Cholesterol Worships a New Idol

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The growing worldwide epidemic of cardiovascular disease suggests that new therapeutic strategies are needed to complement statins in the lowering of cholesterol levels. In a recent paper in Science, Tontonoz and colleagues have identified Idol as a protein that can control cholesterol levels by regulating the stability of the low-density lipoprotein receptor; inhibiting the activity of Idol could provide novel approaches for the treatment of cardiovascular disease.

Cholesterol plays important roles in diverse biological systems including the control of membrane fluidity, the formation of caveolae and developmental signaling (Brown and Goldstein, 1997). Like many things in life, however, too much cholesterol can be harmful. Elevated levels of cholesterol in the blood drive the development of atherosclerosis and too much cholesterol in the gallbladder contributes to the formation of gallstones. Not surprisingly cholesterol levels are tightly regulated and reflect a delicate balance between dietary uptake, endogenous synthesis and removal from the body (Brown and Goldstein, 1997). In spite of these exquisite regulatory mechanisms, diets rich in cholesterol and increasingly sedentary life styles have begun to overwhelm the ability to control cholesterol levels in populations around the world. The American Heart Association estimates that over half of the adults in the USA have blood cholesterol levels that put them at risk for developing cardiovascular disease. In China, recent studies put this number at close to 25% (He et al., 2004).

The ground-breaking work of Brown and Goldstein defined the pathway by which cholesterol functions as a negative regulator to control its own uptake and synthesis (Brown and Goldstein, 1997). The genes encoding the enzymes required for the biosynthesis of cholesterol as well as the gene encoding the low-density lipoprotein receptor (LDLR), which mediates the uptake of cholesterol, are coordinately regulated at the level of transcription with the sterol response element binding proteins (SREBPs). The three SREBPs (SREBP1a, SREBP1c and SREBP2) are synthesized as inactive precursor proteins that are proteolytically processed into mature forms that translocate to the nucleus and activate gene expression. When intracellular cholesterol levels are high, the proteolytic processing of SREBPs is blocked, gene expression is down-regulated and consequently, the synthesis and uptake of additional cholesterol are reduced. Alongside these transcriptional control mechanisms, the stability and trafficking of LDLRs have been shown to be modulated by the LDLR adaptor protein 1 (LDLRAP/ARH) (Stolt and Bock, 2006) and by the proprotein convertase subtilisin/kexin 9 (PCSK9) (Costet et al., 2008).

The other side of the cholesterol equation, removal, is also regulated at the level of transcription by the liver X receptors (LXRs). LXRα (NR1H3) and LXRβ (NR1H2) are members of the steroid and thyroid hormone receptor superfamily of ligand-activated transcription factors that regulate gene expression in response to the direct binding of hormones and other lipophilic small molecules. LXRα was originally cloned from a liver cDNA library and found to be highly expressed in the liver, kidney, intestine and macrophages. In contrast, LXRβ is more ubiquitously expressed. The first link between LXRs and lipid metabolism came from the identification of cholesterol derivatives including 22(R)-hydroxycholesterol, 24(S)-hydroxycholesterol and 24(S),25-epoxycholesterol as ligands that directly bind to both LXRα and LXRβ and increase their transcriptional activity by promoting the release of trans-acting corepressors and interaction with trans-acting coactivators. Several synthetic LXR agonists have also been described (Tontonoz and Mangelsdorf, 2003).

Gene expression analysis of mice treated with LXR agonists identified the ATP binding cassette transporter ABCA1 as a direct LXR target gene (Tontonoz and Mangelsdorf, 2003). ABCA1 is required for the process of reverse cholesterol transport (RCT) whereby cells efflux internal cholesterol to acceptor proteins to form nascent high-density lipoprotein (HDL) particles. Loss of functional ABCA1 results in Tangier disease, a condition in which patients have extremely low levels of circulating HDL and an increased risk for developing atherosclerosis (Tall et al., 2008). Treatment of cells with LXR agonists results in induction of the ABCA1 gene, increased levels of ABCA1 protein and an increase in cholesterol efflux. Importantly, binding sites for LXR have been identified in the promoter of the ABCA1 gene and in the control regions of genes encoding other proteins involved in RCT including ABCG1 and apolipoprotein E (Tontonoz and Mangelsdorf, 2003). Thus activation of LXR promotes a mobilization of cellular cholesterol from peripheral cells to HDL (the good cholesterol) for transport to the liver.

In the liver, LXRs control the expression of two additional ATP binding cassette
transports, ABCG5 and ABCG8, which excrete cholesterol out of the liver and into the intestine (Tontonoz and Mangelsdorf, 2003). In mice Cyp7a1, which encodes cholesterol 7α hydroxylase, the rate-limiting enzyme in the conversion of cholesterol to bile acids is also a LXR target (Tontonoz and Mangelsdorf, 2003).

Importantly, high levels of intracellular cholesterol lead to an increase in the amount of endogenous cholesterol-derived LXR agonists. Linking LXR activity to intracellular cholesterol levels allows the LXRs to coordinately couple cholesterol efflux from peripheral cells with excretion in the liver leading to a net loss of cholesterol from the body. The importance of the LXRs lies in the observation that LXR ligands reduce atherosclerosis in mouse models of cardiovascular disease (Levin et al., 2005; Bischoff et al., 2009).

A recent study in Science has now brought the two sides of cholesterol equation together (Zelcer et al., 2009). Zelcer et al. reasoned that if activation of LXR promotes the removal of cholesterol, it might make sense to simultaneously limit the uptake of cholesterol when LXRs are active. Indeed, the authors demonstrate that treatment of cells with LXR agonists reduces cholesterol uptake by decreasing the protein levels of LDLR. Conversely, knockdown of LXRs increases cholesterol uptake and leads to elevated LDL levels. Interestingly, no change in LDLR mRNA levels was observed, indicating that LXRs influence LDLR levels indirectly. Using gene expression profiling to uncover novel LXR target genes, the authors chose to focus on a protein they named Inducible Degradator of the LDLR (Idol). Examination of the Idol primary sequence identified a band 4.1/Ezrin/Radixin/Moesin (FERM) domain that has been shown to mediate interactions with the cytoplasmic domains of membrane-spanning proteins (Zelcer et al., 2009). Idol, however, appears to be unique among FERM domain-containing proteins, in that it also contains a RING domain at the C-terminus that has been proposed to function as an E3-ubiquitin ligase. Overexpression of Idol in cell culture models significantly enhances the ubiquitination of LDLR and decreases LDLR levels. This activity of Idol is lost when a mutation predicted to disrupt the E3 ligase activity is introduced into the RING domain. In contrast, shRNA-dependent knockdown of Idol increases LDLR levels and increases cholesterol uptake. The activity of Idol appears to be relatively specific for LDLR as no change in the levels of transfected LDL-related protein 1 (LRP1) or amyloid precursor protein was observed. Nevertheless, future studies will need to thoroughly examine the specificity of Idol in order to better understand the implications of regulating the Idol-LDLR pathway in vivo (see below).

To determine if the LXR-Idol-LDLR pathway functions in vivo, mice were treated with the synthetic LXR agonist GW3965. GW3965 induced Idol expression and reduced LDLR protein levels in a reciprocal pattern. Similarly, Idol levels were decreased and LDLR levels were increased in tissues isolated from LXR knockout mice. Although strong regulation of Idol expression was detected in macrophages and in the intestine, perhaps somewhat unexpectedly activation of LXR had only a modest effect on Idol expression and LDLR levels in the liver. Since the liver is considered the major site of cholesterol regulation in the body, what are we to make of the seemingly failure of LXR to induce Idol at this site? GW3965 has a relatively short half-life and is rapidly cleared from the liver raising the possibility that a more sustained activation of LXR is required to induce Idol. Indeed, Idol mRNA levels are decreased and LDLR protein levels are increased in the livers of LXR knockout mice suggesting that LXRs can control Idol expression levels in hepatocytes. It would be interestingly to see if T0901317, a synthetic LXR agonist that accumulates in the livers of mice to high levels, is a more effective inducer of hepatic Idol expression. Importantly, LXR agonists have been shown to elevate LDL cholesterol levels in non-human primates (Groot et al., 2005) raising the possibility that the LXR-Idol pathway will have important implications for strategies targeting the LXRs for drug development.

To address the function of Idol in the liver, Zelcer et al. bypassed LXR and used adenovirus to overexpress Idol in this tissue. Overexpression of Idol reduced liver LDLR levels and produced a significant increase in plasma LDL cholesterol. Thus, Idol can function in animals to influence plasma cholesterol levels. Taken together, these results suggest that Idol is an important regulator of cholesterol metabolism and raise the interesting possibility that targeting the E3 ligase activity of Idol with small molecule inhibitors could provide a novel strategy for lowering cholesterol in humans.

References