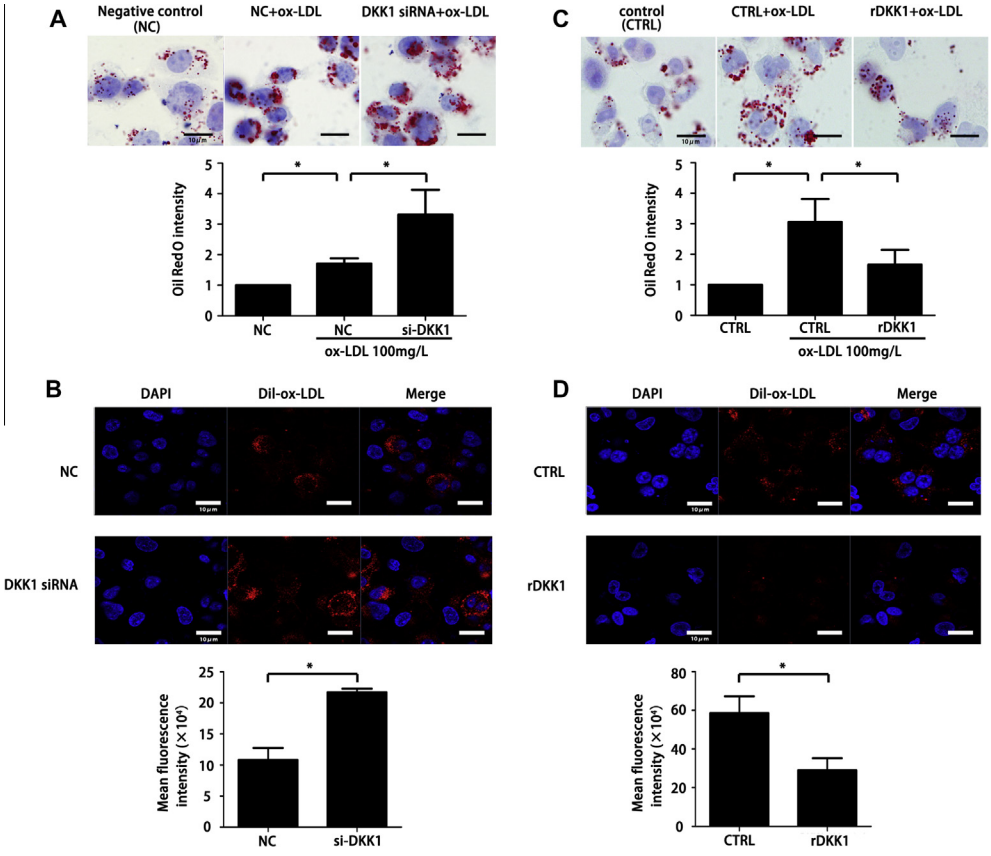

Fig. 3. Effect of DKK1 on foam cell formation in macrophages. (A) Cells were transfected with DKK1 siRNA or scrambled siRNA, then incubated with 100 mg/L ox-LDL. Internalization of ox-LDL was detected by Oil Red O staining. (B) Confocal microscopy of macrophages transfected with DKK1 siRNA, then Dil-ox-LDL (red) added into the cell medium overnight. DAPI was used to stain nuclei. (C) Cells were incubated with recombinant DKK1 (rDKK1; 100 ng/mL) for 12 h, then 100 mg/L ox-LDL was added to examine foam cell formation. (D) Macrophages were pretreated with rDKK1 (100 ng/mL) for 12 h, then incubated with Dil-ox-LDL overnight in the dark. Unpaired *t*-test, $n = 3$.

was greater with DKK1 siRNA knockdown than the negative control (Fig. 3A, $P < 0.05$). To confirm our findings, confocal microscopy revealed that DKK1 siRNA knockdown significantly increased intracellular DiI-ox-LDL level (Fig. 3B, $P < 0.05$).

Furthermore, we pretreated cells with 100 ng/mL rDKK1 for 12 h, then 100 mg/L ox-LDL. Intracellular lipid droplet accumulation was decreased (Fig. 3C, $P < 0.05$) as was intracellular DiI-ox-LDL level (Fig. 3D, $P < 0.05$), so DKK1 inhibited lipid accumulation and foam cell formation in macrophages.

3.4. Effect of DKK1 on Wnt/β-catenin pathway in macrophages

To test the inhibition of DKK1 on the Wnt/β-catenin pathway in macrophages, we examined the level of total β-catenin and its phosphorylation after DKK1 siRNA transfection or rDKK1 pretreatment. DKK1 siRNA knockdown increased the expression of β-catenin and decreased its phosphorylation in macrophages (Fig. 4A, $P < 0.05$), which was in contrast with the effects of rDKK1 (Fig. 4B, $P < 0.05$). Then we treated cells with β-catenin siRNA to mimic the inhibition of DKK1 on the Wnt//β-catenin pathway. Intracellular lipid droplet accumulation and DiI-ox-LDL level were both decreased with β-catenin siRNA knockdown (Fig. 4C and D, $P < 0.05$), so DKK1 suppressed lipid accumulation in part via a Wnt/β-catenin pathway.

3.5. DKK1 affects the expression of LOX-1 and ABCA/G1 via a Wnt and STAT3 pathway, respectively

LOX-1 and ABCA/G1 are closely related to lipid metabolism, which has an important role in the progression of atherosclerosis. We found that rDKK1 abolished the ox-LDL-increased expression of LOX-1 (Fig. 5A, $P < 0.05$) and DKK1 siRNA knockdown increased the expression (Supplementary Fig. S2). We treated cells with β-catenin siRNA to mimic the inhibition of the Wnt pathway by DKK1. β-catenin siRNA knockdown blocked the ox-LDL-increased expression of LOX-1, which resembled the rDKK1 effect (Fig. 5B, $P < 0.05$).

A STAT-binding site in the ABCA1 promoter at −350 bp [19] offers a potential link of the STAT3 pathway to ABCA/G1. However, whether DKK1 affects the expression of ABCA/G1 or regulation through the STAT3 pathway has not been reported. Phosphorylation of STAT3 was increased in macrophages incubated with rDKK1 since 10 min (Supplementary Fig. S3, $P < 0.05$). To address whether the upregulation of ABCA/G1 expression by DKK1 was mediated through an STAT3 pathway, we used Stattic, a selective STAT3 inhibitor, to inhibit STAT3 phosphorylation (Fig. 5C, $P < 0.05$), and found a significantly impaired effect of rDKK1 on ABCA/G1 expression (Fig. 5D, $P < 0.05$). siRNA knockdown of STAT3 expression resulted in abolished upregulation of ABCA/G1 expression by rDKK1 (Fig. 5E, $P < 0.05$).

4. Discussion

In the present study, we found that ox-LDL induced the expression of DKK1 by decreasing the repression of LXRα on β-catenin. Here we first report the regulation of DKK1 in macrophages by ox-LDL. Moreover, we provide evidence that DKK1 inhibits the accumulation of lipids in macrophages by decreasing LOX-1 expression via the Wnt/β-catenin pathway and increasing ABCA/G1 expression via the STAT3 pathway. Thus, DKK1 in macrophages may be a negative mediator in lipid metabolism.

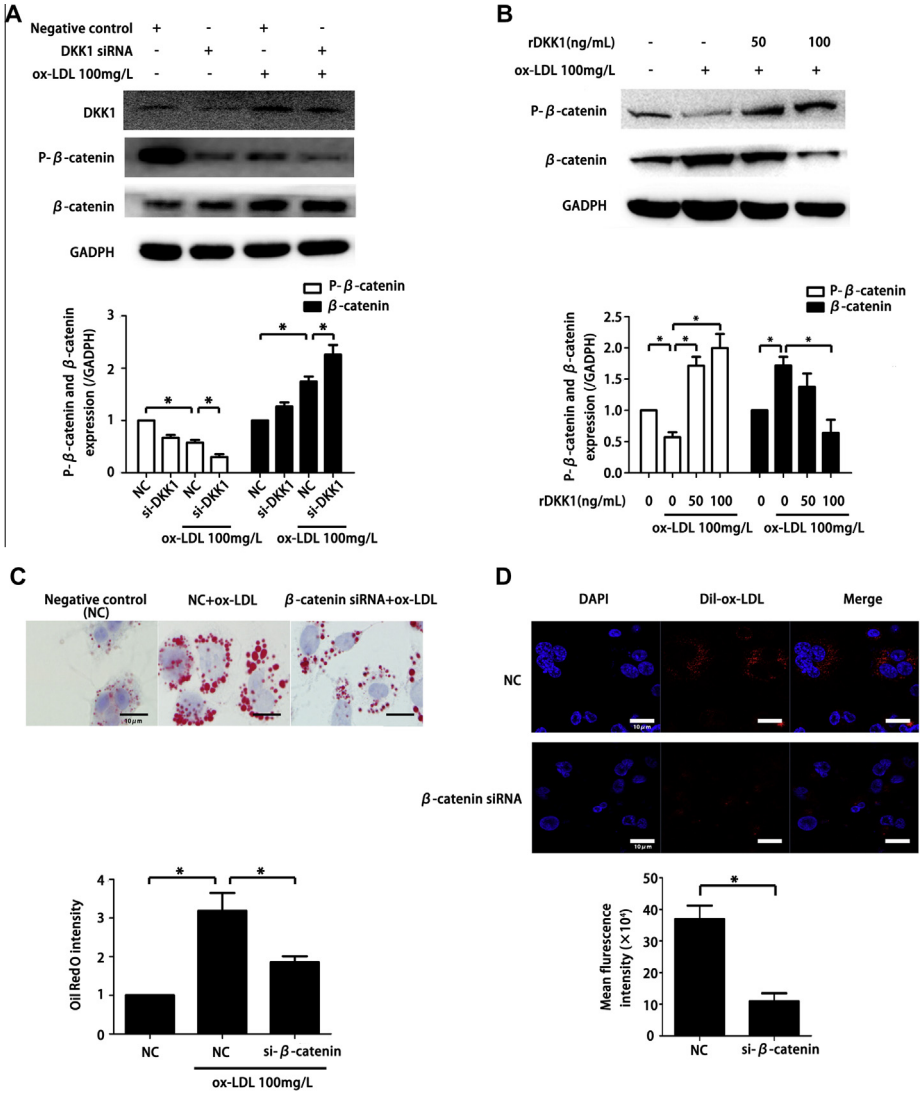

Fig. 4. Wnt/β-catenin mediates the regulation of DKK1 in lipid metabolism of macrophages. (A) Western blot analysis of the effect of DKK1 siRNA on protein expression of DKK1 and total and phosphorylated β-catenin. (B) Western blot analysis of total and phosphorylated β-catenin in cells with rDKK1 treatment and incubated with or without ox-LDL (100 mg/L). (C and D) Oil Red O staining and confocal microscopy of cells transfected with β-catenin siRNA, then ox-LDL or Dil-ox-LDL added into the cell culture medium. One-way ANOVA or unpaired *t*-test, $n = 3$.

DKK1 plays a pivotal role in the development and pathogenesis of the cardiac and vascular system. DKK1 level was found decreased in obesity-prone rat models, with more lipid accumulation in the decidual zones than in obesity-resistant groups. Overexpression of DKK1 in JEG3 cells decreased lipid accumulation and mRNA content of peroxisome proliferator-activated receptor δ [20]. Previous study found that ox-LDL induced senescence of retinal pigment epithelial cells and increased the expression of β-catenin [21]. However, whether DKK1 was involved in ox-LDL-induced macrophage foam-cell formation was unknown. Here, we found that ox-LDL increased both the protein and mRNA levels of DKK1 in macrophages.

DKK1 blocks the canonical Wnt/β-catenin pathway via the combination with LRP5/6 and Kremen on the membrane, which accelerates phosphorylation of β-catenin in an ubiquitin–proteasome–mediated manner [22,23]. At the same time, DKK1 is a target gene of the canonical Wnt/β-catenin pathway, for a negative feedback loop [24]. LXR belongs to the nuclear receptor superfamily of ligand-activated transcription factors and has two isoforms, LXRα and LXRβ. LXRs are activated by forming heterodimers with retinoid X receptor and bind to the LXR response element found in the promoter region of target genes [25]. LXRα has a central role in lipid metabolism against cholesterol overload by inducing the transcription of lipid-related genes such as ABCA1 and ABCG1. Previous studies of LXRs mainly focused on lipid metabolism and inflammation, whereas LXRs are closely related to the Wnt pathway. In colon cancer HCT116 cells, LXRs regulated β-catenin activity rather than expression by direct binding [18]. Although we found that ox-LDL did not affect the expression of LXRα (Supplementary Fig. S4), the effects of LXRα and β-catenin on DKK1 induced by ox-LDL were confirmed by using gene silencing or pharmacological agonists, whereas gene silencing of LXRβ had no such an effect (Supplementary Fig. S1C). In addition, LXRα bound with β-catenin in macrophages, which was attenuated by ox-LDL treatment (Supplementary Fig. S1A). Thus, our results show that ox-LDL upregulated DKK1 by decreasing the repression of LXRα on β-catenin.

DKK1 has wide and complex effects on cell proliferation and differentiation. DKK1 is involved in many human systemic diseases, such as rheumatic arthritis, Alzheimer disease [26] and cancers. Nevertheless, the role of DKK1 in different cancers is diverse. A high level of DKK1 in multiple myeloma [27] and human

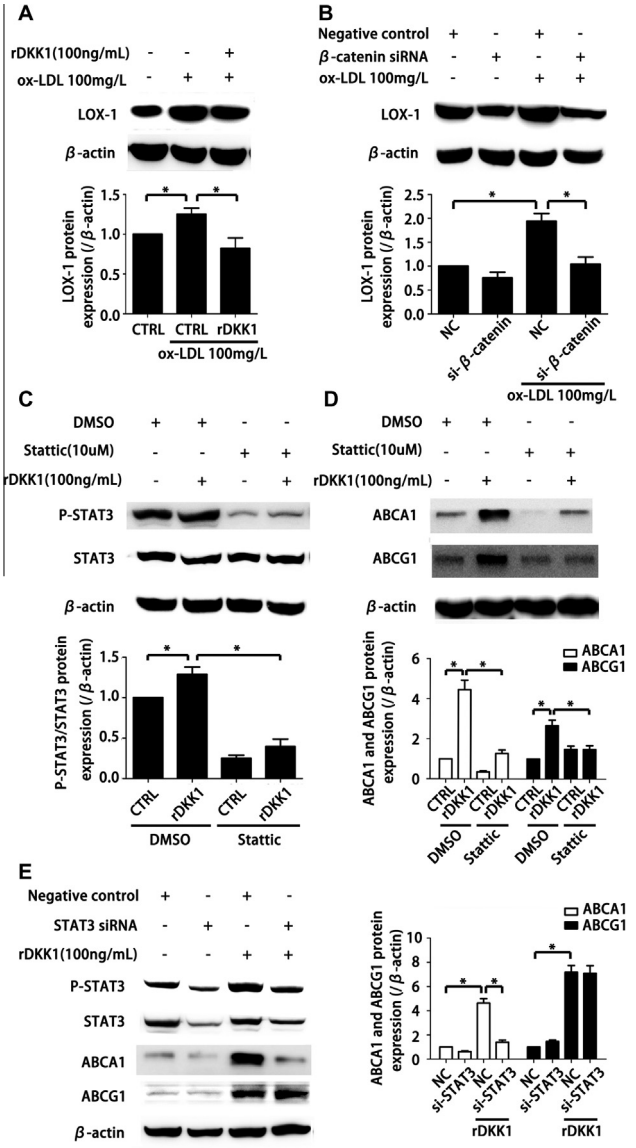

Fig. 5. Wnt and STAT3 pathways mediate the regulation of LOX-1 and ABCA/G1 by DKK1, respectively. Western blot analysis of protein level of LOX-1 in macrophages (A) incubated with rDKK1 (100 ng/mL) for 12 h and (B) with β-catenin siRNA knockdown before adding 100 mg/L ox-LDL. (C and D) Western blot analysis of p-STAT3, total STAT3, ABCA1 and ABCG1 levels in cells pretreated with DMSO or Stattic (10 μM) for 1 h before and during stimulation with rDKK1 and (E) in cells transfected with STAT3 siRNA or negative control, and rDKK1 added into the supernatant. One-way ANOVA with LSD post hoc test, $n = 3–6$.

hepatocellular carcinomas [28] was found related to poor prognosis. High serum DKK1 level in patients with multiple myeloma was related to osteolytic lesions [6]. However, inhibiting oncogenic Wnt/β-catenin induced DKK1 as a suppresser in colon cancer [28]. Regardless, the function of DKK1 in lipid metabolism of macrophages is still unknown. We found that DKK1 inhibited intracellular lipid accumulation and Dil-ox-LDL uptake. Our findings suggest that DKK1 inhibition of lipid metabolism involves inhibiting the canonical Wnt/β-catenin pathway.

Cholesterol accumulation in macrophages results from imbalanced cholesterol influx and efflux. As main receptors and regulators for lipid metabolism, LOX-1 transports ox-LDL into differentiated macrophages and ABC transporters use ATP as an energy source for efflux of intercellular cholesterol. Both ABCG1 and secreted frizzled-related protein 2 were upregulated on

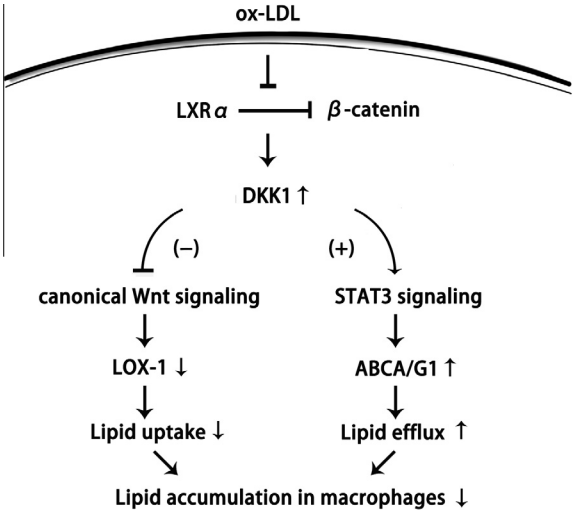

Fig. 6. An intrinsic mechanism for DKK1 inhibiting lipid accumulation in macrophages. Ox-LDL increases the levels of DKK1 by decreasing the repression of LXRα on β-catenin. DKK1 activates the STAT3 pathway and blocks the Wnt/β-catenin pathway, which increases the levels of ABCA/G1 and decreases that of LOX-1. This process accelerates lipid efflux and attenuates lipid uptake, for reduced lipid accumulation in macrophages.

microarray assay of lymphedema tissue, which indicates a potential cross-talk between the ABC family and Wnt proteins [29]. Here, we found that DKK1 reduced the expression of LOX-1 and increased that of ABCA/G1, which agrees with DKK1 inhibiting lipid accumulation in macrophages.

The phenomenon that DKK1 influences the receptors of lipid metabolism raised the question of the signaling pathway involved. The TCF/LEF-1 binding site in the LOX-1 promoter mediates the canonical Wnt/β-catenin pathway in hepatic stellate cells [30], and DKK1 leads to the phosphorylation and destabilitation of β-catenin to block the canonical Wnt pathway. Here, by using gene silencing of β-catenin, we found that DKK1 repressed the level of LOX-1 via a Wnt/β-catenin pathway. Mammalian Wnt5a orthologs may be STAT3-target genes [31]. STAT3 may be a direct target of a β-catenin–TCF/LEF complex [32]. Duplin, a negative regulator of the Wnt pathway, represses STAT3 activity [33]. This evidence shows a cross-link between STAT3 and the Wnt pathway. Moreover, STAT3 signaling in the ABCG1-deficient endothelium was found responsible for monocyte–endothelial cell interactions [34]. ABCA1 contains a well-conserved STAT3 element located in its first intron [35]. Hence, we focused on whether DKK1 influenced the expression of ABCA/G1 in macrophages via a STAT3 signaling pathway. Indeed, we found DKK1-increased ABCA/G1 level abolished by silencing STAT3 expression or inactivating p-STAT3.

We found that DKK1 enhances the capacity of human macrophages to maintain cholesterol homeostasis. People on a high-fat diet are at increased risk of hypercholesterolemia and atherosclerosis. Macrophages in the arterial wall internalize ox-LDL and are transformed into foam cells, which lead to atherosclerotic plaque formation and progression [36]. Intracellular excessive lipid accumulation may increase the expression of DKK1 in macrophages, which inhibits the phagocytosis of lipids, especially ox-LDL, in human macrophages, for a negative feedback. Our data show the beneficial effects of DKK1 on lipid metabolism, which has an important role in atherosclerosis. Previous study showed that DKK1 enhances the inflammatory interaction between platelets and endothelial cells [14]. Therefore, DKK1 may have various effects on atherosclerosis depending on the circumstances and stages. The significance of DKK1 in atherosclerosis warrants further investigation.

In summary, our study suggests that ox-LDL increases the expression of DKK1 in macrophages by decreasing the repression of LXRα on β-catenin (Fig. 6). DKK1 affects lipid metabolism to decrease the level of LOX-1 and increase that of ABCA/G1 via Wnt/β-catenin and STAT3 pathways, respectively. Ox-LDL induces the expression of DKK1 in macrophages, which inhibits lipid accumulation in macrophages.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.febslet.2014.11.023.

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