Review

NPC1L1 and cholesterol transport

Jenna L. Betters, Liqing Yu*

Department of Pathology Section on Lipid Sciences, Wake Forest University School of Medicine, Winston-Salem, NC 27157-1040, USA

ABSTRACT

The polytopic transmembrane protein, Niemann–Pick C1-Like 1 (NPC1L1), is enriched in the apical membrane of small intestine absorptive enterocytes where it mediates extracellular sterol transport across the brush border membrane. It is essential for intestinal sterol absorption and is the molecular target of ezetimibe, a potent cholesterol absorption inhibitor that lowers blood cholesterol in humans. NPC1L1 is also highly expressed in human liver. The hepatic function of NPC1L1 may be to limit excessive biliary cholesterol loss. NPC1L1-dependent sterol uptake seems to be a clathrin-mediated endocytic process and is regulated by cellular cholesterol content. Recently, NPC1L1 inhibition has been shown to have beneficial effects on components of the metabolic syndrome, such as obesity, insulin resistance, and fatty liver, in addition to atherosclerosis.

© 2010 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.

1. Introduction

Elevated blood cholesterol levels contribute to atherosclerotic coronary heart disease. Cholesterol homeostasis in the body is mainly balanced by its intestinal absorption, endogenous biosynthesis, and biliary/intestinal excretion. While much is known about cholesterol biosynthesis and its regulation [1,2], the mechanisms for cholesterol absorption and excretion were poorly understood until recently. Early in the 21st century, the heterodimer of two ATP-binding cassette (ABC) transporters G5 and G8 (ABCG5/G8) was demonstrated to be crucial for biliary and perhaps intestinal secretion of cholesterol and non-cholesterol sterols [3–6]. In 2004, Niemann–Pick C1-Like 1 (NPC1L1) was shown to play an essential role in intestinal cholesterol absorption [7]. Discovery of NPC1L1 has greatly enhanced our understanding of whole-body cholesterol metabolism, and specifically intestinal cholesterol absorption, a process that can be blocked by the potent cholesterol absorption inhibitor ezetimibe. A growing body of data is implicating a role for NPC1L1 and NPC1L1-dependent intestinal cholesterol absorption in metabolic diseases such as non-alcoholic fatty liver disease, insulin resistance, diabetes, and obesity in addition to atherosclerotic coronary heart disease. This review will examine the recent studies on the role of NPC1L1 in sterol transport and diseases.

2. NPC1L1 as an intestinal cholesterol transporter

Cholesterol in the intestinal lumen is mainly derived from biliary secretion and dietary intake. Intestinal epithelial sloughing and direct secretion of cholesterol from enterocytes may also partially contribute to the luminal cholesterol pool where cholesterol is solubilized in micelles enriched with bile acids and phospholipids. Intestinal cholesterol absorption is an integrated process, including at least three major steps: (1) solubilization in micelles, (2) transport across the apical membrane of absorptive enterocytes, and (3) mobilization to chylomicrons for secretion into the lymph and blood via the basolateral membrane of enterocytes. In humans, the fractional intestinal cholesterol absorption ranges from 29% to 80% [8]. Despite this large variation, intestinal cholesterol absorption is a major pathway controlling whole-body cholesterol homeostasis, and therefore represents an attractive drug target.

The search for intestinal cholesterol absorption inhibitors led to the development of ezetimibe (commercially known as Zetia) by Schering-Plough Research Institute and Merck Co. (NJ, USA). The drug was approved by the US Food and Drug Administration to treat hypercholesterolemic patients before its molecular target was known. Because ezetimibe inhibition of intestinal cholesterol absorption in animals results in a compensatory upregulation of endogenous cholesterol biosynthesis [9,10], the drug is often used in combination with a statin that blocks cholesterol synthesis by inhibiting the rate-limiting enzyme 3-hydroxy-3-methyl-glutaryl-CoA reductase [11]. Ezetimibe monotherapy or coadministration with a statin efficiently lowers plasma total cholesterol and...
low-density lipoprotein cholesterol (LDL-C) in humans [10]. The mechanism for cholesterol transport across the apical membrane of enterocytes was thought to be passive diffusion before the discovery of ezetimibe [12]. The pharmacological potency of ezetimibe demonstrated that specific proteins rather than passive diffusion are implicated in this process. In the search for molecular targets of ezetimibe, Altman and colleagues at Schering-Plough Research Institute (NJ, USA) identified NPC1L1, an apically-localized sterol transporter in the small intestine, using a genome-wide bioinformatics screening approach [7]. They further showed that genetic deletion of NPC1L1 in mice reduces intestinal cholesterol absorption to the levels seen in ezetimibe-treated mice, and that ezetimibe treatment results in no further reduction of intestinal cholesterol absorption in mice lacking NPC1L1 [7,9]. This study clearly demonstrated that NPC1L1 is in the ezetimibe-sensitive pathway and definitively established the critical role of NPC1L1 in intestinal cholesterol absorption.

3. Conserved domains in NPC1L1 protein

NPC1L1 is a polytopic transmembrane protein of 1332 amino acids. It shares sequence homology with Niemann–Pick C1 (NPC1) [13], a protein that is mutated in the lipid storage disorder Niemann–Pick disease type C1 [14,15] (Fig. 1). Like its homolog, NPC1L1 was predicted to have a typical signal peptide and 13 membrane-spanning domains [7,13,16–18], and this prediction is consistent with recent experimental data [19]. NPC1L1 also has a conserved N-terminal “NPC1” domain, and extensive N-linked glycosylation sites [7,13,17] (Fig. 1). Interestingly, the crystal structure of the cysteine-rich N-terminal domain of NPC1 exposed a sterol-binding pocket [20]. Because the N-terminus of NPC1L1 encompasses a similar cysteine-rich globular domain, it is likely that this region also binds sterols [21].

Five of the 13 membrane-spanning helices of NPC1L1 constitute a predicted sterol sensing domain (SSD) encompassing ~180 amino acids [7,13] (Fig. 1). This SSD is conserved in several other membrane proteins involved in cholesterol transport, metabolism, or regulation [22], including NPC1 [13–15]; 3-hydroxy-3-methylglutaryl CoA reductase, the rate-limiting enzyme in the cholesterol biosynthetic pathway [1,23]; sterol regulatory element-binding protein (SREBP)-cleavage activating protein (SCAP), a protein that controls the transport and proteolytic activation of SREBPs, which are membrane-bound transcription factors governing the synthesis of cholesterol and other lipids [1,2,23]; and Patched, a membrane receptor for the cholesterol-linked signaling peptide Hedgehog [24]. The functional significance of the SSD in these proteins remains elusive.

Biochemical evidence suggests that the SSD may be involved in sterol binding. For example, cholesterol was shown to directly bind to the purified SSD-containing membrane region of SCAP through receptor–ligand interaction; thus, SCAP was defined as a receptor for free cholesterol in the endoplasmic reticulum [25]. The requirement of a functional SSD for NPC1 binding to a photo-activatable cholesterol analog was reported years ago [26], although a sterol-binding pocket was recently localized to its cysteine-rich N-terminal domain [20].

Fig. 1. Amino acid sequence and predicted topological structure of human Niemann–Pick C1-Like 1 (NPC1L1). The conserved N-terminal “NPC1” domain is shown with red residues. Two potential YXXØ endocytic motifs are outlined in blue squares. Residues in dark circles denote the sterol-sensing domain. The luminal portion of NPC1L1 also has extensive N-linked glycosylation sites which are highlighted in green. The N-terminal 21 amino acids are assumed to be the signal peptide and are not shown in this figure.
The subcellular localization of SSD-containing proteins is often regulated by cellular cholesterol content [22,27]. The SSD may regulate sterol-dependent trafficking of these proteins. In animals, NPC1L1 mainly resides at the apical membrane of enterocytes and hepatocytes [7,28–31], a membrane region that is exposed to unesterified free cholesterol. In cultured hepatoma cells, we showed that NPC1L1 can localize to both the plasma membrane and endocytic recycling compartment (ERC), mirroring the distribution of free cholesterol in the cell, and its subcellular location is regulated by cellular cholesterol availability [29]. Under normal culture conditions, NPC1L1 is enriched in the ERC, which has been shown to be a cholesterol-rich region of cells [32]. When cholesterol is depleted in cultured cells with cyclodextran, NPC1L1 is translocated to the apical subdomain of the plasma membrane from the ERC. Conversely, when cholesterol is replenished, NPC1L1 is internalized, which is coupled with cellular cholesterol uptake [29]. These findings are further supported by subsequent cell biology studies using the same cell line [33]. Future studies are required to examine the role of NPC1L1’s SSD in the sterol-regulated trafficking of NPC1L1.

4. Molecular mechanisms for NPC1L1-dependent cholesterol uptake

Several pieces of evidence suggest that the molecular mechanism for NPC1L1-dependent cholesterol uptake may be cholesterol-regulated clathrin-mediated endocytosis [29,33–35]. First, NPC1L1 protein cycles between the cell surface and intracellular compartments in a cholesterol-dependent manner [29]. Second, this cholesterol-regulated NPC1L1 translocation is coupled to cholesterol uptake [29], which can be blocked by potassium depletion [34], a treatment that inhibits clathrin-mediated endocytosis [36]. Third, caveolin-1, a structural protein of caveolae, is dispensable for intestinal cholesterol absorption [37], demonstrating that caveolae-mediated endocytosis is not the cellular basis for NPC1L1-dependent cholesterol uptake. Fourth, NPC1L1 co-immunoprecipitates with the μ2 subunit of the adaptor protein (AP) complex AP2, and with clathrin heavy chain [33], two proteins necessary for clathrin-dependent endocytosis.

A cytosolic tyrosine-based sorting signal YXXØ (tetrapeptide) has been shown to facilitate the clathrin-mediated endocytosis of many plasma membrane proteins via interaction with the μ subunit of AP2 [38]. In YXXØ motif, the tyrosine (Y) residue is functionally indispensable. The Ø represents a residue with a bulky hydrophobic side chain. The X residues vary highly. The endocytic YXXØ signals are most often 10–40 residues away from the membrane-spanning domains, but not at the carboxy-termini of proteins [38]. Intriguingly, NPC1L1 has two potential YXXØ motifs (Y721QRL and Y836APF) [21] (Figs. 1 and 2). The Y721QRL sequence is within the SSD region and the two motifs are conserved in NPC1L1 proteins from drosophila to mammals, implying their functional significance. It is tempting to speculate that one of these motifs regulates NPC1L1-dependent cholesterol uptake through interaction with proteins of the clathrin-mediated endocytic pathway in a cholesterol-dependent manner (Fig. 2). In this case, NPC1L1 appears to function as a free cholesterol receptor in the

Fig. 2. Proposed model of NPC1L1-dependent cholesterol uptake. NPC1L1 is enriched at the plasma membrane when cellular cholesterol levels are low. Extracellular cholesterol is recruited to the NPC1L1-containing plasma membrane microdomain by binding to NPC1L1 or other mechanisms. When cholesterol content increases to a threshold in the microdomain, it is sensed by NPC1L1’s SSD, which then triggers NPC1L1 protein conformational changes and subsequent internalization of the NPC1L1/cholesterol-containing membrane microdomain via clathrin-mediated endocytosis. Two potential YXXØ motifs known to facilitate the clathrin-mediated endocytosis of many plasma membrane proteins via interaction with the μ subunit of AP2 are highlighted in blue squares. NPC1L1 and its sterol cargo are dissociated in the sorting endosome and/or the endocytic recycling compartment, freeing NPC1L1 to be recycled back to the plasma membrane to take up additional cholesterol, particularly during cholesterol deprivation. Ezetimibe interacts with the second extracellular loop of NPC1L1, causing conformational changes of NPC1L1 protein, thereby inhibiting cholesterol uptake.
plasma membrane, resembling SCAP which is a receptor for free cholesterol in the endoplasmic reticulum [25].

How does NPC1L1 efficiently transport cholesterol into a cell? One plausible possibility is that NPC1L1 recruits extracellular free cholesterol through its N-terminus to its cell membrane location, which may create a RAFT-like plasma membrane microdomain. The cholesterol content in this microdomain is sensed by NPC1L1’s SSD. When cholesterol is enriched to a threshold in this membrane region, the entire microdomain is endocytosed to facilitate cholesterol uptake.

For intestinal cholesterol absorption, free cholesterol has to be sorted to the endoplasmic reticulum for incorporation into cholesterol ester, which will then be packaged into chylomicrons. How is NPC1L1-derived free cholesterol sorted to the endoplasmic reticulum? Is it sorted out directly from the early endosome or from the endocytic recycling compartment? What will carry it to its destination? Answers to these questions would greatly enhance the molecular understanding of intestinal cholesterol absorption. Based on cell culture studies [29,33–35], NPC1L1 sorted to the endocytic recycling compartment can be recycled back to the cell surface, a process that appears to involve an endocytic recycling triple complex consisting of the microfilament-interacting motor myosin Vb, the small GTPase Rab11a, and the adaptor Rab11-FIP2 [39]. Thus, it is likely that free cholesterol is dissociated from NPC1L1 in the endocytic recycling compartment or earlier endocytic stages.

5. Substrate specificity of NPC1L1

Evidence indicates that NPC1L1 can absorb both animal and plant sterols because both sterol classes are dramatically reduced in NPC1L1 knockout mice [7,9,40,41] and in ezetimibe-treated sitosterolemia patients [42] and mice [43]. But NPC1L1 does not seem to absorb all sterols equally. Uptake of sitosterol versus cholesterol from various donors is significantly lower in cells over-expressing NPC1L1 [33,34,44], suggesting that NPC1L1 has a greater affinity for cholesterol than sitosterol, a major plant-derived sterol in the diet. It was thought that the heterodimer ABCG5/G8 was the major discriminator of animal-derived sterols from plant-derived phytosterols because mutations in these genes cause a genetic disorder, sitosterolemia that is characterized by massive accumulation of phytosterols in the body [3,4]. However, the rank order of intestinal sterol absorption rates (cholesterol > cholesteryl ester, a process that appears to involve an endocytic recycling triple complex consisting of the microfilament-interacting motor myosin Vb, the small GTPase Rab11a, and the adaptor Rab11-FIP2 [39]. Thus, it is likely that free cholesterol is dissociated from NPC1L1 in the endocytic recycling compartment or earlier endocytic stages.

5. Substrate specificity of NPC1L1

Evidence indicates that NPC1L1 can absorb both animal and plant sterols because both sterol classes are dramatically reduced in NPC1L1 knockout mice [7,9,40,41] and in ezetimibe-treated sitosterolemia patients [42] and mice [43]. But NPC1L1 does not seem to absorb all sterols equally. Uptake of sitosterol versus cholesterol from various donors is significantly lower in cells over-expressing NPC1L1 [33,34,44], suggesting that NPC1L1 has a greater affinity for cholesterol than sitosterol, a major plant-derived sterol in the diet. It was thought that the heterodimer ABCG5/G8 was the major discriminator of animal-derived sterols from plant-derived phytosterols because mutations in these genes cause a genetic disorder, sitosterolemia that is characterized by massive accumulation of phytosterols in the body [3,4]. However, the rank order of intestinal sterol absorption rates (cholesterol > cholesteryl ester, a process that appears to involve an endocytic recycling triple complex consisting of the microfilament-interacting motor myosin Vb, the small GTPase Rab11a, and the adaptor Rab11-FIP2 [39]. Thus, it is likely that free cholesterol is dissociated from NPC1L1 in the endocytic recycling compartment or earlier endocytic stages.

5. Substrate specificity of NPC1L1

Evidence indicates that NPC1L1 can absorb both animal and plant sterols because both sterol classes are dramatically reduced in NPC1L1 knockout mice [7,9,40,41] and in ezetimibe-treated sitosterolemia patients [42] and mice [43]. But NPC1L1 does not seem to absorb all sterols equally. Uptake of sitosterol versus cholesterol from various donors is significantly lower in cells over-expressing NPC1L1 [33,34,44], suggesting that NPC1L1 has a greater affinity for cholesterol than sitosterol, a major plant-derived sterol in the diet. It was thought that the heterodimer ABCG5/G8 was the major discriminator of animal-derived sterols from plant-derived phytosterols because mutations in these genes cause a genetic disorder, sitosterolemia that is characterized by massive accumulation of phytosterols in the body [3,4]. However, the rank order of intestinal sterol absorption rates (cholesterol > cholesteryl ester, a process that appears to involve an endocytic recycling triple complex consisting of the microfilament-interacting motor myosin Vb, the small GTPase Rab11a, and the adaptor Rab11-FIP2 [39]. Thus, it is likely that free cholesterol is dissociated from NPC1L1 in the endocytic recycling compartment or earlier endocytic stages.
the two species. Bile acids in human bile are more hydrophobic compared to those from rodents. Hydrophobic bile acids are cytotoxic and can cause liver injury and cholestasis. Membrane cholesterol content could be an important factor that renders the canalicular membrane resistant to the cytotoxic effects of concentrated bile acids [81]. Thus, canalicular NPC1L1 protein, by retrieving free cholesterol in the canalicular bile back to the canalicular membrane may protect hepatocytes against cytotoxicity induced by hydrophobic bile acids concentrated in human bile.

8. Hepatic NPC1L1 as a target of ezetimibe: good or bad?

When liver-specific NPC1L1 transgenic mice are treated with ezetimibe, biliary cholesterol excretion is restored [30]. This finding demonstrated that hepatic NPC1L1 is a target of ezetimibe in mice in addition to intestinal NPC1L1. This action of ezetimibe may permit more biliary cholesterol to be excreted into bile, thus promoting the final step in the reverse cholestery transport pathway. But excess cholesterol build-up in the bile may negatively impact health by leading to gallstone formation. An increase in gallstone disease, however, was not observed in humans treated with ezetimibe. Conversely, ezetimibe has been shown to prevent cholesterol gallstone formation in mice [82], hamsters [83], and a few human subjects [84]. Inter-individual variation exists in hepatic [30] and perhaps intestinal NPC1L1 expression levels. The relative expression level of NPC1L1 in intestine and liver, and differences in the ezetimibe efficiency in inhibiting intestinal cholesterol absorption may ultimately determine whether ezetimibe increases or decreases biliary cholesterol. In individuals with lower expression of NPC1L1 in liver relative to intestine, if ezetimibe efficiently inhibits intestinal cholesterol absorption, it may actually reduce the amount of cholesterol transported to the liver for hepatobiliary secretion.

9. NPC1L1 and diseases

Cholesterol is an important biological component of cell membranes, and is a precursor for bile acid and steroid hormone biosynthesis. However, high levels of blood cholesterol are associated with atherosclerotic coronary heart disease. By lowering blood cholesterol levels, NPC1L1 inhibition should have beneficial effects on this disease. Indeed, ezetimibe treatment reduces cholesterol absorption, lowers plasma cholesterol, and inhibits the development and progression of atherosclerosis in apolipoprotein (apo) E knockout mice [85]. Likewise, NPC1L1/apoE double knockout mice have a significant reduction in cholesterol absorption and plasma cholesterol levels, and almost complete protection from the development of atherosclerosis [86]. In humans, ezetimibe monotherapy or coadministration with a statin significantly reduces blood LDL-C in primary hypercholesterolemic subjects [10]. A large clinical trial, IMPROVE-IT (Improved Reduction of Outcomes: Vytorin Efficacy International Trial), is underway to determine whether this reduction is translated to prevention of atherosclerosis and cardiovascular events [10]. An earlier clinical trial ENHANCE (Ezetimibe and Simvastatin in Hypercholesterolemia Enhances Atherosclerosis Regression) failed to show benefit of Vytorin (ezetimibe plus simvastatin) over simvastatin in improving the carotid artery intima-media thickness (IMT), though the combined therapy versus statin alone resulted in more reduction in plasma LDL-C, in 720 heterozygous familial hypercholesterolemic patients who had been treated with a statin prior to enrollment in the trial [87]. But in this trial, patient selection and methodology may have confounded the outcomes. The IMPROVE-IT trial should provide a definitive answer to whether the combined therapy improves cardiovascular events.

Unexpectedly, ezetimibe treatment or NPC1L1 deficiency was recently shown to improve many metabolic disorders besides hypercholesterolemia in rodents, although similar effects have yet to be examined in humans. For instance, ezetimibe treatment improved hepatic steatosis and insulin sensitivity in leptin receptor-deficient Zucker obese rats [88,89]. Ezetimibe also reduced hepatic steatosis in mice on the methionine choline-deficient diet [90] or diets containing high amounts of cholesterol [91,92]. In a study focusing on obesity and diabetes, ezetimibe treatment or NPC1L1 deficiency was shown to attenuate weight gain and insulin resistance in mice fed a high fat diet, though it remains to be
NPC1L1 protein possesses multiple conserved domains, including a SSD that is found in many other proteins involved in cholesterol metabolism and regulation. NPC1L1 mediates intestinal cholesterol absorption and it may also limit hepatobiliary cholesterol secretion and reduces fractional absorption of dietary cholesterol. J. Clin. Invest. 110, 671–680.

10. Conclusions

Determined whether these interventions influence hepatic steatosis [50]. In another study focusing on the role of NPC1L1 in protein trafficking and diet-induced hypercholesterolemia, NPC1L1 knock-out mice were shown to be resistant to hepatic steatosis induced by the Paigen diet, a lithogenic diet that contains high amounts of bile acids plus cholesterol and fat [40]. Despite these interesting observations, the mechanism by which NPC1L1 deficiency or ezetimibe treatment alleviates hepatic steatosis, insulin resistance, and obesity remain largely unexplored. Ezetimibe was reported to decrease reactive oxygen species production, c-Jun N-terminal kinase (JNK) activation, and endoplasmic reticulum stress in the livers of Zucker obese fatty rats, as well as hepatocytes in vitro [89], but it is unclear if this was a result of, or the mechanisms for, reduced hepatic accumulation of cholesterol and fat in ezetimibe-treated animals and hepatocytes. Nonetheless, the aforementioned findings together suggest that NPC1L1 plays an important role in the pathogenesis of metabolic disorders, and its physiological role extends beyond simple facilitation of intestinal cholesterol absorption. Inhibition of NPC1L1 or NPC1L1-dependent intestinal cholesterol absorption could be a potential preventative and therapeutice approach for metabolic diseases such as non-alcoholic fatty liver disease, insulin resistance, type 2 diabetes, and central obesity.

Acknowledgements

Dr. Jenna L. Betters is supported by a Ruth L. Kirschstein National Research Service Award (NRSA) (#1F32DK084582-01) provided by the National Institute of Diabetes and Digestive and Kidney Diseases. Dr. Liqing Yu is supported by a Scientist Development Grant (#0635261N) from the American Heart Association, and by intramural funds from Wake Forest University Health Sciences.

Reference


