The E3 ubiquitin ligase inducible degrader of the LDL receptor/myosin light chain interacting protein in health and disease

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Purpose of review
The RING E3 ubiquitin ligase inducible degrader of the LDL receptor (IDOL, also known as MYLIP) promotes ubiquitylation and subsequent lysosomal degradation of the LDL receptor (LDLR), thus acting to limit uptake of lipoprotein-derived cholesterol into cells. Next to the LDLR, IDOL also promotes degradation of two related receptors, the very LDL receptor (VLDLR) and apolipoprotein E receptor 2 (APOER2), which have important signaling functions in the brain. We review here the emerging role of IDOL in lipoprotein and energy metabolism, neurodegenerative diseases, and the potential for therapeutic targeting of IDOL.

Recent findings
Genetic studies suggest an association between IDOL and lipoprotein metabolism in humans. Studies in rodents and nonhuman primates support an in-vivo role for IDOL in lipoprotein metabolism, and also uncovered an unexpected role in whole-body energy metabolism. Recent evaluation of IDOL function in the brain revealed a role in memory formation and progression of Alzheimer’s disease. The report of the first IDOL inhibitor may facilitate further investigations on therapeutic strategies to target IDOL.

Summary
IDOL is emerging as an important determinant of lipid and energy metabolism in metabolic disease as well as in Alzheimer’s disease. IDOL targeting may be beneficial in treating these conditions.

Keywords
cholesterol metabolism, inducible degrader of the LDL receptor, lipoprotein receptors, metabolic syndrome, neurodegenerative disease

INTRODUCTION
Cholesterol metabolism has a well established role in development of cardiovascular disease, with an elevated level of circulating LDL representing a major risk factor for cardiovascular disease development. Lipoprotein metabolism is now recognized as an important determinant of other human maladies, including obesity and neurodegenerative diseases. This emphasizes the need to tightly coordinate lipoprotein metabolism at the cellular and organismal level, a process in which lipoprotein receptors play a crucial role.

The LDL receptor (LDLR), through its ability to enhance hepatic clearance of LDL from the circulation governs LDL metabolism [1,2]. Accordingly, mutations in the LDLR are the most frequent cause of familial hypercholesterolemia. It follows that the level and activity of the LDLR needs to be tightly controlled and for this both transcriptional and posttranscriptional mechanisms are used. Transcriptional regulation of the LDLR is primarily under control of the Sterol Regulatory Element-Binding Protein (SREBP) family of transcription factors [1,3,4]. Two potent posttranscriptional regulators of the LDLR that promote its lysosomal degradation have been described; the secreted proprotein convertase subtilisin/kexin type 9 (PCSK9) (for reviews see [5,6]), and the E3 ubiquitin ligase inducible degrader of the LDLR [IDOL, also known as the myosin light chain interacting protein (MYLIP)]
KEY POINTS

- Variation in IDOL is associated with lipid traits in humans.
- Rodent and nonhuman primate models of Idol/IDOL demonstrate an in-vivo role in lipoprotein metabolism.
- Loss of Idol in mice improves metabolic regulation during normal physiological aging and in response to a high-fat diet.
- Idol is implicated in learning and memory formation.
- Loss of Idol in mice reduces Alzheimer’s-disease-like symptoms.

CLONING AND CHARACTERIZATION OF INDUCTIBLE DEGRADER OF THE LDL RECEPTOR/MYOSIN LIGHT CHAIN INTERACTING PROTEIN

MYLIP (referred to further as IDOL) was cloned from a brain cDNA library in search of novel neuronal cytoskeletal regulators [9]. IDOL is ubiquitously expressed, with particular high expression in the hippocampus [10**,11]. The molecular cloning of IDOL revealed it is a cytosolic protein of ~50 kDa (445 amino acids) that consists of two major protein domains; a C-terminal really interesting new gene (RING) domain and an N-terminal FERM domain. FERM domains are present in proteins, such as ezrin, radixin and moesin, and known to regulate plasma membrane–cytoskeleton interactions [12]. Accordingly, studies of IDOL’s FERM domain indicated predominant localization to the plasma membrane [13]. The presence of the E3 ligase RING domain in IDOL is unique among human FERM proteins, and suggests that IDOL may influence the function or level of membrane proteins via targeted ubiquitination.

Ubiquitination – the conjugation of the small protein ubiquitin to target proteins – is a process requiring the sequential activity of an E1-activating, an E2-conjugating, and an E3-ligating enzyme [14]. Target specificity is largely dictated, in the case of RING E3s like IDOL, by the E3 ligase itself. In 2009, the LDLR was identified as a direct ubiquitination target of IDOL [7]. Together with the E2 UBC13 or members of the UBE2D E2 family [13,15], IDOL promotes ubiquitination of a conserved lysine residue in the short intracellular tail of the receptor, a mark that targets the LDLR for subsequent lysosomal degradation through a clathrin-independent pathway [16,17]. Expression of IDOL is transcriptionally regulated by the sterol-sensing liver X receptor (LXR) transcription factors [7]. LXRs are members of the ligand activated nuclear receptor family of transcription factors, and respond to elevated levels of oxysterols and intermediates of the biosynthetic pathway by increasing expression of their target genes, amongst them IDOL [18]. Induction of the LXR–IDOL axis leads to decreased LDLR protein and associated cellular LDL uptake, and as such its activation can be viewed as a feedback mechanism to prevent further cellular cholesterol accumulation when its levels are adequate (Fig. 1). Posttranscriptional regulation also plays an important role in controlling IDOL levels and activity, as similar to other E3 ligases, IDOL promotes its own auto-ubiquitylation and rapid proteasomal degradation [7,19**]. Intriguingly, we have reported that degradation of the LDLR by IDOL is subject to negative regulation by the de-ubiquitinating enzyme USP2 [19**]. It remains to be seen whether this is because of direct removal of ubiquitin from the LDLR, which redirects the receptor away from the degradation pathway, or that removal of ubiquitin from IDOL directly inhibits its activity.

In addition to the LDLR, IDOL also promotes ubiquitination-dependent degradation of the closely related receptors, the very LDL receptor (VLDLR) and apolipoprotein E receptor 2 (APOER2) [20], which are notably also targeted by PCSK9 [21]. These two receptors play an important role in the Reelin signaling pathway and are required during development for proper neuronal migration and positioning [22]. To date, no other ubiquitination targets have been conclusively established for IDOL. This narrow and specific target specificity is rather unique among E3s, as typically E3s are promiscuous and have a broader spectrum of ubiquitination targets. IDOL’s specificity is dependent on recognition between the FERM domain and a conserved motif in the intracellular tail of the LDLR, VLDLR, and APOER2 [13,23*], which is further enhanced by proper orientation of IDOL with the negatively charged surface of the plasma membrane [23*]. Akin to the LDLR, genetic or pharmacologic manipulation of the LXR–IDOL axis regulates the level of the VLDLR and APOER2 [10**,20]. However, whether cholesterol metabolism and LXRs are relevant physiological signals for regulating these receptors in vivo is unclear at present (see below). In view of its target specificity and the known functions of its targets in lipoprotein metabolism and neuronal function, IDOL has been studied in these contexts in recent years.
The mechanism underlying regulation of the LDLR by IDOL has been extensively studied in cell-based systems and suggests an important role in regulation of lipoprotein metabolism in vivo [8]. This notion was supported early-on by genome-wide association studies (GWAS) that reported an association between genetic variation in IDOL and circulating LDL levels in humans [24–27]. One of the single nucleotide polymorphisms (SNPs) identified in these studies, rs9370867, which encodes an IDOL Asn342Ser variant, was subsequently shown to have a significant association with plasma cholesterol levels in a dyslipidemic Mexican population and to affect LDLR degradation in vitro [28]. However, this association was not observed in subsequent Brazilian, Italian, and Dutch cohorts [29–31], putting the significance of this variant in question. Genetic variation in IDOL has also been associated with the response to lipid-lowering statins in the Brazilian cohort, as well as in the JUPITER trial [32,33]. Yet a recent report suggests that for one of the reported IDOL SNPs (rs6924995), the association with statin response may be linked to the nearby long noncoding RNA RPI-13D10.2 (RP1), and not to IDOL itself [34]. Additional genetic support for the potential role of IDOL in human lipoprotein metabolism comes from our studies in a Dutch cohort in which mutations in other cholesterol-related genes have been excluded [31]. In this study, we identified an IDOL loss-of-function variant in a limited number of carriers, which was associated with reduced levels of circulating LDL. Taken together, human genetic studies, although not conclusive, support the involvement of IDOL in human lipoprotein metabolism and its targeting in cholesterol-lowering strategies.

The development of Idol mouse models has greatly facilitated the study of its in-vivo functions. Several studies have demonstrated that transient or stable hepatic transgenic expression of IDOL/Idol dramatically increases LDL levels in mice and hamsters [7,35,36,37], and consequently can promote atherosclerosis development [35]. Similarly, increasing hepatic IDOL expression in nonhuman primates by pharmacological LXR activation increased circulating LDL levels in an IDOL-dependent manner [38]. Conversely, cell-based assays, including experiments conducted in Idol(−/−) mouse embryonic stem cells [39], demonstrated that loss of IDOL expression increases the level of the LDLR and concomitantly enhances LDL uptake [7,16,17]. However, different from these experiments, initial assessment of global loss of Idol in mice unexpectedly showed no significant effect on circulating total cholesterol or LDL-cholesterol [38]. This was proposed to be a result of...
species-dependent hepatic IDOL activity; in mice, IDOL is highly active in peripheral tissues yet its activity in the liver is muted, whereas in nonhuman primates (and presumably humans [31]) hepatic IDOL activity is prominent. In line with this, antisense oligonucleotide (ASO)-based IDOL silencing increased hepatic LDLR and attenuated the rise in circulating levels of LDL following treatment with an LXR ligand in nonhuman primates [38]. However, in contrast to the initial report in mice [38], we have recently found a significant decrease in circulating cholesterol in high-fat-diet-fed Idol knock-out mice, emphasizing the need for further studies on the role of IDOL in lipoprotein metabolism in vivo [40**,41]. Surprisingly, despite the potential involvement of IDOL in LDL metabolism, no studies have been reported to date on the role of Idol ablation or silencing in coronary artery disease.

Beyond cholesterol homeostasis, we recently addressed the role of IDOL in broader lipoprotein and energy metabolism [40**]. Our study was the first to report that mice lacking Idol are protected from age-induced and diet-induced weight gain, have reduced circulating triglyceride levels, improved glycemic control, and decreased neutral lipids accumulation in the liver and brown adipose tissue compared with their wildtype controls. Protection from these metabolic syndrome-associated comorbidities was remarkably not a result of a marked increase in energy expenditure. Rather, we speculated that sustained brown-adipose activity and enhanced sympathetic signaling in this tissue may underlie the improved metabolic function of Idol+/− mice. We also observed an increase in locomotor activity in Idol+/− mice, and although our study was not designed to address this issue, this could suggest involvement of IDOL in regulation of metabolism through central nervous system circuitry (see below).

THE ROLE OF INDUCIBLE DEGRADER OF THE LDL RECEPTOR IN THE CENTRAL NERVOUS SYSTEM AND NEURODEGENERATIVE DISEASE

Cholesterol transport within the brain is primarily carried out by ApoE-containing lipoproteins. The finding that the e4 isoform of ApoE is the most robust genetic risk factor for Alzheimer’s disease in humans suggests that disturbed central cholesterol homeostasis has a causal role in its pathophysiology [42,43]. All three IDOL targets are ApoE receptors, expressed in the brain, and have an established role in transport of cholesterol in this organ. Accordingly, the level of these receptors is increased in the brain in the absence of Idol [10**,44], in contrast to the lack of change in their levels in Pcsk9 knockout mice [45]. In line with this, ablation of Idol reduced ApoE protein levels in the brain, beta-amyloid (Aβ) plaque deposition, and neuroinflammation in a mouse Alzheimer’s disease model [44]. This beneficial outcome has been attributed to enhanced LDLR-mediated clearance of Aβ-carrying ApoE particles by resident microglia cells, yet the possibility that Aβ clearance across the brain–blood barrier was also increased was not evaluated [46]. Moreover, as functional learning and memory in the Alzheimer’s disease setting was not reported it remains to be seen whether loss of IDOL improves these parameters (discussed in [47]).

The high expression of Idol in the hippocampus [10**,11], may suggest an important role for IDOL in learning and memory formation. Neuronal expression of Idol has been recently reported to influence dendritic spinogenesis and spine morphogenesis [10**]. This is because of the ability of IDOL to titrate the levels of ApoER2 and couple synaptic activity to Rac1 activation. This coupling, which results in cytoskeletal remodeling important for spine formation and dynamics, is reminiscent of the original report showing that IDOL over-expression can inhibit neurite outgrowth in the PC12 neuroendocrine cell line [9]. As a result, absence of Idol and dysregulation of ApoER2 levels leads to impaired long-term potentiation (a proxy for memory formation) in primary neurons and hippocampal slices, and impaired learning and memory formation in mice lacking Idol. This represents a potential detrimental consequence of dysregulated IDOL activity in neurons. In that respect, we have previously reported that sterol-dependent regulation of Idol expression in neurons is blunted in comparison to other cell types [20], suggesting that other signals or mechanism may be more important for this in this lineage. Indeed, neuronal activation [10**], as well as pro-Nerve growth factor and brain-derived neurotrophic factor [48,49] seem to regulate Idol expression. The underlying transcriptional mechanism has not been addressed, but it may involve sterol-independent regulation of IDOL, as we have recently reported [50*].

THERAPEUTIC TARGETING OF INDUCIBLE DEGRADER OF THE LDL RECEPTOR AND OUTLOOK

IDOL plays a role in multiple tissues and cell types, and our current understanding of IDOL function suggests that targeting its expression or activity could be therapeutically beneficial in regimens to treat hypercholesterolemia, metabolic syndrome-associated morbidities, and potentially also Alzheimer’s disease (Fig. 2). The beneficial effect of IDOL-directed
ASOs in nonhuman primates demonstrates the feasibility of this approach [38], even though small-molecule IDOL inhibitors would be preferable. It is, therefore, encouraging that the first peptide-based inhibitor of IDOL, which interferes with its dimerization, has been reported [51]. Targeting the interaction between IDOL and its targets or its association with the membrane also offer opportunities to inhibit IDOL activity. Although challenging, availability of the high-resolution structure of IDOL will facilitate these developments. Given the strong effect Idol has on memory and learning in mice [10], caution should be taken during development of IDOL inhibitors. We point out that whereas the current focus lies in developing IDOL inhibitors, increasing IDOL activity may be a tractable approach to treat glioblastoma [52,53], and potentially other tumors, which are dependent on LDLR-mediated lipoprotein uptake. Despite an increase in our understanding of IDOL several pertinent questions remain:

(1) How does Idol loss improve metabolic syndrome-associated comorbidities and in which tissue(s) is IDOL activity pertinent for this? Importantly, does this translate to the human setting?

(2) How is IDOL transcriptionally regulated in different cell types and tissues?

(3) What is the consequence of IDOL inhibition or ablation on atherosclerosis?

(4) What is the specific contribution of each of the three IDOL targets to its overall in-vivo function?

(5) Can effective and safe small-molecule inhibitors be developed for in-vivo use and can these inhibitors act either independently or complementary to statins and PCSK9 inhibitors?

(6) Is IDOL involved in learning and memory disabilities in humans?

For addressing some of these questions, generation of tissue-specific Idol models will be instrumental. The growing interest in therapeutic targeting of IDOL and the recent description of the first pharmacological IDOL inhibitor ensure that the coming years of IDOL research will be exciting ones.

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Conflicts of interest
There are no conflicts of interest.

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


Similar to [35], this study uses AAV-IDOL as a model to evaluate hepatic IDOL activity.


This recent study implicates IDOL in whole-body lipid and energy metabolism and demonstrates that loss of IDOL protects against age-induced and diet-induced metabolic alterations.


This report identifies an LXR-independent mechanism to regulate liver x receptor degradation with a cyclic peptide that disrupts the homodimerization of IDOL E3 ubiquitin ligase. Chem Sci 2018; 9:5957–5966.

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