Fooling IDOL to Help Control Cholesterol

Andrew J. Brown, Joanne Hsieh

Forty years ago, Akira Endo discovered that a fungal metabolite inhibits 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR), the rate limiting step of the mevalonate pathway and therefore cholesterol biosynthesis, and initiated the development of statins to treat hypercholesterolemia. Subsequent work by the group of Michael Brown and Joseph Goldstein identified the low density lipoprotein receptor (LDLR) as the central player in the elaborate homeostatic controls that occur in response to statin-induced cellular cholesterol depletion. In a bid to acquire more cholesterol, hepatocytes (to which the drugs are mostly targeted) respond by transcriptionally upregulating LDLR via the sterol regulatory element binding protein (SREBP) transcription factors, thereby clearing more circulating LDL, and thus lowering blood cholesterol levels.1 Statins are effective, both clinically and economically, but decades of its widespread use have made apparent some infrequent side effects, including myopathy and an increased onset of diabetes. In addition to producing cholesterol, the mevalonate pathway is also essential to protein isoprenylation and glycosylation, which may account for both the beneficial and adverse side effects of statins. A drug that posttranscriptionally regulates cholesterol metabolism in response to nutritional and "extracellular" LDLR levels is available at http://circres.ahajournals.org (circulation research, 2016;118:371-373).

The LDLR protein abundance is also regulated by the inducible degrader of LDLR (IDOL), which is upregulated in conditions of sterol excess as the IDOL gene is a transcriptional gene target for the liver X receptor (LXR).2 While at an earlier stage of translational development than PCSK9, human genetic evidence suggests IDOL also has an important role in the regulation of human LDL levels.4 IDOL belongs to a class of enzymes called E3 ubiquitin ligases, which can target proteins for destruction by ubiquitylation (also called ubiquitination). Ubiquitylation plays key regulatory roles in all aspects of cholesterol metabolism including cholesterol efflux, synthesis, and uptake (reviewed in 5). The FERM domain of IDOL interacts with the LDLR at the plasma membrane, while the RING domain conjugates a chain of ubiquitin moieties via an isopeptide bond to the cytoplasmic tail of LDLR.3 With respect to regulated turnover of proteins, ubiquitylation is usually associated with degradation by the proteasome, but IDOL is unusual in that it marks the LDLR for degradation by the lysosome.3 E3 ubiquitin ligases are often deemed “druggable targets,” as they confer the necessary substrate specificity, with the human genome encoding a rich diversity of E3 ubiquitin ligases (more than 600, compared with about 40 E2 ubiquitin-conjugating enzymes and just two E1 ubiquitin-activating enzymes). A small molecule inhibitor of an enzyme that could be more affordable, but unfortunately, PCSK9’s degradative effect on LDLR is not attributable to its subtilase activity2 and PCSK9 has not proven amenable to a small molecule approach.3

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circadian cues, particularly in the liver which has the greatest impact on circulating LDL levels. While hepatic expression of the USP2-45 isoform oscillates profoundly throughout the diurnal cycle, USP2-69 levels are relatively constant throughout the day.7 Such a temporal expression pattern would leave little opportunity to alleviate the USP2-mediated antagonism of IDOL, and the tripartite complex model would imply that IDOL is constantly engaged in a futile reaction, in which its ubiquitylation of LDLR is immediately reversed by USP2. However, tumor necrosis factor α (TNFα) has been reported to downregulate a USP2 isoform in hepatocytes,8 so possibly, USP2-inhibited degradation of LDLR is a pathway that is affected in inflammatory conditions. In summary, USP2 activity leads to the deubiquitylation and stabilization of cell surface LDLR in an IDOL-dependent manner, but it remains uncertain exactly why such a phenomenon is accompanied by an extended IDOL protein half-life.

While it is clear that USP2 has an effect on LDLR ubiquitylation, stability and activity, this paper did not directly demonstrate that LDLR is a substrate of USP2’s isopeptidase activity. As the authors themselves acknowledge, it remains entirely possible that the decreased LDLR ubiquitylation is a consequence of lower IDOL activity. USP2 overexpression was shown to decrease IDOL ubiquitylation, but it remains unknown which type of ubiquitin linkages on IDOL are edited by USP2. Lys48-linked ubiquitin chains signal for the proteasomal degradation that is typically associated with protein polyubiquitylation, but ubiquitin can be linked via six other lysine residues to signal distinct and diverse biological processes (reviewed in 9). For example, the DUB A20 removes Lys63-linked ubiquitin chains from TNF receptor associated factor 6 (TRAF6), which inhibits TRAF6’s E3 ubiquitin ligase activity and consequently tempers NFκB signaling.10 Therefore, future work that characterizes the ubiquitin chain conjugated to IDOL could impart valuable information on how exactly USP2 regulates IDOL. Moreover, although it seems counterintuitive, it is feasible that the enhanced IDOL stability is secondary to a decrease in its E3 ubiquitin ligase activity. As has been shown for the Casitas B-lineage Lymphoma (CBL) ligases, polyubiquitylation of the stem cell factor receptor (KIT) results in codegradation of both the substrate and the RING domain-containing E3 ubiquitin ligase.11 Having E3 ubiquitin ligase degradation contingent on its activity has been speculated to self-limit the degradation of the substrate receptor, thereby allowing membrane receptor levels to recover.12 While such a scenario may be difficult to envisage for IDOL and LDLR, given that the former is degraded by the proteasome and the latter via the lysosomal pathway,3 two different

**Figure. The role of ubiquitylation in the regulation of LDLR.** Boxed items are treatments currently approved to lower LDL cholesterol. (1) Statin treatment targets HMGCR, the rate-limiting enzyme of the mevalonate pathway, lowering cellular cholesterol and activating SREBP-2. (2) SREBP-2 is also inhibited by an E3 ubiquitin ligase MARCH6, and transcriptionally induces LDLR expression, which increases LDL uptake. (3) LDLR is bound by extracellular PCSK9, leading to its lysosomal degradation. Anti-PCSK9 monoclonal antibodies including evolocumab and alirocumab are approved to block the PCSK9-induced LDLR degradation to maintain LDL uptake. (4) The sterol sensor LXR transcriptionally induces IDOL which, (5) with its cognate E2 ubiquitin ligase UBE2D, ubiquitylates LDLR for lysosomal degradation. (6) LDLR’s subsequent deubiquitylation by USP8 is necessary to sort it into the lysosomal degradation pathway. (7) Inhibition of DUB activity has been shown to induce IDOL expression via an unknown transcription factor binding site (TFBS). (8) MARCH6 also inhibits HMGCR, lowering cholesterol biosynthetic intermediates that would activate LXR and IDOL transcription. The colored proteins depict the new pathway described in this issue by Nelson, Sorrentino et al. (9) USP2’s DUB activity decreases the ubiquitylation of both IDOL and LDLR, protecting the former from proteasomal degradation and the latter from lysosomal degradation. USP2 activity therefore maintains membrane expression of LDLR and LDL uptake.
degradation systems have also been suggested in the case of CBL and KIT. Nevertheless, whether USP2 regulates LDLR ubiquitylation directly as a tripartite complex with IDOL, or indirectly by deubiquitylating IDOL to modulate its activity, is a question that can only be answered by the very difficult task of reconstituting the enzymatic reactions in vitro.

USP2-mediated regulation of IDOL activity is ultimately the more attractive scenario because, as mentioned earlier in this editorial, USP2 is one of only about 100 DUBs, implying it has a considerable number of substrates apart from IDOL and LDLR. One of USP2’s targets is fatty acid synthase, raising concerns of fatty liver with hepatocyte-targeted USP2 activators. USP2 activation has been associated with increased blood glucose, therefore precluding it as a pharmacological target in diabetics. Moreover, USP2 is a well-described oncogene (reviewed in (4)), suggesting that there are multiple limitations in using USP2 activation as a strategy to control LDL-cholesterol levels. However, with six-fold more E3 ubiquitin ligases, inhibiting the ubiquitylation of IDOL could offer a therapeutic approach to lower LDL cholesterol while minimizing undesirable side effects. Inhibiting IDOL itself may appear to be the most direct approach, but IDOL’s RING domain works in conjunction with E2 ubiquitin ligases from the UBE2D family to ubiquitylate directly as a tripartite complex with IDOL, or indirectly by deubiquitylating IDOL to modulate its activity, suggesting that there are multiple limitations in counteracting the E3-ubiquitin ligase IDOL. The role of ubiquitylation-mediated protein turnover in controlling all aspects of cholesterol homeostasis is becoming more widely appreciated. A role has previously been described for USP8, in which its DUB activity directly decreases membrane LDLR downstream of IDOL by sorting it for lysosomal degradation. This current work elucidates a completely distinct function for a DUB on LDLR regulation, as USP2 exemplifies the emerging concept that DUBs can in turn regulate E3 ligase activity. Other recent work from the Zelcer group adds to the already complex picture of cholesterol regulation (Figure). This group also reported that general pharmacological inhibition of cellular DUB activity induced gene expression of IDOL in an LXRI-independent manner, as well as an interesting interplay between IDOL and another E3 ubiquitin ligase (MARCH6), which together uncouple cholesterol uptake (via LDLR) from cholesterol synthesis in liver cells. Clearly, there is still much to learn about the role of the ubiquitylation-deubiquitylation balance in cholesterol homeostasis, with the making and breaking of ubiquitin chains generally expanding the repertoire of regulation.

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Disclosures

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