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Mylip makes an Idol turn into regulation of LDL receptor

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Abstract High blood low-density-lipoprotein (LDL) cholesterol is a serious health problem among an increased number of patients in the Western world. Statins and other cholesterol lowering drugs have proven to be beneficial as therapy but are not optimal and show adverse effects in some patients. The LDL receptor is a crucial determinant of cholesterol metabolism in the body and amenable for drug interventions. Novel insights into the physiology of this receptor come from studies on the ubiquitination and degradation of LDL receptor by the ubiquitin ligase Mylip/Idol that is induced in cells by the nuclear receptor, LXR. This may open up new possibilities in the future to influence LDL receptor levels and cholesterol metabolism pharmacologically in various diseases.

Keywords LDL receptor · Protein degradation · Ubiquitination · Ubiquitin ligase · Mylip

Introduction

Increased plasma levels of low-density-lipoprotein cholesterol (LDL) represent a known risk for cardiovascular disease and atherosclerosis. LDL is taken up mainly into hepatocytes by the LDL receptor (LDL-R) which is a major drug target in molecular medicine and disease prevention. The abundance of LDL-R on the cell surface is modulated by transcriptional and posttranscriptional events that have been extensively studied. A new twist to this comes from a recent work showing that liver LDL-R is influenced by the ubiquitin ligase, Mylip/Idol that is induced by the sterol-responsive nuclear receptor, LXR [1]. This shows a new level of LDL-R regulation by protein ubiquitination that may facilitate development of novel strategies for treatment of high blood cholesterol in various diseases.

LDL receptor regulation and cholesterol metabolism

Cellular proteins have a certain half-life determined by the rate of synthesis and degradation that occurs in the lysosomes or by ubiquitination via the proteasomal system. For cell surface receptors that are often involved in cell signaling, endocytosis and receptor recycling are additional events determining the functional half-life in the cell. The LDL-R is a classical example of a membrane protein that is transported to the plasma membrane via the endoplasmic reticulum-Golgi pathway and then taken up via endocytosis followed by recycling or degradation [2]. In familial hypercholesterolemia, different mutations in LDL-R decrease the levels of functional receptors, which elevates blood LDL cholesterol [3] and increases the risk for cardiovascular disease [4]. Statins are cholesterol lowering drugs that are widely used to treat patients with lipid disorders [5] and to reduce cardiovascular complications [6]. Statins act by reducing serum LDL levels by upregulating LDL-R activity in liver, but these drugs have also other metabolic effects in cells [5–7]. Normally the

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LDL-R levels are tightly controlled by nuclear factors including the sterol regulatory element binding protein (SREBP) factors that enhance its transcription and thereby decrease blood LDL levels [8]. The LDL-R is posttranscriptionally regulated by the proprotein convertase subtilisin/kexin type 9 (PCSK9) protein that binds to the receptor and alters its stability and trafficking thereby increasing its lysosomal degradation [9–11]. The mechanism for this involves the interaction of PCSK9 with EGF-like repeats (EGF-A) in the LDL-R that somehow interferes with the recycling of this complex after endocytosis [11–13]. PCSK9 expression is under the control of the SREBP transcription factors, and its physiological importance in cholesterol metabolism has been demonstrated in both gene-modified mice and in humans with PCSK9 mutations [7, 10]. Thus, overexpression or gain-of-function mutations in PCSK9 lead to high blood LDL cholesterol, whereas a reduced expression or loss-of-function mutations in PCSK9 cause low blood cholesterol and protection from heart disease, as reviewed recently [7, 10]. It has been further shown recently that blocking of PCSK9 binding to the LDL-R in monkey by infusing humanized murine mAB increased hepatic LDL-R with a reduction in plasma LDL cholesterol [14]. Most importantly, this beneficial effect was synergistic to that obtained with the use of statins. This is important since cholesterol-lowering drugs that increase LDL-R also elevate PCSK9 levels, thus blunting their beneficial effects on cholesterol clearance in various situations. Targeting the LDL-R from different angels and using a combination of drugs would improve the treatment of high blood LDL cholesterol with hopefully less side effects in patients [5, 7].

Nuclear receptor LXR induces Mylip/Idol

LXR is sterol-responsive nuclear receptor that decreases LDL-R levels and promotes cholesterol efflux from cells following the induction of the ABCG1 and ABCA1 transport proteins [15]. LXR also has other target genes important in fat and cholesterol metabolism and can influence glucose homeostasis and tissue inflammation [15–17]. Recent work on genes induced by the LXR in liver and macrophages pin-point the ubiquitin ligase, Mylip, as a novel regulator of LDL-R [1]. This was surprising as Mylip was originally identified as a protein in neurons with a FERM (4.1 band, ezrin, radixin and moesin) homology domain at aminoterminus and a RING Zinc finger ubiquitin ligase domain at the carboxyterminal end [18]. The FERM-containing proteins interact with the cytoplasmic part of transmembrane proteins and link these to the cytoskeleton in cell signaling [19]. Different FERM protein members have physiological functions ranging from actin and RhoGTPase binding to phosphatase activities; however, Mylip is the only one possessing an ubiquitin ligase motif. Mylip was shown to interact with the myosin regulatory light chain (MRLC), and the protein was therefore named MRLC interacting protein, MIR [18]. Later, the protein was renamed Mylip, following the nomenclature for this gene family. Functional data revealed that overexpression of Mylip inhibited nerve growth factor-driven neurite outgrowth in neuronal PC12 cells that was associated with the downregulation of MRLC by Mylip-induced protein ubiquitination [18, 20]. In line with this, Mylip is expressed in neurons in hippocampus and in cerebellum during brain development [21]. Mylip is also abundantly expressed in almost all human tissues, suggesting additional functions and targets for this protein. Biological substrates for Mylip have subsequently been found and include the saposin-like protein, MSAP, which affects neurite outgrowth and glioma cell motility [22, 23]. Mylip is further involved in platelet-derived growth factor (PDGF) signaling in fibroblasts via a rapid increase in the expression of Mylip and the association of Mylip-mRNA with the heteronuclear RNA binding protein-K (hnRNP-K) in these cells [24].

Mylip/Idol in the regulation of LDL-R and LDL uptake

Zelcer et al. have now extended these studies by showing that LDL-R is a target for protein ubiquitination by Mylip. Data showed that LXR agonists induced Mylip in macrophages and liver, which led to ubiquitination of the LDL-R and enhanced receptor degradation, probably via the lysosomes [1]. In view of this, Mylip was alternatively named inducible degrader of the LDL-R (Idol) and in the following, the name Mylip/Idol is used. Specifically Mylip/Idol targeted LDL-R and not other transporters involved in cholesterol metabolism. Overexpression of Mylip/Idol by adenoviral vectors reduced LDL-R abundance and the uptake of LDL into cells increasing plasma cholesterol in infected mice [1]. Downregulation of endogenous Mylip/Idol by RNA silencing increased LDL-R levels and enhanced LDL uptake showing that this is a physiological mechanism to regulate the LDL-R. The relevance of the LXR-Mylip/Idol pathway was further substantiated in mice treated with LXR agonists that reduced LDL-R levels in a tissue-specific manner depending on the degree of Mylip/Idol induction [1].

Future Perspectives

This study is important in showing a novel mechanism for regulation of LDL-R by protein ubiquitination to control

plasma LDL uptake. The LXR-Mylip/Idol-LDL-R pathway may be amenable for interventions and serve as novel target for drug development in disease. As Mylip/Idol is a rather unstable protein [1, 24], this could be done by targeting its mRNA expression levels or protein stability in a screen for small molecular compounds. The work also raises new questions related to the role of Mylip/Idol in LDL-R metabolism and in cell physiology. Thus, it would be important to know how the ubiquitination of LDL-R by Mylip/Idol is linked to the regulation of this receptor by the PCSK9 system and whether these two act in an additive or even synergistic manner in decreasing LDL-R? Further, does the binding to PCSK9 make the LDL-R a more favorable substrate for protein ubiquitination by Mylip/Idol? Where precisely in the cell does the ubiquitination takes place and is the LDL-R poly- or monoubiquitinated, as the latter would affect receptor trafficking and internalization [25]? Finally, are there additional proteins involved in the Mylip/Idol–LDL-R interaction? Binding proteins for Mylip/Idol with antagonistic activities have been identified [22, 24], and these may play a role also in LDL-R metabolism. Along this line, MSAP was recently shown to bind human hepatic lipase that affects lipoprotein and LDL metabolism and is involved in atherosclerosis [26]. Given the involvement of Mylip/Idol in PDGF signaling, it would be important to study the role of this growth factor in LDL-R regulation in different cells. Elevated PDGF has been associated with increased cell fibrosis and thickening of cell layers in the vessel wall that links it to the development of atherosclerosis [27]. PDGF receptor interacts with the LDL-R-related protein [28], and this protein is known to modulate PDGF action and counteract atherosclerosis [27]. It would be interesting to know whether PDGF influences LDL-R and cholesterol metabolism through upregulation of Mylip/Idol or related factors in various diseases. Clinically, in atherosclerosis and lipid disorders, the levels and subtypes of HDL and of total lipids are also important as are tissue inflammation reactions that all have to be taken into account in such studies.

With the work on LDL-R degradation [1], the ubiquitin ligase Mylip/Idol has clearly entered the field of molecular medicine and lipid metabolism. Elevated blood LDL-cholesterol is a severe health problem in the Western world, and current drug therapies are not optimal. In view of the importance of LDL-R for regulation of blood cholesterol, we may in the near future expect more exciting news on the regulation of this receptor at the molecular level in various diseases. The present work also encourages further studies on the normal physiology of the Mylip/Idol in different cells and its role in ubiquitination of possible other membrane receptors.

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