Post-transcriptional regulation of lipoprotein receptors by the E3-ubiquitin ligase inducible degrader of the low-density lipoprotein receptor

Vincenzo Sorrentino and Noam Zelcer

Purpose of review
The hepatic low-density lipoprotein receptor (LDLR) pathway is essential for clearing circulating LDL and is an important therapeutic target for treating cardiovascular disease. Abundance of the LDLR is subject to both transcriptional and nontranscriptional control. Here, we highlight a new post-transcriptional mechanism for controlling LDLR function via ubiquitination of the receptor by the E3-ubiquitin ligase inducible degrader of the LDLR (IDOL).

Recent findings
IDOL is a recently identified transcriptional target of the liver X receptors. Acting as an E3-ubiquitin ligase IDOL promotes ubiquitination of the LDLR, thereby marking it for lysosomal degradation. The determinants required for degradation of the LDLR by IDOL have been largely identified. IDOL also targets two related lipoprotein receptors, the very low-density lipoprotein receptor and apolipoprotein E receptor 2. Despite several similarities, the IDOL, and PCSK9 pathways for controlling LDLR abundance seem independent of each other. Genome-wide association studies have recently identified IDOL as a locus influencing variability in circulating levels of LDL, thereby highlighting the possible role of IDOL in human lipoprotein metabolism.

Summary
Transcriptional induction of IDOL by liver X receptor defines a new post-transcriptional pathway for controlling LDLR abundance and LDL uptake independent of sterol regulatory element binding proteins. Targeting IDOL activity may offer a novel therapeutic approach complementary to statins for treating cardiovascular disease.

Keywords
inducible degrader of the low-density lipoprotein receptor, low-density lipoprotein cholesterol, low-density lipoprotein receptor, liver X receptors, PCSK9, ubiquitin E3 ligase

INTRODUCTION

Autosomal dominant hypercholesterolemia, a disease characterized by reduced hepatic LDL clearance, elevated plasma cholesterol levels and accelerated atherosclerosis, is caused by mutations in the low-density lipoprotein receptor (*LDLR*), *APOB*, and *PCSK9* genes [1▪]. A rare form of autosomal recessive hypercholesterolemia is a result of mutations in the LDLR adaptor protein 1 [autosomal recessive hypercholesterolemia (*ARH*)] [2]. The convergence of these disease-causing mutations on the LDLR pathway emphasizes its centrality in controlling the plasma level of LDL.

In 2009, the sterol-regulated E3-ubiquitin ligase inducible degrader of the LDLR (IDOL) was identified as a new post-transcriptional regulator of the LDLR [3▪▪]. IDOL decreases LDLR abundance and attenuates LDL uptake into cells, analogous to the described effects of PCSK9. However, the mechanisms employed by IDOL and PCSK9 to achieve this outcome are distinct. In this review, we aim to summarize our current understanding of IDOL

Department of Medical Biochemistry, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

Correspondence to Noam Zelcer, PhD, Department of Medical Biochemistry, Academic Medical Center, University of Amsterdam, Meibergdreef 15, 1105AZ Amsterdam, The Netherlands. Tel: +31 20 5665131; fax: +31 20 6915519; e-mail: n.zelcer@amc.uva.nl

Curr Opin Lipidol 2012, 23:000–000

DOI:10.1097/MOL.0b013e3283532947

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

KEY POINTS

- The E3-ubiquitin ligase IDOL is a direct transcriptional target of the liver X receptors (LXR).
- IDOL promotes ubiquitination and subsequent lysosomal degradation of the LDLR, very low-density lipoprotein receptor, and apolipoprotein E receptor 2.
- Post-transcriptional regulation of lipoprotein receptors via ubiquitination is an ancient and conserved mechanism to control their function.
- Recent genome-wide association studies identify IDOL as a locus that contributes to variation in circulating levels of LDL in humans.
- The LXR–IDOL–LDLR pathway defines a complementary pathway to the sterol regulatory element binding protein one for controlling lipoprotein metabolism and a potential target for treating dyslipidemia.

function and discuss its relation to PCSK9 in controlling activity of the LDLR pathway.

IDENTIFICATION AND TRANSCRIPTIONAL REGULATION OF IDOL

The level of cellular cholesterol reflects the net balance of biosynthesis, efflux, and uptake of cholesterol. This balance is largely governed by the coordinated actions of two transcription factor families: the sterol regulatory element binding proteins 1 and 2 (SREBP) and the ligand-dependent transcription factors liver X receptors (LXR) α and β [4,5]. Activation of SREBP, when cellular sterol levels decline, leads to increased expression of the LDLR and of the genes required for cholesterol biosynthesis. Increased sterol levels, on the contrary, lead to activation of LXRs by their cognate oxysterol ligands. Once activated, LXR act primarily to reduce cellular cholesterol by increasing expression of a set of genes (e.g., *ABCA1*) that promote cholesterol efflux.

IDOL was identified following the observation that in addition to promoting cholesterol efflux, LXR also decreased LDL binding and uptake in a variety of cell types [3▪▪]. Activation of LXR in these cells markedly reduced LDLR abundance without changing expression of the *LDLR*. This post-transcriptional event was rapid and lost in fibroblasts and macrophages lacking LXR, thus, implicating an LXR target gene as a mediator of this outcome. Using transcriptional profiling, myosin regulatory light chain interacting protein (*MYLIP*) was identified as an LXR target and a candidate gene for LDLR degradation. In view of its adopted function, we renamed it IDOL. Subsequent studies confirmed that IDOL is a direct transcriptional target of both human and mouse LXR *in vitro* and *in vivo*.

IDOL PROMOTES UBIQUITINATION AND SUBSEQUENT DEGRADATION OF THE LOW-DENSITY LIPOPROTEIN RECEPTOR

The IDOL gene, originally cloned as *MYLIP* by Lindholm and colleagues [6], encodes an open reading frame of 445 amino acids with two distinct protein domains: an N-terminal FERM and a C-terminal Really Interesting New Gene (RING) (Fig. 1). The FERM domain forms the signature for members of the ERM family of proteins and is known to mediate the interaction between membranes and membrane proteins [7]. The RING domain of IDOL is similar to that found in other RING-containing E3-ubiquitin ligases [8]. A series of gain-of-function experiments in cells and mice indicated that IDOL reduced LDLR abundance and LDL uptake, thereby mimicking the effect of LXR activation [3▪▪]. Conversely, loss of IDOL function brought about the opposite outcome. Conclusive evidence demonstrating that LXR requires IDOL to downregulate the LDLR was obtained by studying

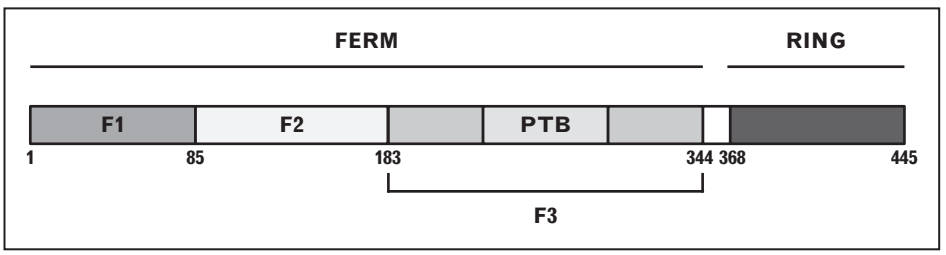

FIGURE 1. Domain structure of the E3-ubiquitin ligase IDOL. IDOL is a bimodular protein, with an N-terminal FERM domain and a C-terminal RING domain separated by a short linker region. The FERM contains the three subdomains F1, F2, and F3 found in other FERM-containing proteins. Binding to the intracellular tail of the LDLR occurs at the phosphotyrosine-binding (PTB) domain embedded within the F3 region. Numbers correspond to amino acid residues in human IDOL.

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

Idol$^{(-/-)}$ embryonic stem cells [9▪▪]. These cells have elevated levels of LDLR and increased LDL uptake under basal and sterol-depleted growth conditions, and are unable to downregulate the receptor in response to a synthetic LXR ligand.

Post-transcriptional downregulation of the LDLR by IDOL is consistent with it acting as an E3-ubiquitin ligase. Modification of proteins via covalent attachment of ubiquitin is a three-step cascade involving ubiquitin-activating (E1), ubiquitin-conjugating (E2), and ubiquitin-ligating (E3) enzymes. The pairing of E2 and substrate by RING-containing E3 determines the specificity in ubiquitination [8]. Accordingly, IDOL promoted the covalent attachment of poly-K63 ubiquitin chains to conserved residues in the intracellular tail of the LDLR [3▪▪,10▪]. This particular ubiquitin linkage is an established lysosomal targeting signal for several membrane receptors and likely explains how IDOL directs the LDLR toward this degradation pathway [11]. Similar to other E3-ubiquitin ligases, IDOL also mediates its own ubiquitination [3▪▪]. However, unlike lysosomal degradation of the LDLR, autoubiquitination leads to rapid proteasomal degradation of IDOL. Together with transcriptional regulation of IDOL by LXR, autoubiquitination may also serve to control its activity.

Recent studies highlight the distinct contribution of the RING and FERM domains to IDOL activity. The RING domain supports ubiquitination of the LDLR [10▪,12▪]. Of the approximately 40 distinct E2s present in humans, Zhang *et al.* [12▪] identified the UBE2D family (UBE2D1–4) as the physiological E2 partners of IDOL. The structures of the RING and of the RING-UBE2D1 complex also revealed that, like other RING-containing ligases, the IDOL RING forms a homodimer. Dimerization of intact IDOL was also confirmed by pull-down experiments in cells. An intriguing possibility is that dimerization of IDOL serves to cluster LDLR on the plasma membrane prior to ubiquitination, akin to the role ARH plays in endocytosis of the LDLR.

Binding of IDOL to the LDLR depends on the FERM domain. IDOL's FERM contains an apparent insertion not found in other FERM-containing proteins (Fig. 1). Secondary structure prediction identified this region as a phosphotyrosine-binding domain (PTB), analogous to that present in the LDLR-family adaptor proteins DAB1 and ARH [2,13]. By implementing modeling and structure-guided mutagenesis, we and Calkin *et al.* collectively identified residues in the PTB domain and in the proximal section of the cytosolic tail of the LDLR that contribute to the binding interface [10▪,14▪]. These results also highlight a difference between the mode of IDOL and ARH binding to the LDLR. Namely, unlike ARH, the NPxY endocytosis motif is dispensable for binding and subsequent degradation of the LDLR by IDOL.

In addition to the mentioned above, membrane context is important for proper positioning of IDOL vis-à-vis the LDLR. This can explain why fusing the intracellular tail of the LDLR to another unrelated membrane receptor confers sensitivity to IDOL [15▪], whereas doing the same with a soluble protein does not [14▪]. Biochemically, the FERM domain associates with membrane-mimicking liposomes [14▪]. Our imaging studies of living HepG2 cells indicate that the IDOL–LDLR interaction occurs primarily at the plasma membrane [10▪]. This is consistent with biotinylation studies demonstrating that LDLR in the plasma membrane is highly sensitive to IDOL-dependent degradation [9▪▪]. The endocytic route that is used to bring ubiquitinated LDLR from the plasma membrane to the lysosome is unknown. Furthermore, an intracellular pathway for degradation of the LDLR by IDOL exists [3▪▪,9▪▪], and its relative contribution to IDOL activity remains to be determined. Our current understanding of IDOL regulation and activity is summarized in Fig. 2.

IDOL TARGETS THE VERY LOW-DENSITY LIPOPROTEIN RECEPTOR AND APOLIPOPROTEIN E RECEPTOR 2 FOR DEGRADATION

In addition to targeting the LDLR, IDOL targets two closely related LDLR family members, the very low-density lipoprotein receptor (VLDLR) and apolipoprotein E receptor 2 (APOER2) [15▪]. Given that all the structural determinants defined for degradation of the LDLR by IDOL are conserved in these related receptors, this could have been anticipated. Accordingly, activation of the LXR pathway or overexpression of IDOL reduced VLDLR and APOER2 in several cell types including glioblastoma cells, fibroblasts, and primary rat hippocampal neurons [15▪]. Furthermore, LXR activation *in vivo* reduced VLDLR content in mouse white adipose tissue [15▪], and to a lesser degree in the brain [16]. The mechanism underlying downregulation of these receptors, ubiquitination of a conserved lysine in the intracellular tail of these receptors, is identical to that used by IDOL to target the LDLR [15▪]. Although the role of the VLDLR and APOER2 in lipid metabolism is under debate, their essential role in relaying the extracellular signal of Reelin in the developing brain is well documented [17]. In agreement with reduced VLDLR and APOER2, activation of the LXR–IDOL pathway reduced binding of Reelin to cells and inhibited the rapid phoshorylation of DAB1 in

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

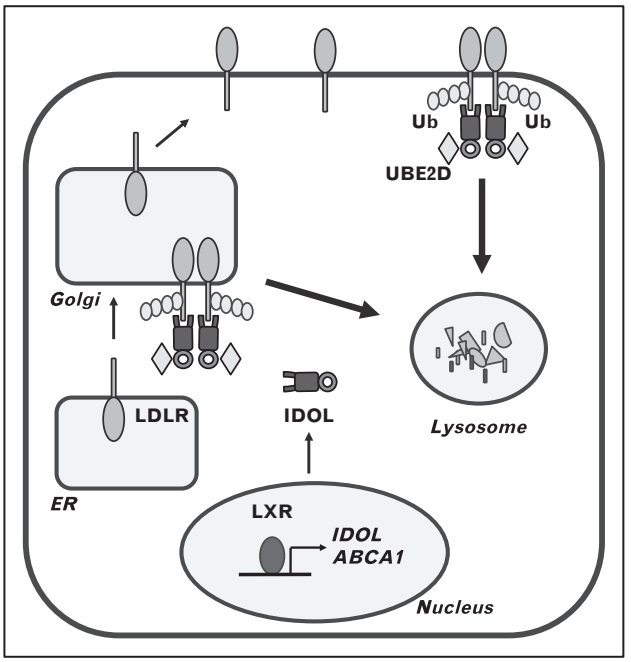

FIGURE 2. The LXR-IDOL-LDLR axis mediates ubiquitination and degradation of the LDLR. Increased cellular sterol leads to transcriptional activation of the liver X receptor (LXR) pathway and increased expression of cholesterol efflux genes (e.g., *ABCA1*) and of inducible degrader of the low-density lipoprotein receptor (IDOL). Working as a homodimer, and in cooperation with a UBE2D family member E2-ubiquitin conjugating enzyme, IDOL binds and enhances the covalent attachment of K63-linked ubiquitin chains to the intracellular tail of the LDLR. This marks ubiquitinated LDLR for lysosomal degradation. IDOL acts on LDLR present in the plasma membrane, but an intracellular route has been described as well. The endocytic route that ubiquitinated LDLR follows is still undefined and is therefore not indicated.

response to receptor ligation in fibroblasts reconstituted with a functional Reelin pathway [15▪]. The physiological significance of targeting the VLDLR and APOER2 by IDOL is unknown. Nevertheless, disturbed cholesterol metabolism in the brains of $LXR\alpha\beta^{(-/-)}$ mice results in aberrant anatomical structures and neuronal function [18]. Whether disturbed Reelin signaling contributes to these aberrancies has not been studied.

The structural determinants controlling degradation of the LDLR, VLDLR, and APOER2 by IDOL can be found in lipoprotein receptors from diverse species. Remarkably, IDOL degraded lipoprotein receptor (LpR) and LpR1, the main lipoprotein receptors of the migrating locust and fruit fly, respectively [14▪,15▪]. Furthermore, the distant fly homolog of IDOL, defense regulator 1 (Dnr1), degraded human LDLR and VLDLR. Even though the overall sequence homology between IDOL and Dnr1 is limited, the residues needed for binding the LDLR by IDOL are conserved in Dnr1, explaining why it is able to degrade the mammalian lipoprotein receptors [15▪]. It seems, therefore, that ubiquitination of lipoprotein receptors is an ancient and highly conserved post-transcriptional mechanism for controlling their function. The myosin regulatory light chain protein (MRLC) has been also proposed as an IDOL target in neuronal cells [6] and cardiomyocytes [19]. However, in the course of studying IDOL, we have not observed morphological changes in cells or MRLC degradation but it remains possible that cell-type-specific factors may confer additional IDOL functions.

PHYSIOLOGICAL ROLE OF IDOL IN LOW-DENSITY LIPOPROTEIN METABOLISM AND POTENTIAL THERAPEUTIC IMPLICATIONS

Mice lacking IDOL have been recently generated, but their metabolic phenotype has not been reported yet [9▪▪]. Therefore, we are limited to drawing conclusions from animal models in which IDOL mRNA expression is altered. In $LXR\alpha\beta^{(-/-)}$ mice IDOL expression is reduced and this is mirrored by increased LDLR protein in the intestine, macrophages, and to a lesser extent in the liver [3▪▪]. Reciprocally, pharmacological dosing of mice with an LXR ligand increased IDOL mRNA expression and decreased LDLR abundance in the intestine and macrophages, but not in the liver. The latter may reflect the modest hepatic increase in IDOL mRNA expression following this dosing regimen. A larger magnitude of increase in IDOL mRNA expression was observed in livers of $Npc^{(-/-)}Apoe^{(-/-)}$ mice when compared with control counterparts [20▪]. Concomitantly, the mutant mice had lower LDLR levels and decreased VLDLR clearance. However, interpretation of this result is confounded by the fact that in addition to IDOL, expression of *Pcsk9* was elevated as well. As is the case for IDOL, hepatic *ABCA1* expression is only modestly increased by LXR activation. Nevertheless, ABCA1 activity in theliver is indispensible for generating nascent HDL particles [21]. Along this line, forced expression of IDOL in the liver indicates that the cellular machinery required to target the LDLR is present [3▪▪]. Therefore, the consequence of hepatic loss of IDOL activity on lipoprotein metabolism remains to be seen.

The potential role of IDOL in human LDL metabolism has also garnered attention after several genome-wide association studies identified *IDOL/MYLIP* as a new genetic locus influencing variation

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

in the levels of circulating LDL in humans [22▪▪–24▪▪]. Studying a dyslipidemic population of Mexican origin, Weissglas-Volkov *et al.* [25▪▪] found an association between the N342S IDOL polymorphism and elevated levels of total cholesterol. Functional characterization of the two encoded alleles revealed that the Asn-encoding one displayed enhanced LDLR degradation and diminished LDL uptake, thereby providing a plausible explanation for the association. Our sequencing efforts identified several rare IDOL variants and preliminary analysis suggests that some may represent loss of function mutants.

From a therapeutic standpoint, it is worth noting that statins, the mainstay of hypercholesterolemia treatment, reduce IDOL mRNA in hepatocytes [3▪▪,26▪]. Intriguingly, a recent genome-wide association study suggested that variation in IDOL might account for variability in the response to rosuvastatin [27▪]. However, this association did not reach genome-wide significance and requires replication. Nevertheless, it is interesting to speculate that in addition to increasing expression of *LDLR* mRNA, statins may also elevate LDLR protein by reducing IDOL activity. In this context, we would like to emphasize that cells still retain substantial residual IDOL activity when treated with statins. Inhibition of IDOL activity in cells enhances the statin-mediated increase in LDLR protein and LDL uptake [3▪▪,9▪▪]. This points to the complementary and independent nature of these pathways and lends support for the development of therapeutic strategies aimed at inhibiting IDOL activity to complement current statin-based treatment of dyslipidemia.

In contrast to the desired goal in treating hypercholesterolemia, a recent study demonstrated that enhancing the LXR–IDOL pathway in glioblastomas was beneficial [28▪]. Glioblastoma cells are dependent on uptake of LDL for survival. Accordingly, increased IDOL activity in glioblastoma cells diminished LDLR content and inhibited cell growth in-vitro and in an in-vivo xenograft model. Whether this applies to other tumor types, needs to be tested.

INTEGRATION OF THE IDOL AND PCSK9 PATHWAYS FOR DEGRADATION OF THE LOW-DENSITY LIPOPROTEIN RECEPTOR

The outcome of the LXR–IDOL pathway – lysosomal degradation of the LDLR – resembles that achieved by PCSK9. A large body of work [29] indicates that *PCSK9* is coregulated together with the *LDLR* by SREBP2. Once secreted, PCSK9 binds to the extracellular epidermal growth factor-A domain of the LDLR and directs the receptor toward lysosomal degradation (Fig. 3). IDOL and PCSK9 share several similarities. Their substrate specificity overlaps and both promote degradation of the LDLR, VLDLR, and APOER2 [15▪,30]. Furthermore, they preferentially target the plasma membrane pool of LDLR for degradation, even though intracellular routes have been described for both [3▪▪,9▪▪,31,32]. However, whereas PCSK9 binds the extracellular domain of the LDLR, IDOL binds the protruding intracellular tail of the receptor.

How PCSK9 routes the LDLR toward lysosomal degradation remains a gap in understanding its mechanism of action. Given the similarities, a possible scenario would have IDOL working downstream of PCSK9 binding to the LDLR. However, this does not seem to be the case. PCSK9 retains its ability to degrade the LDLR in *Idol*$^{(-/-)}$ cells [9▪▪].

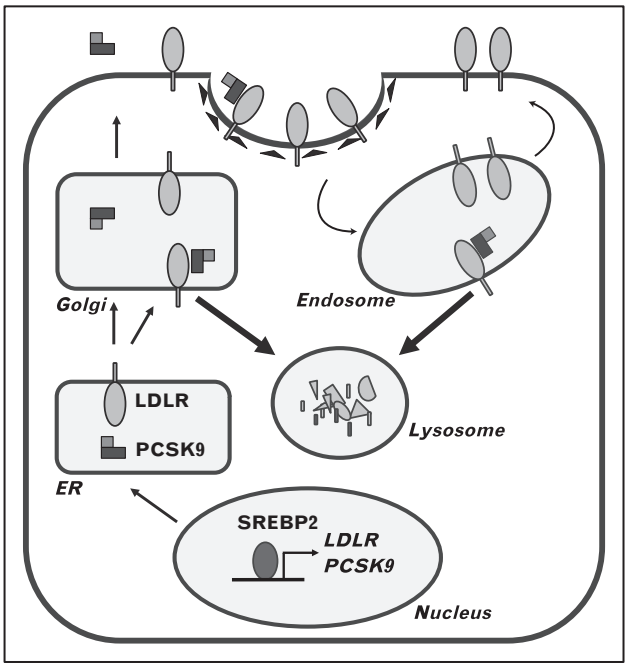

FIGURE 3. The SREBP–PCSK9 pathway controls abundance of the LDLR. Activation of the sterol regulatory element binding proteins (SREBP) pathway under conditions of low intracellular sterol levels increases expression of low-density lipoprotein receptor (*LDLR*) and *PCSK9* mRNAs. Autocatalytic processing of PCSK9 in the endoplasmic reticulum releases its prodomain (light grey), which remains associated with PCSK9 (dark grey). The active protein is secreted and binds the extracellular domain of the LDLR on the cell surface. The LDLR/PCSK9 complex is internalized via clathrin-dependent endocytosis. Association of PCSK9 with the LDLR prevents recycling of LDLR from endosomes to the cell surface, and directs the receptor toward lysosomal degradation. There is also evidence for an intracellular pathway in which PCSK9 binds the LDLR in the *trans*-golgi network and targets LDLR for degradation in lysosomes.

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

Furthermore, studies from the Leren group demonstrated that an LDLR lacking intracellular ubiquitination sites or even lacking the intracellular tail remains sensitive to extracellular PCSK9 [33▪,34▪]. Cumulatively, these studies indicate that the IDOL and PCSK9 pathways for degrading the LDLR are independent.

This conclusion raises the question as to why two independent pathways for post-translation regulation of the LDLR exist? One explanation may be that these pathways are differentially used in a tissue-specific manner. The expression of annexin A2, an endogenous inhibitor of PCSK9, in peripheral tissues might limit PCSK9 to targeting hepatic LDLR [35]. In contrast, IDOL is highly active in peripheral tissues. A second explanation may relate to the kinetics of these two pathways. The IDOL pathway allows cells to rapidly respond to increasing sterol levels, as the mechanism is catalytic, independent of the SREBP pathway, and circumvents the long half-life of the LDLR.

CONCLUSION

The ubiquitin system is emerging as an important facet of lipid metabolism. The stability and function of several key metabolic proteins, including HMGCR, INSIGs, SQLE, and CD36 is tightly regulated by ubiquitination [36,37▪,38▪,39]. Post-transcriptional control of LDLR function by IDOL extends this notion. The LXR–IDOL–LDLR axis defines a pathway, independent of SREBP2, to control the LDLR and current evidence supports investigating strategies to inhibit IDOL activity as a complement to statin-based therapy. A major challenge in the coming years will be to elucidate the contribution of IDOL to lipoprotein metabolism in animal models and in humans. The integration of the IDOL and PCSK9 pathways is gradually changing our view of the LDLR from a constitutive receptor toward a complex regulated membrane protein. Understanding the mechanisms governing the LDLR pathway may allow development of novel strategies for treating dyslipidemia.

Acknowledgements

We thank Ana Santos, Jessica Nelson, Lilith Scheer, Boris Bleijlevens, and Irith Koster for their suggestions and critical reading of the study.

Conflicts of interest

This study was supported by a Career Development Award from the Human Frontier Science Program Organization (HFSPO) and by a VIDI grant from the Netherlands Organization of Scientific Research (NWO) to NZ. The authors declare no competing financial interests.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ▪ of special interest
- ▪▪ of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000–000).

1. ▪ Calandra S, Tarugi P, Speedy HE, *et al.* Mechanisms and genetic determinants regulating sterol absorption, circulating LDL levels, and sterol elimination: implications for classification and disease risk. J Lipid Res 2011; 52:1885–1926.

This is an excellent recent review summarizing the genetics and mechanisms governing LDL metabolism in humans.

2. Garcia CK. Autosomal recessive hypercholesterolemia caused by mutations in a putative LDL receptor adaptor protein. Science 2001; 292:1394–1398.
3. ▪▪ Zelcer N, Hong C, Boyadjian R, Tontonoz P. LXR regulates cholesterol uptake through Idol-dependent ubiquitination of the LDL receptor. Science 2009; 325:100–104.

This is the first study that identifies IDOL as an LXR target gene and a post-transcriptional regulator of the LDLR.

4. Horton JD, Goldstein JL, Brown MS. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. J Clin Invest 2002; 109:1125–1131.
5. Im S-S, Osborne TF. Liver X receptors in atherosclerosis and inflammation. Circ Res 2011; 108:996–1001.
6. Olsson PA, Korhonen L, Mercer EA, Lindholm D. MIR is a novel ERM-like protein that interacts with myosin regulatory light chain and inhibits neurite outgrowth. J Biol Chem 1999; 274:36288–36292.
7. Bretscher A, Edwards K, Fehon RG. ERM proteins and merlin: integrators at the cell cortex. Nat Rev Mol Cell Biol 2002; 3:586–599.
8. Deshaies RJ, Joazeiro CAP. RING domain E3 ubiquitin ligases. Annu Rev Biochem 2009; 78:399–434.
9. ▪▪ Scotti E, Hong C, Yoshinaga Y, *et al.* Targeted disruption of the idol gene alters cellular regulation of the low-density lipoprotein receptor by sterols and liver X receptor agonists. Mol Cell Biol 2011; 31:1885–1893.

This article establishes the strict requirement of IDOL for LXR-mediated down-regulation of the LDLR. Additionally, this study indicates that LDLR at the plasma membrane is the prime target of IDOL.

10. ▪ Sorrentino V, Scheer L, Santos A, *et al.* Distinct functional domains contribute to degradation of the low density lipoprotein receptor (LDLR) by the E3 ubiquitin ligase inducible degrader of the LDLR (IDOL). J Biol Chem 2011; 286:30190–30199.

A detailed analysis of the structural determinants that establish the interaction between IDOL and the LDLR. This study also shows that IDOL promotes poly-K63 ubiquitin chain linkage on the LDLR.

11. Mukhopadhyay D, Riezman H. Proteasome-independent functions of ubiquitin in endocytosis and signaling. Science 2007; 315:201–205.
12. ▪ Zhang L, Fairall L, Goult BT, *et al.* The IDOL-UBE2D complex mediates sterol-dependent degradation of the LDL receptor. Genes Dev 2011; 25:1262–1274.

This study presents a detailed structural analysis of IDOLs RING domain and identifies the UBE2D family of E2-conjugating enzymes as physiological partners of IDOL

13. Stolt PC, Jeon H, Song HK, *et al.* Origins of peptide selectivity and phosphoinositide binding revealed by structures of disabled-1 PTB domain complexes. Structure 2003; 11:569–579.
14. ▪ Calkin AC, Goult BT, Zhang L, *et al.* FERM-dependent E3 ligase recognition is a conserved mechanism for targeted degradation of lipoprotein receptors. Proc Natl Acad Sci USA 2011; 108:20107–20112.

This study, together with that in reference [10▪], provides a detailed analysis of how IDOL binds the intracellular tail of the LDLR.

15. ▪ Hong C, Duit S, Jalonen P, *et al.* The E3 ubiquitin ligase IDOL induces the degradation of the low density lipoprotein receptor family members VLDLR and ApoER2. J Biol Chem 2010; 285:19720–19726.

This article demonstrates that in addition to the LDLR, IDOL also targets the VLDLR and APOER2 receptors. This study, together with reference [14▪], provides evidence that ubiquitination is an evolutionary conserved mechanism for controlling lipoprotein receptor function.

16. Suon S, Zhao J, Villarreal SA, *et al.* Systemic treatment with liver X receptor agonists raises apolipoprotein E, cholesterol, and amyloid-β peptides in the cerebral spinal fluid of rats. Mol Neurodegener 2010; 5:44.
17. Trommsdorff M, Gotthardt M, Hiesberger T, *et al.* Reeler/disabled-like disruption of neuronal migration in knockout mice lacking the VLDL receptor and ApoE receptor 2. Cell 1999; 97:689–701.
18. Wang L. Liver X receptors in the central nervous system: from lipid homeostasis to neuronal degeneration. Proc Natl Acad Sci U S A 2002; 99:13878–13883.
19. Kararigas G, Bito V, Tinel H, *et al.* Transcriptome characterization of estrogen-treated human myocardium identifies Myosin regulatory light chain interacting protein as a sex-specific element influencing contractile function. J Am Coll Cardiol 2012; 59:410–417.

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

20. Ishibashi M, Masson D, Westerterp M, *et al.* Reduced VLDL clearance in ■ Apoe($^{-/-}$)Npc1($^{-/-}$) mice is associated with increased Pcsk9 and Idol expression and decreased hepatic LDL-receptor levels. J Lipid Res 2010; 51:2655–2663.
This study suggests that increased expression of IDOL in liver of mice harboring a combined loss of ApoE and Npc1 decreases hepatic LDLR.
21. Timmins JM, Lee J-Y, Boudyguina E, *et al.* Targeted inactivation of hepatic ABCA1 causes profound hypoalphalipoproteinemia and kidney hypercatabolism of apoA-I. J Clin Invest 2005; 115:1333–1342.
22. Chasman DI, Paré G, Mora S, *et al.* Forty-three loci associated with plasma ■■ lipoprotein size, concentration, and cholesterol content in genome-wide analysis. PLoS Genet 2009; 5:e1000730.
This genome-wide association study identifies IDOL (also known as MYLIP) as a locus that contributes to variation of circulating LDL levels in humans.
23. Teslovich TM, Musunuru K, Smith AV, *et al.* Biological, clinical and population ■■ relevance of 95 loci for blood lipids. Nature 2010; 466:707–713.
This genome-wide association study identifies IDOL (also known as MYLIP) as a locus that contributes to variation of circulating LDL levels in humans.
24. Waterworth DM, Ricketts SL, Song K, *et al.* Genetic variants influencing ■■ circulating lipid levels and risk of coronary artery disease. Arterioscler Thromb Vasc Biol 2010; 30:2264–2276.
This genome-wide association study identifies IDOL (also known as MYLIP) as a locus that contributes to variation of circulating LDL levels in humans.
25. Weissglas-Volkov D, Calkin AC, Tusie-Luna T, *et al.* The N342S MYLIP ■■ polymorphism is associated with high total cholesterol and increased LDL receptor degradation in humans. J Clin Invest 2011; 121:3062–3071.
This study provides the first evidence that a common polymorphism in IDOL contributes to variation in levels of total cholesterol in plasma.
26. Dong B, Wu M, Cao A, *et al.* Suppression of Idol expression is an additional ■ mechanism underlying statin-induced up-regulation of hepatic LDL receptor expression. Int J Mol Med 2011; 27:103–110.
This study suggests that in addition to increasing expression of LDLR, mRNA statins may also increase abundance of LDLR by suppressing IDOL expression.
27. Chasman DI, Giulianini F, Macfadyen J, *et al.* Genetic determinants of statin ■ induced LDL-C reduction: the JUPITER Trial. Circ Cardiovasc Genet 2012. [Epub ahead of print]. doi:10.1161/CIRCGENETICS.111.961144.
This is a recent study suggesting that IDOL may contribute to variation in response to rosuvastatin.
28. Guo D, Reinitz F, Youssef M, *et al.* An LXR agonist promotes GBM cell death ■ through inhibition of an EGFR/AKT/SREBP-1/LDLR-dependent pathway. Cancer Discov 2011; 1:442–456.
This study demonstrates that promoting the LXR–IDOL pathway is effective in limiting growth of glioblastoma cells.
29. Horton JD, Cohen JC, Hobbs HH. PCSK9: a convertase that coordinates LDL catabolism. J Lipid Res 2009; 50 (Suppl):S172–S177.
30. Poirier S, Mayer G, Benjannet S, *et al.* The proprotein convertase PCSK9 induces the degradation of low density lipoprotein receptor (LDLR) and its closest family members VLDLR and ApoER2. J Biol Chem 2008; 283:2363–2372.
31. Lagace TA, Curtis DE, Garuti R, *et al.* Secreted PCSK9 decreases the number of LDL receptors in hepatocytes and in livers of parabiotic mice. J Clin Invest 2006; 116:2995–3005.
32. Poirier S, Mayer G, Poupon V, *et al.* Dissection of the endogenous cellular pathways of PCSK9-induced low density lipoprotein receptor degradation: evidence for an intracellular route. J Biol Chem 2009; 284:28856–28864.
33. Holla ØL, Strøm TB, Cameron J, *et al.* A chimeric LDL receptor containing the ■ cytoplasmic domain of the transferrin receptor is degraded by PCSK9. Mol Genet Metab 2010; 99:149–156.
The study demonstrates that the intracellular domain of the LDLR is not essential for PCSK9-mediated degradation of the LDLR. Together with [9■■], this study indicates that the IDOL and PCSK9 pathways are independent.
34. Strøm TB, Holla ØL, Tveten K, *et al.* Disrupted recycling of the low density ■ lipoprotein receptor by PCSK9 is not mediated by residues of the cytoplasmic domain. Mol Genet Metab 2010; 101:76–80.
The study demonstrates that the intracellular domain of the LDLR is not essential for PCSK9-mediated degradation of the LDLR. Together with [9■■], this study indicates that the IDOL and PCSK9 pathways are independent.
35. Mayer G, Poirier S, Seidah NG. Annexin A2 is a C-terminal PCSK9-binding protein that regulates endogenous low density lipoprotein receptor levels. J Biol Chem 2008; 283:31791–31801.
36. Gong Y, Lee JN, Lee PCW, *et al.* Sterol-regulated ubiquitination and degradation of Insig-1 creates a convergent mechanism for feedback control of cholesterol synthesis and uptake. Cell Metab 2006; 3:15–24.
37. Gill S, Stevenson J, Kristiana I, Brown AJ. Cholesterol-dependent degradation ■ of squalene monooxygenase, a control point in cholesterol synthesis beyond HMG-CoA reductase. Cell Metab 2011; 13:260–273.
Recent example of posttranscriptional regulation of key proteins in lipid metabolism via ubiquitination.
38. Kim K-Y, Stevens MV, Akter MH, *et al.* Parkin is a lipid-responsive regulator of ■ fat uptake in mice and mutant human cells. J Clin Invest 2011; 121:3701–3712.
Recent example of posttranscriptional regulation of key proteins in lipid metabolism via ubiquitination.
39. DeBose-Boyd RA. Feedback regulation of cholesterol synthesis: sterol-accelerated ubiquitination and degradation of HMG CoA reductase. Cell Res 2008; 18:609–621.

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.