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Review Article

Review Article

Potential function of hepatic Niemann–Pick C1-like 1: cholesterol homeostasis regulation of the canalicular lipid bilayer membrane

Hongtan Chen[1], Pingfan Mo[1] and Guoqiang Xu[1,*]

[1]Department of Gastroenterology, The First Affiliated Hospital of Zhejiang University, Hangzhou, Zhejiang, P. R. China

*Corresponding author. Department of Gastroenterology, The First Affiliated Hospital of Zhejiang University, 79 Qingchun Road, Hangzhou, Zhejiang 310006, P. R. China. Tel: +86-13957121569; Fax: +86-13957121569; Email: 1193065@zju.edu.cn

Abstract

Niemann–Pick C1-like 1 (NPC1L1) is distributed in the human liver and intestine but only slightly expressed in the mouse liver. While it is well established that intestinal NPC1L1 is crucial for the absorption of exogenous cholesterol, the physiological and pathological roles of canalicular membrane-localized NPC1L1 in human hepatic cholesterol transport remain unclear. In this review, we discussed the potential function of human hepatic NPC1L1 and proposed that the disparity in NPC1L1 abundance between humans and mice in the liver may be attributable to their distinct bile hydrophobicity. Human hepatic NPC1L1 might interact with other proteins in the canalicular membrane, regulate membrane cholesterol homeostasis, and contribute to the stability of the canalicular lipid bilayer membrane in response to the greater detergent properties of human bile salts. We hoped to provide novel perspectives on hepatic NPC1L1 for future investigations.

Keywords: NPC1L1; liver; cholesterol; caveolin-1; canalicular membrane

Introduction

The Niemann–Pick C1-like 1 (NPC1L1) is primarily expressed in the small intestine in mice and plays a crucial role in the absorption of dietary cholesterol [1]. Unlike in mice, NPC1L1 is expressed in both human liver and small intestine [2]. The current study suggests that hepatic NPC1L1 in humans plays an important role in the transport of cholesterol from bile to the liver [3], which indicates that hepatic NPC1L1 may potentially regulate the cholesterol homeostasis of the hepatobiliary system. However, some studies have found that the expression of hepatic NPC1L1 was inhibited when mice were fed with lithogenic diet, as the latter would lead to changes in the bile acid environment of mice, making it closer to the human bile acid environment [4–6]. Therefore, there are still controversies regarding the physiological and pathological role played by hepatic NPC1L1 in the human body.

We reviewed previous studies of hepatic NPC1L1 and other apical plasma membrane (PM)-localized proteins, as well as the differences in biliary environment between humans and mice, aimed to hypothesize the possible pathophysiological processes and dynamic regulation of hepatic NPC1L1 in humans.

Summary of NPC1L1

The protein structure of NPC1L1

NPC1L1 belongs to the resistance-nodulation-division protein family and possesses a complex structure characterized by multiple extracellular domains and transmembrane domains. In the extracellular domain of NPC1L1, the N-terminal domain contains a cholesterol-binding cavity, whereas the middle luminal domain harbors a binding site for ezetimibe (EZE), which can inhibit the function of NPC1L1 and thereby reduce the absorption of cholesterol from diet [7, 8]. The transmembrane domain consists of 13 transmembrane helices, among which the sterol-sensing domain (SSD) is thought to be connected to the N-terminal domain by an internal tunnel, which is the mode of transport for cholesterol from the N-terminal domain to the PM adjacent to the SSD, providing a pathway for captured cholesterol to enter the lipid bilayer [9, 10].

NPC1L1 expression

The tissue expression profile of NPC1L1 differs markedly between human and rodent models [2]. In humans, NPC1L1 protein levels are prominent in the small intestine and liver; however, NPC1L1 is predominantly confined to the small intestine in rodents, with minimal to no detectable expression observed in the liver. This divergent tissue distribution of NPC1L1 represents an important species difference that must be considered when extrapolating findings from preclinical rodent research to the human pathophysiological context, particularly for mechanisms related to liver cholesterol transport and regulation. Further exploration of how these alternate NPC1L1 expressions impact cholesterol homeostasis between animal models and humans remains an area for future investigation.

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NPC1L1 expression regulation

Bile salt (BS) in humans is more hydrophobic than those in rodents [11, 12] and can inhibit the function of hepatic NPC1L1 [4–6]. In contrast to that in mice and rats, the hydrophobicity of BS in rabbits is closer to that in humans [13, 14]. Therefore, utilizing a genetically engineered rabbit model with intrinsic hepatic-specific NPC1L1 expression, which naturally exists in a hydrophilic BS environment, may provide valuable insights into the role of hepatic NPC1L1.

The expression of hepatic NPC1L1 basically comes from the research evidence of the intestinal NPC1L1, which is regulated by various factors, such as cholesterol transporters, nuclear receptors, and biomolecules. Sterol regulatory element binding protein 2 (SREBP2) can bind to the NPC1L1 promoter and enhance NPC1L1 expression, whereas the presence of hepatocyte nuclear factor 4α (HNF4α) can cooperate with SREBP2 and further elevate promoter activity [15–17]. Nuclear receptors, such as peroxisome proliferator-activated receptor alpha (PPARα) and delta (PPARδ), can restrict cholesterol absorption in the gut [18, 19]. Activation of PPARδ, or treatment with the drug fenofibrate, a US Food and Drug Administration-approved PPARα agonist drug, can reduce NPC1L1 expression in the intestine [20, 21]. Polyunsaturated fatty acids (PUFAs) can also downregulate NPC1L1 expression through the SREBP2 and LXR/RXR pathways [22]. Interestingly, the cholic acid induced the degradation of hepatic NPC1L1 through the FGF15/19-FGFR4 pathway, which did not occur in the intestinal NPC1L1 [4, 5]. Furthermore, hepatic NPC1L1 can be degraded via the endoplasmic reticulum-associated degradation pathway, which can be impeded by the inactivation of valosin-containing protein [23].

Distribution of NPC1L1 and interacting proteins

There are dynamic changes in the subcellular distribution of NPC1L1. Previous study has found that NPC1L1 is primarily located in cellular compartments rich in cholesterol and can translocate to the PM when the cholesterol content in the PM is reduced, which can exert its function [24]. In humans, NPC1L1 is widely observed on PMs in contact with external spaces, including the canalicular membrane in the liver and the brush border membrane in the intestine [24, 25].

Hepatocyte PMs are divided into basolateral membranes and canalicular membranes, with the latter forming bile ducts and central lumens [26]. The localization of hepatic NPC1L1 to canalicular membranes is essential for its cholesterol transport function, which relies on transcytosis [27]. A previous study demonstrated the significance of protein interactions involving hepatic NPC1L1, low-density lipoprotein receptor-related protein 6 (LRP6), and flotillin-2 for transcytosis and the subsequent subcellular localization of hepatic NPC1L1 in canalicular membranes [27].

NPC1L1-mediated cholesterol transport

Several studies have revealed that NPC1L1 internalizes cholesterol into intracellular compartments through the clathrin/AP2 complex and microfilament-dependent vesicular endocytosis [28–31]. EZE can block the cholesterol transport tunnel of NPC1L1, and this drug's structure–activity relationships have been reported in detail [31, 32]. Further cryo-electron microscopy (cryo-EM) investigations revealed the mechanism of NPC1L1-mediated cholesterol transport and the EZE binding site. These studies have demonstrated that the cholesterol-binding cavity in the N-terminal domain of NPC1L1 connects with the SSD through a tunnel, providing a pathway for transporting captured cholesterol to the PM lipid bilayer surrounding the SSD structure. EZE, in turn, can obstruct the central area of this tunnel [33, 34]. Thus, hepatic NPC1L1 may potentially increase the abundance of membrane cholesterol after the captured cholesterol is deposited, diffused, and restored to the lipid bilayer, which EZE can disrupt this process.

Hepatic NPC1L1 and the integrity of the canalicular membrane

Hepatic NPC1L1 potentially regulates the integrity of the canalicular membrane

When bile is excreted through the bile ducts, the hydrophobic BS present in the bile canaliculus of humans possesses detergent properties toward the lipid bilayer PM of hepatocytes. Hepatic NPC1L1 mediates the reabsorption of cholesterol into the liver, which in turn may regulate the cholesterol content of hepatocyte PMs. Higher cholesterol content in the canalicular membrane can strengthen the membrane density and reduce the destructive effects of BS detergents [35, 36]. Thus, hepatic NPC1L1 may help to resist the detergent properties of BS. Because of the hydrophilic BS in mice, it is possible that hepatic NPC1L1-mediated cholesterol sequestration may be more significant in humans [37]. Thus, the different detergent properties of BS between mice and humans may lead to the different requirements of membrane cholesterol, which may cause the substantial variation in hepatic NPC1L1 and require further investigation. Moreover, previous studies have demonstrated that hepatic NPC1L1 may undergo ubiquitination and degradation when mice were fed a lithogenic diet that better simulated the hydrophobic BS environment in the human bile canaliculus, which suggested the adjustment of BS to hepatic NPC1L1 [4, 5].

Canalicular efflux transporters help maintain the integrity of the canalicular membrane

ATP-binding cassette (ABC) transporter family members contribute to bile formation and are uniformly located in the hepatocyte apical membrane in the liver, which partially resides in the canalicular membrane [38–40]. The presence of abundant membrane cholesterol, leading to a thick lipid bilayer, is essential for maintaining the function of transporters, such as BSEP (bile-salt export pump), MDR2 (multidrug resistance protein 2), and MRP2 (multidrug resistance-associated protein 2), which transport toxic substances and BS between the endoplasmic reticulum and PM in the liver [41–43]. Moreover, Ismair *et al.* found that the canalicular membrane of polarized hepatocytes contains caveolin-1 (CAV1)-positive BS resistance microdomains enriched in cholesterol, sphingomyelin, and CAV1, which play a crucial role in protecting the PM against the detergent effects of BS and maintaining the integrity of canalicular bile [44]. Guyot and Stieger further discovered the existence of BS-resistant microdomains in canalicular membrane, which may be the same entities of BS-resistant microdomains due to their similar composition [45]. Therefore, hepatic NPC1L1 potentially resists the detergent properties of BS by regulating the cholesterol restoration in the BS-resistant microdomains. Meanwhile, the storage of cholesterol in the PM promotes resistance to detergent-induced cholestasis and liver injury by enhancing the function of canalicular efflux transporters. This process may also potentially be regulated by hepatic NPC1L1 [46, 47]. In fact, the EZE treatment, as the inhibitor of hepatic NPC1L1, has been associated with cholestatic-like liver injury [48, 49].

In contrast to hepatic NPC1L1, ABCG5/8 mediates the cholesterol efflux from hepatocytes to bile, which requires a relatively

loose PM lipid bilayer structure [50, 51]. Thus, ABCG5/8 may not frequently colocalize with other canalicular efflux transporters and hepatic NPC1L1 simultaneously due to their dynamic subcellular distributions and distinct lipid bilayer structure requirements (Figure 1). A possible hypothesis is that the diet-induced high concentration of BS increases the PM location of ABCG5/8 by loosening the canalicular membranes [52–54] and promoting the degradation of hepatic NPC1L1 [4], preventing the excessive absorption and accumulation of dietary cholesterol in hepatocytes and subsequent liver diseases [55].

Hepatic NPC1L1, caveolae, and cavolin-1

Lipid rafts and hepatic NPC1L1

Lipid rafts are highly ordered lipid microdomains within the PM with enriched cholesterol compositions that mediate membrane protein trafficking and regulate membrane fluidity [56]. Caveolae, the subdomains of lipid rafts, are flask-shaped invaginations of the PM enriched in cholesterol and sphingolipids that participate in several important cellular processes, including signal transduction, cholesterol homeostasis, and vesicular trafficking [57, 58]. Intestinal NPC1L1 plays a role in the uptake of cholesterol from the circulation and its sequestration into intestinal caveolae, which is necessary for the formation of caveolae [59, 60].

Association between caveolin-1 and hepatic NPC1L1

The stability of caveolae structures and the expression/stability of the CAV1 protein both appear to be dependent on the cell membrane content of free (unesterified) cholesterol [61]. Previous study has shown that while CAV1 is the principal structural protein of caveolae, it is not essential for the NPC1L1-mediated transport of cholesterol in the intestine [62]. Considering that cholesterol is necessary for the caveolae, it is suggested that hepatic NPC1L1, which is responsible for the uptake of free cholesterol from the bile duct, may play a crucial role in maintaining the structure of caveolae, which is independent of CAV1 itself [63].

There may exist a potential intricate regulatory mechanism between hepatic NPC1L1 and CAV1 proteins without caveolae structure in the canalicular membrane. Previous study demonstrated that CAV1 undergoes constant shuttling between intracellular compartments and the PM [64]. Flotillins are lipid raft scaffolding proteins with ubiquitous and conserved expression characteristics [65]. Flotillin-1-mediated vesicle exocytosis may support the intracellular trafficking of CAV1, while flotillin-2 may facilitate the transport and degradation of CAV1 in the absence of flotillin-1 [66]. In polarized HepG2 cell lines, hepatic NPC1L1 colocalizes with flotillin-2 in the canalicular membrane, which can be blocked by the competitive binding of LDL–LDLR-clathrin complexes with LRP6 [27]. Meanwhile, membrane microdomains containing the CAV1 protein are crucial for maintaining the integrity of the lipid bilayer structure in the hepatocyte PM [67]. Importantly, CAV1 can bind to cholesterol, which helps to decrease the solubilizing effects of BS on the phospholipids in the PM, which in turn promotes the stability of canalicular efflux transporters [68–70]. Given the close cellular localization between NPC1L1 and CAV1 within the cell membrane, it has been hypothesized that hepatic CAV1, independent of its role in caveolae structure, may potentially assist hepatic NPC1L1 in withstanding the detergent-like properties of BS. This functional interaction between NPC1L1 and CAV1 could be an important mechanism by which the liver maintains cholesterol and BS homeostasis in the face of the disruptive effects of hydrophobic BS [13, 14].

In summary, the interplay between NPC1L1 and CAV1 at the level of the hepatocyte PM appears to be crucial for preserving the integrity of the lipid bilayer and supporting the proper functioning of key canalicular transporters. This represents an

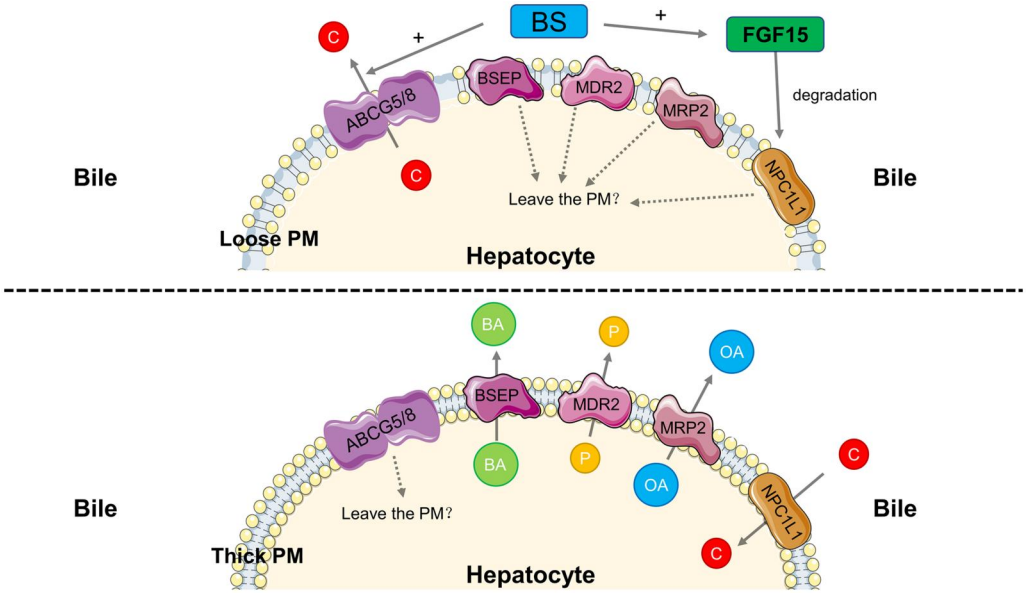

Figure 1. Dynamic subcellular distributions and functions of hepatic NPC1L1 and efflux transporters in canalicular membrane. (Upper panel) Increasing concentration of BS can loosen the lipid bilayer and act as the receptor for ABCG5/8-mediated cholesterol transport and thereby increase the PM location and function of ABCG5/8, as well as promote the degradation of NPC1L1 through the FGF15 pathway, whereas the PM location of BSEP, MDR2 and MRP2 would be decreased due to the insufficient membrane cholesterol. (Lower panel) Decreasing concentration of BS promotes the PM location of NPC1L1, BSEP, MDR2, and MRP2, which recovers the membrane cholesterol contents and thickens the lipid bilayer. PM = plasma membrane, C = cholesterol, BS = bile salt, P = phospholipid, OA = organic anion, NPC1L1 = Niemann–Pick C1-like 1, ABCG5/8 = cholesterol efflux pump, ATP-binding cassette, subfamily G, member 5/8, FGF15 = fibroblast growth factor 15, BSEP = bile-salt export pump, MDR2 = multidrug resistance protein 2, MRP2 = multidrug resistance-associated protein 2.

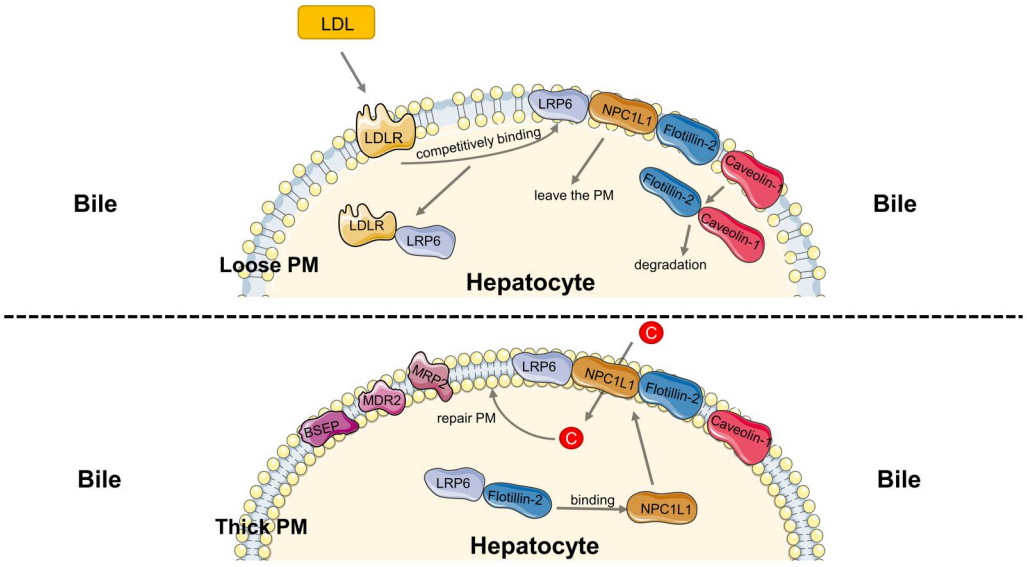

Figure 2. Dynamic subcellular distributions of hepatic NPC1L1 and caveolin-1 in canalicular membrane. (Upper panel) Increasing concentration of plasma LDL can form the LDL-LDLR-clathrin complex, which subsequently competitively bind with LRP6 and promote the transport of NPC1L1, flotillin-2, and caveolin-1 protein from PM to cytoplasm, and finally inhibits the function of NPC1L1. (Lower panel) Free LRP6 protein forms the LRP6-flotillin-2-NPC1L1 complex, which maintains the PM location of NPC1L1 and caveolin-1 and thereby recovers the membrane cholesterol contents and PM location of BSEP, MDR2, and MRP2. NPC1L1 = Niemann–Pick C1-like 1, LDL = low-density lipoprotein, LDLR = low-density lipoprotein receptor, PM = plasma membrane, C = cholesterol, LRP6 = low-density lipoprotein receptor-related protein 6, BSEP = bile-salt export pump, MDR2 = multidrug resistance protein 2, MRP2 = multidrug resistance-associated protein 2.

important area of investigation in understanding the complex regulatory networks governing cholesterol and BS metabolism in the liver.

Rhythmicity of hepatic NPC1L1

Hydrophobic BS is known to disrupt the function of NPC1L1 located on the canalicular membrane of hepatocytes [4–6]. Meanwhile, previous study has reported that when the liver increases its uptake of LDL-cholesterol from the peripheral circulation, the NPC1L1 protein on the canalicular membrane can be mediated by the flotillin-2 protein to translocate into the cytoplasm and undergo degradation [66]. Based on these observations, we hypothesized that this mechanism may represent a protective strategy employed by hepatocytes to avoid the excessive accumulation of cholesterol. By dynamically regulating the localization and stability of NPC1L1 in response to changes in cholesterol availability, the liver can maintain tight control over its cholesterol and bile acid homeostasis, preventing the potentially harmful effects of cholesterol overload. This proposed regulatory interplay between NPC1L1, flotillin-2, and cholesterol uptake pathways in the liver provides an intriguing potential mechanism by which hepatocytes can fine-tune their cholesterol-handling capacity. Further investigation into this hypothesis could yield important insights into the complex mechanisms governing liver cholesterol metabolism and provide new avenues for therapeutic intervention in cholesterol-related liver diseases (Figure 2).

NPC1L1 and liver disease

Previous studies have demonstrated that EZE can reduce serum alanine aminotransferase levels and hepatic cholesterol and improve lipid deposition and liver fibrosis in obese rats. Moreover, knockdown of the NPC1L1 gene prevented high-fat diet-induced nonalcoholic fatty liver disease/nonalcoholic steatohepatitis (NAFLD/NASH) in mice [71–74]. Consistent with these findings, clinical studies have revealed that EZE can reduce liver damage and attenuate hepatic steatosis in patients with NAFLD [75, 76]. Meanwhile, researchers have demonstrated using genetically engineered mice expressing hepatic NPC1L1 and found that hepatic NPC1L1 can increase NAFLD risk by promoting diet-induced liver steatosis and reabsorption of specific biliary oxysterols [77–79].

Conclusions

Most studies on NPC1L1 function are conducted in mouse models that do not express hepatic NPC1L1. Compared with mice, the hydrophobic BS in humans suggests that hepatic NPC1L1 may participate in resistance to hepatocyte PM destruction caused by detergent properties of human bile. Investigating whether CAV1 protein without a caveolae structure can interact with hepatic NPC1L1 would be interesting. Additionally, further research is needed to fully understand the mechanisms by which hepatic NPC1L1 regulates the function and stability of canalicular efflux transporters in the liver, thereby maintaining the integrity of canalicular membranes. Advanced technologies, such as liver organoids, may provide a better understanding of these mechanisms and their implications for developing therapies for liver diseases.

Authors' Contributions

G.X. conceived and designed the project. P.M. and H.C. drafted the manuscript. All authors read and approved the final version of the manuscript.

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Conflicts of Interest

The authors declare no competing interests.

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