RESEARCH

Effects of statins on the inducible degrader of low-density lipoprotein receptor in familial hypercholesterolemia

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Abstract

The inducible degrader of low-density lipoprotein receptor (IDOL) is an E3 ubiquitin ligase involved in the post-transcriptional regulation of LDL receptor (LDLR). Statins lower plasma LDL by activating transcription of hepatic LDLR expression, and we have determined whether statins modulate IDOL expression and influence LDLR protein abundance. IDOL expression in monocytes and serum IDOL level was determined in statin-treated familial hypercholesterolemia (FH) patients and compared with control subjects. Serum IDOL level was also evaluated in a group of untreated FH patients before and after the initiation of statin. The mechanism underlying the inhibitory effect of statin on IDOL expression was investigated *in vitro*. In statin-treated FH patients, serum IDOL level and its expression in monocytes was reduced compared with control ($P < 0.05$). In contrast, untreated FH patients had higher serum levels of IDOL and proprotein convertase subtilisin/kexintype 9 (PCSK9) than control ($P < 0.05$), and serum IDOL level decreased after statin therapy ($P < 0.05$) whereas an increase was observed in PCSK9 level ($P < 0.01$). *In vitro*, atorvastatin significantly decreased IDOL abundance in a dose-dependent manner in cultured macrophages and hepatocytes with a concomitant increase in LDLR expression. The transcription of IDOL was restored by adding either an LXR agonist T0901317 or oxysterol 22(R)-hydroxycholesterol, indicating that statin inhibited IDOL expression by reducing LXR activation. The LXR-IDOL-LDLR axis can be modulated by statins *in vitro* and *in vivo*. Statins inhibit IDOL expression by reducing LXR activation and upregulate LDLR, and statins exert the opposite effect on IDOL and PCSK9.

Key Words

- ubiquitin-protein ligases
- lipid metabolism
- statin therapy
- familial hypercholesterolemia
- low-density lipoprotein

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Introduction

Inducible degrader of LDL receptor (IDOL), an E3 ubiquitin ligase, has recently been identified as a novel post-translational regulator of the LDL receptor (LDLR) in addition to proprotein convertase subtilisin/kexin type 9 (PCSK9) (1). IDOL, also known as the myosin light chain-interacting protein (MYLIP), was cloned from a brain cDNA library and consists of two major protein domains (2). The N-terminal FERM domain binds the intracellular tail of the LDLR and the C-terminal really interesting new gene (RING) domain is responsible for E3 ligase activity. Ubiquitination of the intracellular tail of LDLR by IDOL targets the receptor for lysosomal degradation and results in degradation of the LDLR. Adenovirus-mediated acute over-expression of IDOL in mouse liver promoted

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degradation of LDLR and raised plasma LDL levels (3), whereas knockdown of IDOL in hepatocytes increased protein levels of LDLR and promoted the uptake of LDL (1). Studies in non-human primates show that gain or loss of IDOL function leads to a corresponding increase and reduction in plasma LDL levels, respectively (4). In addition to experimental data, genetic studies have provided further evidence to support the notion that IDOL may potentially be involved in LDL metabolism (5, 6, 7). A recent study in children with untreated familial hypercholesterolemia (FH) has shown that plasma concentration of IDOL was elevated and was significantly associated with total and LDL cholesterol levels (8).

IDOL is ubiquitously expressed, and unlike the LDLR and PCSK9 genes, IDOL is not regulated by sterol regulatory element-binding protein (SREBP). IDOL is regulated by the sterol-responsive nuclear receptor liver X receptors (LXRα and LXRβ) (1), and LXR-dependent induction of IDOL is a complementary but distinct pathway for sterol-dependent inhibition of cellular cholesterol uptake through the LDLR. Activation of LXR mainly acts to limit cellular cholesterol accumulation by reducing LDLR abundance via IDOL and increases the expression of genes that promote cholesterol efflux (9). In addition to regulation by LXR, it has recently been suggested that statins may potentially influence IDOL expression *in vitro* (10). Statins are most widely used in lipid-lowering therapy. By inhibiting the rate-limiting enzyme in cholesterol synthesis, HMG-CoA reductase, statins decrease plasma LDL by inducing intracellular cholesterol depletion and upregulation of the hepatic LDLR expression through activation of the SREBP2 pathway (11). Statins also concomitantly induce PCSK9 expression which attenuates the impact of statins on LDLR abundance in hepatocytes (12). Although the effect of statins on IDOL expression has been examined in cultured hepatocytes (10), the potential underlying mechanism(s) have not been elucidated. Whether statins affect IDOL expression *in vivo* is unclear. We have therefore investigated the effects of statins on IDOL expression in human subjects with FH, and determined the underlying mechanism(s) *in vitro*.

Materials and methods

Clinical studies

The study was approved by the Ethics Committee of the University of Hong Kong, and written informed consents were obtained from all subjects. Subjects with FH were recruited from Endocrine clinics and healthy controls were recruited from the community. Diagnosis of FH was based on phenotypic criteria using the Dutch Lipid Clinic Network criteria ('definite' or 'probable' FH) (13), and/or genetic testing. In the cross-sectional study, fasting blood samples were taken for the measurement of lipids, serum levels of IDOL and PCSK9, and IDOL and LDLR expression in circulating monocytes was determined in a random subgroup of treated FH patients and control subjects. The effect of statins on serum levels of IDOL and PCSK9 was then investigated in a prospective manner in a group of untreated FH subjects before and after the initiation of statin therapy. Plasma total cholesterol, HDL cholesterol and triglycerides were determined enzymatically on an analyzer (Hitachi 912; Roche Diagnostics), and LDL cholesterol was calculated by the Friedewald equation.

Serum levels of IDOL and PCSK9 were measured using commercially available ELISA kits (CUSABIO® Biotech Co., MD, USA; and Quantikine, R&D systems, respectively) according to the manufacturer's protocol. Absorbance readings against the concentration of serum IDOL and PCSK9 were captured within 30 min by an ELISA reader at 450 nm. The intra- and inter-assay coefficients of the variant were below 8% for IDOL and PCSK9. Since IDOL is an intracellular protein, we have performed *in vitro* experiments to confirm that IDOL can be secreted. IDOL was measured in cell culture media of HepG2 cells with or without LXR activation by competitive ELISA. IDOL was secreted by both HepG2 cells and THP-1 cells and higher levels were seen after LXR activation (Supplementary Fig. 1, see section on supplementary materials given at the end of this article). We have also performed immunoblot for IDOL directly in serum. Seventy-five micrograms of protein per lane of 1/25 diluted human serum samples were electrophoresed as per the western blot protocol below. Immunoblot showed that IDOL can be detected in serum samples (Supplementary Fig. 2).

Cellular expression of IDOL and LDLR in monocytes from FH subjects and control was measured by flow cytometry. Fifty microliters of anticoagulated whole blood freshly collected from participants was incubated for 10 min room temperature with 2 mL of pharmalysze solution (BD Biosciences). To differentiate monocytes from other leukocytes, anti-human CD14+ IgG conjugated with FITC (ab28061, Abcam) was first added and incubated on ice for 30 min followed by either surface LDLR or intracellular IDOL antibodies staining. Cell surface LDLR was quantified by using an anti-human LDLR antibody (ab204941, Abcam) as the primary antibody. For intracellular IDOL detection, CD14-positive-labelled monocytes were permeabilized and fixed using fixation/permeabilization

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solution kit (BD Biosciences) prior to immunostaining with anti-human IDOL antibody (SAB1403211, Sigma). Stained monocytes were then washed with 1× PBS and subsequently probed with secondary antibody conjugated with APC fluorochrome (F0111 and F0101B, R&D Systems). The samples were analyzed with BD LSR Fortessa Analyzer (BD Biosciences). A fixed number of 10,000 monocytes were analyzed for each sample. Mean fluorescence intensity (MFI) was analyzed with FAScan (BD Biosciences), and the results were presented as the magnitude increase of MFI (MFI units of tested antibody staining minus MFI units of control antibody staining).

Data analysis was performed using IBM Statistical Package for Social Sciences (IBM SPSS version 25.0 software). Kolmogorov–Smirnov test was used to test for data normality. Skewed data were subjected to logarithmical transformation prior to analysis. Student's *t*-test and one-way ANOVA were used to compare variables between two and multiple groups, respectively. *Post hoc* multiple comparisons were performed using the Bonferroni test. Person's correlations were used to test the relationship between variables. Linear stepwise regression analysis was used to investigate relationships between circulating serum IDOL, PCSK9 and plasma LDL cholesterol.

In vitro studies

Human monocytic leukemia THP-1 and HepG2 cell lines (ATCC) were grown in RPMI 1640 medium containing 10% FBS and MEM with Glutamax (Invitrogen) at 37°C in 5% CO_2, respectively. THP-1 monocytes were first differentiated using phorbol 12, 13-dibutyrate (PMA) at 100 nM for 72 h, and both cell cultures were starved for 24 h before being used in experiments. The cells were then incubated in the absence or presence of atorvastatin (0–50 μM, Sigma-Aldrich) for 24 h in each experiment. To investigate whether IDOL expression was regulated by statin, THP-1 macrophages were incubated with mevalonate (200 μM) or LXRα activators (2.5 μM of T0901317 and 2.5 μM of 22(R)-hydroxycholesterol, 22R-HC) in the absence or presence of atorvastatin (25 μM).

Western immunoblotting was performed and 75 μg protein per lane from treated cells in the above experiments was resolved by 7.5% SDS-PAGE, and subsequently electro-transferred onto the PVDF membranes (Millipore). The membranes were blocked in Tris-buffered saline Tween 20 (TBST) with 5% powdered skimmed milk and subjected to immunoblot analysis. Blotted membranes were probed and incubated in 1:500 diluted TBST with 1% of powdered skimmed milk with either rabbit anti-human MYLIP/IDOL (ab74562, Abcam), rabbit anti-human LDLR (ab52818, Abcam), rabbit anti-human LXR-α (TA325122, OriGene, Rockville, MD, USA) or mouse anti-human PCSK9 (ab84041, Abcam) antibodies, respectively. Membranes were washed, then incubated with horseradish peroxidase-conjugated secondary antibodies. Specific bound immunoreactive bands were detected and visualized by an enhanced chemiluminescent advanced detection system (GE Healthcare), which were finally image-captured by ChemiDoc MP imaging system (Bio-Rad).

We have performed RNA silencing experiments to verify the specificity of the anti-IDOL antibody. HepG2 cells were transfected with either IDOL siRNA (27mer siRNA duplexes – SR309214A primer) or scramble control siRNA (SR30005, Origene, Rockville, MD) by Lipofectamine 2000 (Invitrogen). IDOL mRNA expression was quantified by quantitative PCR using IDOL-specific TaqMan probe (Invitrogen). Knockdown of IDOL resulted in a significant reduction in IDOL mRNA expression, and Western blot analysis of cell lysate using the anti-IDOL antibody showed a corresponding reduction in protein expression at a band size of approximately 50 kDa (Supplementary Fig. 3). Human recombinant human IDOL protein (ab198638, Abcam) was used as a positive control to confirm the identification of IDOL in HepG2 cell lysate.

Results

Effect of statins on IDOL in control, untreated and statin-treated FH

Serum IDOL and PCSK9 levels were determined in a group of untreated FH patients, statin-treated FH patients and healthy control, and the results are shown in Table 1. Sixty-four percent and 71% of the untreated and statin-treated FH patients, respectively, were defined by genetic testing and their mutations are summarized in Supplementary Table 1. In untreated FH subjects, serum concentration of IDOL was significantly elevated compared with control, whereas serum IDOL level was significantly reduced in statin-treated FH (Fig. 1). These differences remained significant even after adjusting for age, gender and BMI ($P < 0.05$). In contrast, serum concentration of PCSK9 was significantly increased in both untreated and statin-treated FH patients compared with control subjects (Fig. 1). In untreated FH patients, both serum IDOL and PCSK9 levels correlated with plasma LDL cholesterol ($r = 0.42$, $P < 0.01$; $r = 0.48$, $P < 0.01$, respectively). These relationships were no longer seen in FH patients treated with statins,

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Table 1 Serum IDOL and PCSK9 levels in control, untreated and statin-treated FH subjects.

	Control ($n = 147$)	**Untreated FH** ($n = 55$)	**Statin-treated FH** ($n = 162$)
Age (years)	45 ± 12	38 ± 14[a]	54 ± 13[b,d]
Male/female, *n*	65/82	26/29	72/90
BMI, kg/m^2	23.5 ± 3.5	22.5 ± 3.7	25.1 ± 3.6[a,d]
CVD, *n* (%)	0	0	59 (36.4%)
Tendon xanthomata, *n* (%)	0	1 (1.8%)	20 (12.3%)
Mutation-positive, *n* (%)	0	35 (64%)	115 (71%)
Smoker, *n* (%)	12 (8.1%)	4 (7.2%)	10 (6.1%)
SBP, mmHg	118.0 ± 15.8	121.6 ± 18.3	130.1 ± 18.8[b,c]
DBP, mmHg	74.6 ± 9.9	72.2 ± 11.8	74.3 ± 11.4
TC, mmol/L	4.9 ± 0.9	7.4 ± 1.7[b]	5.3 ± 1.3[a,d]
LDL-C, mmol/L	3.0 ± 0.8	5.4 ± 1.9[b]	3.3 ± 1.2[d]
TG, mmol/L	1.2 ± 0.6	1.2 ± 0.7	1.3 ± 0.7
HDL-C, mmol/L	1.4 ± 0.3	1.4 ± 0.3	1.4 ± 0.4
Statin intensity (high/moderate/low)	0	0	95 / 56 / 11
IDOL, pg/mL	1143 (808–1388)	1419 (915–2148)[a]	1023 (553–1529)[a,d]
PCSK, ng/mL	255 (205–306)	279 (228–353)[a]	556 (417–687)[b,d]

Data are expressed as mean ± S.D. or median (interquartile range).
[a]$P < 0.05$ and [b]$P < 0.001$ vs healthy control. [c]$P < 0.05$ and [d]$P < 0.001$ vs untreated FH.

presumably due to the overriding effect of statins on LDL cholesterol. Multiple regression analysis was performed in the untreated FH subjects to determine the contribution of IDOL in the variation in plasma LDL cholesterol. Age, gender, BMI, the presence or absence of mutation, serum PCSK9 and IDOL levels were included in the model. The major determinants of plasma LDL cholesterol were serum PCSK9 and IDOL, accounting for 21 and 7% of the variance in plasma LDL, respectively ($P < 0.001$), after adjusting for age, gender, BMI and the presence or absence of mutation.

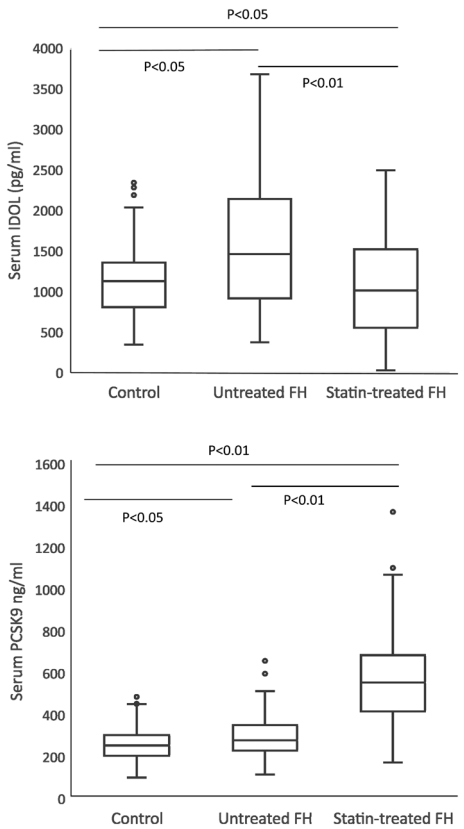

Figure 1
Boxplots of serum levels of IDOL and PCSK9 in control and FH patients.

The effect of statin therapy on the cellular level of expression of IDOL and LDLR in circulating monocytes was examined in a random subgroup of statin-treated FH patients (31 on high-intensity and 23 on moderate-intensity statin) and 45 age- and gender-matched control subjects (Supplementary Table 2).

Plasma LDL cholesterol was significantly higher in FH patients than in control ($P < 0.01$). Both serum level of IDOL (1061 pg/mL (745–1326) vs 1219 (852–1414), respectively, $P < 0.05$) and the expression of IDOL in monocytes was significantly lower (10.3 MFI (6.4–13.0) vs 12.7 (11.0–15.6), respectively, $P < 0.01$) in statin-treated FH patients compared with control (Supplementary Fig. 4). There was a weak correlation ($r = 0.24$, $P = 0.02$) between log(serum IDOL) and log(monocyte IDOL). As expected, cell surface LDLR expression was significantly reduced in FH patients (17.2 MFI (7.2–21.9) vs 18.8 (16.5–26.9), respectively, $P < 0.05$) (Supplementary Fig. 4).

To confirm that statins inhibit IDOL expression, serum levels of IDOL and PCSK9 were measured in 32 FH subjects before and at least 4 months after starting statin therapy. As shown in Table 2, statins exert opposite effects on serum levels of IDOL and PCSK9, and statin therapies decreased serum IDOL levels but increased serum PCSK9. The changes in serum IDOL and PCSK9 levels did not correlate with the magnitude of reduction in LDL cholesterol.

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Table 2 Effect of statins on serum IDOL and PCSK9 levels.

	Baseline	After treatment
Age, years	38 ± 13	–
Men/women, *n*	17/15	–
Statin intensity (high/moderate/low)	–	11/19/2
TC, mmol/L	8.2 ± 1.7	4.8 ± 1.0[b]
LDL-C, mmol/L	6.2 ± 1.6	2.9 ± 1.0[b]
TG, mmol/L	1.2 ± 0.6	1.0 ± 0.7[a]
HDL-C, mmol/L	1.4 ± 0.3	1.5 ± 0.4
IDOL, pg/mL	1419 (1096–2142)	1261 (1048–1650)[a]
PCSK9, ng/mL	203 (165–253)	295 (226–332)[b]

[a]*P*< 0.05 and [b]*P*< 0.001 against baseline, paired Student's *t*-test.

Statin inhibited IDOL expression by reducing LXR activation *in vitro*

The effect of statin on IDOL and LDLR expression was evaluated in THP-1 cells. There was a dose-dependent decrease in IDOL abundance accompanied by an increase in LDLR expression when THP-1 cells were incubated with increasing concentrations of atorvastatin (0–50 μM) (Supplementary Fig. 5A and B, respectively). Similar results were seen when HepG2 cells were treated with atorvastatin (Supplementary Fig. 6A and B). PCSK9 expression was also determined. As reported in previous studies (14), PCSK9 was not expressed in macrophages and atorvastatin increased the expression of PCSK9 in HepG2 cells (Supplementary Fig. 7).

Statins are known to upregulate LDLR and PCSK9 expression via the activation of the SREBP pathway (15). However, IDOL is regulated mainly by the LXR pathway. To explore the mechanism of statin-induced inhibition of IDOL gene expression, we first tested whether the effect of statins could be reversed by the addition of mevalonate. The transcription of IDOL was restored by exogenous mevalonate (Fig. 2). We then evaluated the impact of atorvastatin on LXR signaling as statins have previously been shown to inhibit the synthesis of oxysterols which are endogenous ligands of LXR. Atorvastatin reduced the expression of LXRα (Fig. 3) and other known LXR target genes like ABCA1 and ABCG1 (Supplementary Fig. 8) in a dose-dependent manner. The addition of either an LXR agonist T0901317 or oxysterol 22R-HC was able to overcome the inhibitory effect of statin on IDOL (Fig. 4A and B, respectively) and reduced LDLR expression (Fig. 4C and D, respectively). However, the addition of LXR agonist or oxysterol to statin-treated cells did not lower LDLR expression to levels of control cells presumably because atorvastatin also activated LDLR expression via SREBP. Taken together, these data indicate that statin inhibits IDOL expression by reducing LXR activation.

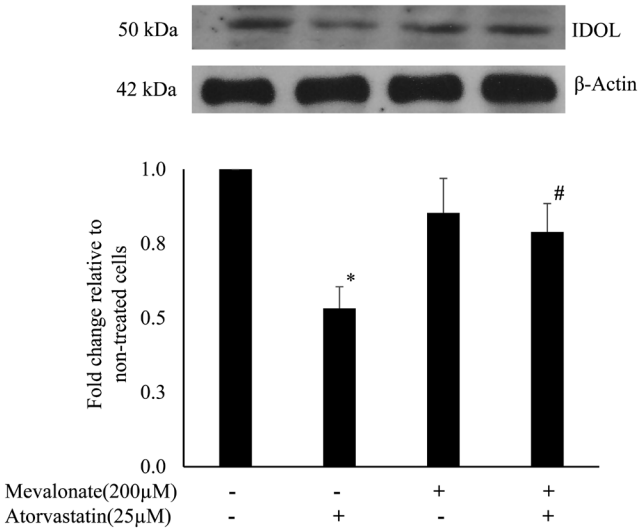

Figure 2
Addition of exogenous mevalonate overcomes atorvastatin-induced reduction in IDOL expression in THP-1 cells. Each bar represents the mean and S.D. of triplicate experiments. **P* < 0.05 compared to non-treated cells, #*P* < 0.05 compared to atorvastatin-treated cells. The statistical significance of differences was determined using ANOVA followed by Bonferroni's comparison.

Discussion

The LDLR pathway plays an important role in the homeostasis of plasma (16) and intracellular cholesterol and IDOL has recently been shown to regulate LDLR at a

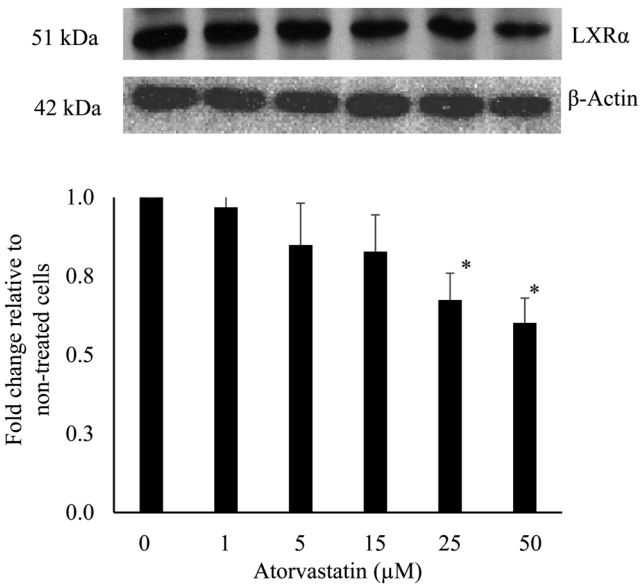

Figure 3
Effect of atorvastatin on LXR expression in THP-1 cells. Each bar represents the mean and S.D. of triplicate experiments. **P* < 0.05 compared to non-treated cells. The statistical significance of differences was determined using ANOVA followed by Bonferroni's comparison.

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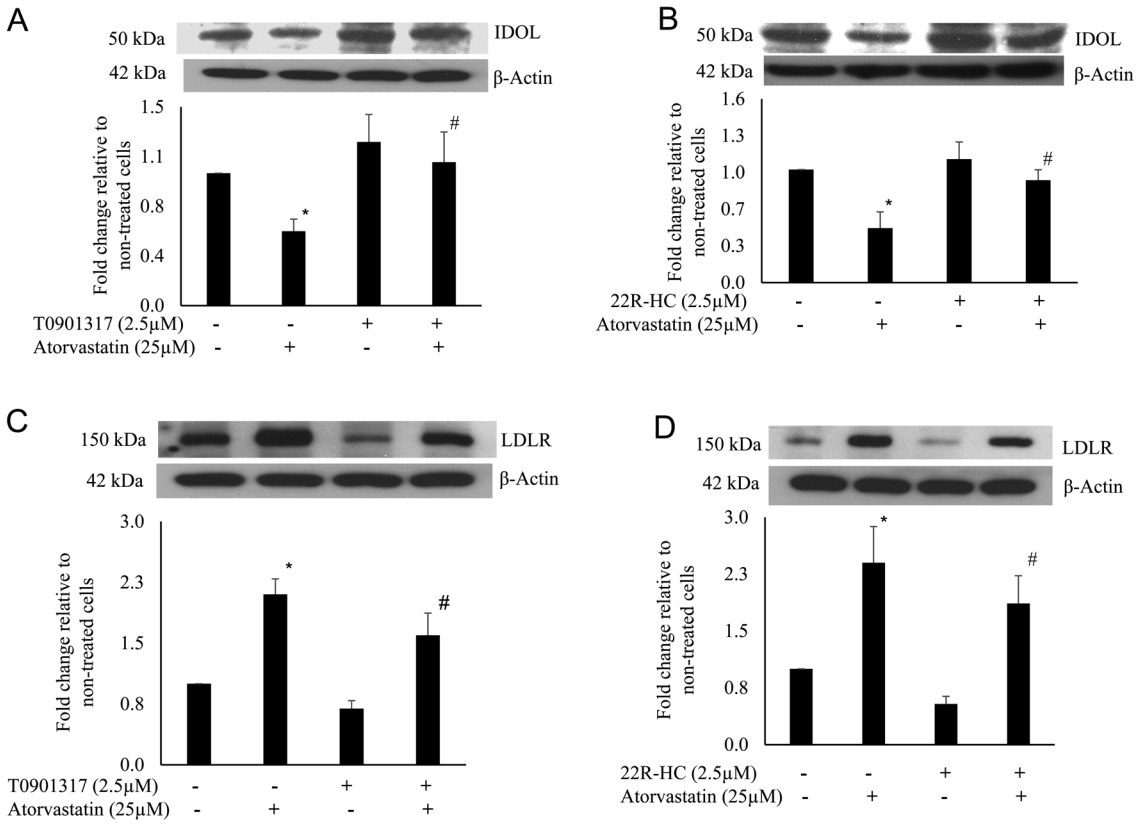

Figure 4

Addition of LXRα agonist (A) T0901317 or (B) 22R-HC normalized IDOL and (C and D) reduced LDLR expression, respectively, in atorvastatin-treated THP-1 cells. Each bar represents the mean and SD of triplicate experiments. *$P < 0.05$ compared to non-treated cells, #$P < 0.05$ compared to atorvastatin-treated cells. The statistical significance of differences was determined using ANOVA followed by Bonferroni's comparison.

post-transcriptional level (1, 4). In addition to its role in cholesterol metabolism, recent data suggest that IDOL may have a broader metabolic role and inactivation of IDOL attenuates metabolic dysfunction in mice (17). Unlike PCSK9 which mainly acts on the liver, IDOL is highly active in peripheral tissues (18). Although genetic studies have suggested an association between IDOL and lipoprotein metabolism, data on IDOL expression and its regulation are still scarce in human subjects. IDOL acts mainly intracellularly by ubiquitination of the intracellular tail of LDLR. A recent study has shown that IDOL can also be detected in the circulation, and it has been suggested that plasma IDOL might be a potential LDLR functional biomarker (8). We have shown that in untreated adult FH patients, serum levels of IDOL were increased compared with control, and there was a significant correlation between serum IDOL level and plasma LDL cholesterol. Both PCSK9 and IDOL were major independent determinants of plasma LDL cholesterol, accounting for up to 30% of the variation in plasma LDL cholesterol in untreated adult FH patients, and our findings are in keeping with those reported in a study of untreated FH children (8). IDOL is a cytosolic protein, and it is unclear how IDOL is secreted into the circulation. Recognition of LDLR by IDOL involves interaction between the FERM domain of IDOL, plasma membrane phospholipids, and the lipoprotein receptor tail (19, 20). Whether binding of IDOL via the FERM domain to plasma membrane that is then shedded off warrants further investigation.

In addition, the present study is the first to demonstrate the inhibitory effect of statins on IDOL *in vivo*. We have shown that in statin-treated FH patients, both serum and intracellular IDOL expression in monocytes was significantly reduced compared to control. Further proof that statins can modulate IDOL expression comes from our longitudinal study comparing serum levels of IDOL in a group of FH patients before and after the initiation of statins. There was a significant reduction in serum IDOL levels after treatment, and statins increased serum PCSK9 while lowering serum IDOL levels. Hence, statins exert opposite effects on IDOL and PCSK9 *in vivo*, and we have further investigated the mechanisms of statin-induced inhibition of IDOL *in vitro*.

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Dong *et al.* reported that statins inhibited IDOL mRNA expression in hepatocytes but the mechanism of statin-induced inhibition of IDOL gene expression was not investigated (10). We have shown that atorvastatin inhibited IDOL expression both in THP-1 cells as well as HepG2 cells, and we have further examined the underlying mechanisms. Experimental and animal studies have so far suggested that IDOL expression is controlled mainly by the LXR signaling pathway (9, 21). By inhibiting the enzyme HMG-CoA reductase, statins deplete intracellular cholesterol and upregulate LDLR and PCSK9 expression by activating the SREBP pathway (22). Our data suggest that the inhibitory effect of statins on IDOL expression is mainly mediated via the LXR pathway, in contrast to the regulation of PCSK9. It has previously been suggested that statins can decrease LXR signaling (23). Forman *et al.* reported that mevastatin or lovastatin reduced the transcriptional activity of LXR/RXR heterodimer *in vitro* and the effect was reversed by mevalonate and by LXR-activating oxysterols (24). Wong *et al*. also showed that statins could inhibit the synthesis of oxysterol in human macrophages and decreased the expression of LXR target genes like *ABCA1* and *ABCG1* (23). We have confirmed that atorvastatin reduced the expression of LXR as well as its target genes *ABCA1* and *ABCG1*. *IDOL* is also an LXR target gene and in our study, restoring LXR activation normalized IDOL expression in statin-treated macrophages *in vitro*. Hence, statins downregulate IDOL expression via reduced LXR activation. Taken together, although the induction of PCSK9 by statins may limit their efficacy in lowering LDL, this may be partially offset by the inhibition of IDOL by statins. In addition to the LXR pathway, it has been shown that MYLIP/IDOL can also be regulated by sterol-independent mechanisms. Expression of MYLIP/IDOL is downregulated by pro-nerve growth factor and upregulated by Reelin in neuronal cells (25, 26). Transcription of MYLIP/IDOL can also be induced by inhibiting deubiquitylase (27).

There are a number of limitations in our study. We have measured IDOL expression in monocytes because these cells are easily accessible. It is difficult to study IDOL in hepatocytes in human subjects as it will require a liver biopsy. IDOL is expressed in a variety of tissues (9), and the contribution of monocyte as a source of circulating IDOL is unclear. There was a weak association between IDOL expression in monocytes and serum levels of IDOL in our study. Relatively little is known about the extracellular role of IDOL in the circulation and the mechanism(s) of its secretion from cells, and whether systemic levels of IDOL reflect IDOL expression in tissue like the liver remains to be determined. Furthermore, the effect of statin on IDOL should ideally be evaluated in a randomized placebo-controlled manner. However, it would be unethical to withhold treatment in patients with FH. Our study cannot address the question of whether IDOL plays a physiological role in determining plasma LDL levels in humans. In our cross-sectional study in FH patients, serum IDOL level was an independent determinant of plasma LDL cholesterol, but the cross-sectional design cannot prove a causal relationship. In our *in vitro* experiments, the effects of statins are seen mainly with the higher concentrations which are greater than the plasma levels achieved in statin-treated subjects and may not be physiological. However, the effects of statins were consistent with our clinical study.

In conclusion, we have shown that the LXR-IDOL-LDLR axis can be modulated by statins *in vitro* and *in vivo*. Statins inhibit IDOL expression by reducing LXR activation and by upregulating LDLR. Serum levels of IDOL and PCSK9, the two degraders of the LDLR protein, are increased in patients with FH, and statins lower IDOL and increase PCSK9 in these subjects.

Supplementary materials

This is linked to the online version of the paper at https://doi.org/10.1530/EC-22-0019.

Declaration of interest

Kathryn Choon-Beng Tan is on the editorial board of journal. Kathryn Choon-Beng Tan was not involved in the review or editorial process for this paper, on which he/she is listed as an author. The other authors have nothing to disclose.

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Author contribution statement

Melody Lok-Yi Chan and Sammy Wing-Ming Shiu: Methodology, Validation, Formal Analysis, Investigation, Resources, Data Curation, Writing – Original Draft, Project Administration. Sammy Wing-Ming Shiu, Anskar Yu-Hung Leung, and Kathryn Choon-Beng Tan: Conceptualization. Melody Lok-Yi Chan and Ching-Lung Cheung: Software. Kathryn Choon-Beng Tan: Validation, Writing – Review and Editing, Visualization, Supervision, Project Administration and Funding Acquisition.

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