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Mini-Review

NPC1L1 Plays a Novel Role in Nonalcoholic Fatty Liver Disease

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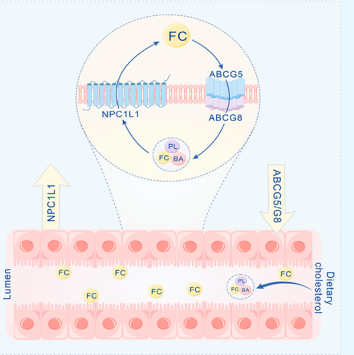
ABSTRACT: Niemann-Pick C1-Like 1 (NPC1L1) is a key protein in the transport of cholesterol, which exists in the brush marginal membrane of the intestinal epithelial cells and the timid duct membrane of the liver. It affects cholesterol absorption and plasma low-density lipoprotein levels. Cholesterol is both an important component of the cell membrane and a precursor of bile acid and steroid hormone synthesis. Abnormal cholesterol metabolism is closely related to nonalcoholic steatohepatitis (NASH). NASH can progress to fibrosis and cirrhosis, with serious consequences. NPC1L1 is involved in the regulation of cholesterol and lipid metabolism and plays an important role in maintaining the balance of cholesterol metabolism in the body. It also plays an important role in some metabolic diseases such as nonalcoholic fatty liver disease, obesity, and hypercholesterolemia. Therefore, it is necessary to elucidate the molecular pathological mechanism of NPC1L1 in the regulation of cholesterol metabolism and the occurrence and development of NASH, which can provide a target for the development of novel drugs for the treatment of NASH and other diseases. More importantly, it helps to accelerate the development of drugs that regulate lipid metabolism at multiple levels and reduce liver steatosis, which is extremely important for the prevention and treatment of NASH and related severe metabolic diseases.



INTRODUCTION

With the improvement of living standards and the change of diet structure and lifestyle, the incidence of nonalcoholic steatohepatitis (NASH) caused by obesity, hyperlipidemia, and diabetes is gradually increasing.[1,2] Some NASH patients even develop liver fibrosis and cirrhosis, which can lead to serious consequences. NASH is a key stage in the disease spectrum of nonalcoholic fatty liver disease (NAFLD), which is the necessary process for the development of simple fatty liver into fatty liver fibrosis and cirrhosis.[3] At present, the pathological mechanism of NASH has not been clarified, and there is a lack of specific clinical treatment methods. Therefore, elucidation of the pathogenesis of NASH, especially the key link and influencing factors in the progression of NASH lesions, is of great significance for the treatment and prevention of NAFLD. Niemann-Pick C1-Like 1 (NPC1L1) is a key protein in intracellular cholesterol absorption, which can regulate the absorption of cholesterol by small intestine cells and the concentration of cholesterol secreted into bile by liver cells.[4] NPC1L1 is involved in the regulation of cholesterol and lipid metabolism and plays an important role in maintaining the balance of cholesterol metabolism in the body. Abnormal cholesterol metabolism is closely related to NASH.[5] By constructing liver-specific expression of an NPC1L1 transgenic pig, researchers have demonstrated that NPC1L1 could promote lipid peroxidation in the liver by regulating the transcription of genes associated with lipid synthesis.[6] It can promote lipid peroxidation in the liver, thus confirming that NPC1L1 plays a key role in lipid metabolism. The study confirmed that the physiological role of NPC1L1 goes beyond simply mediating cholesterol absorption. Inhibiting the expression of NPC1L1 or cutting off the NPC1L1 cholesterol transport pathway can effectively prevent and treat metabolic diseases such as NAFLD and type 2 diabetes mellitus.[7,8] These studies suggest that NPC1L1 is closely related to inflammation and insulin resistance and plays an important role in the signaling pathway associated with inflammation and insulin resistance. Therefore, inhibiting the expression of NPC1L1 or blocking its cholesterol transport pathway will effectively prevent and treat metabolic diseases like nonalcoholic fatty liver disease and type 2 diabetes mellitus. NPC1L1 is anticipated to become a novel drug target for liver disease treatment. This is of great significance for the prevention and treatment of NASH and major metabolic diseases closely related to lipid metabolism.

NPC1L1 IS A KEY PROTEIN IN CHOLESTEROL TRANSPORT

NPC1L1 is a transmembrane protein consisting of 1332 amino acids with 13 transmembrane domains and a wide range of glycosylation sites including an NPC domain and a sterol sensing domain (SSD).[9,10] Altman confirmed that NPC1L1 was located on the brush marginal membrane of intestinal

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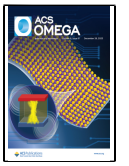

epithelial cells and mainly located in jejunal epithelial cells using immunocombination and in situ hybridization techniques.[11] NPC1L1 plays an important role in the sterol lipid metabolism pathway and is a key protein for the intestinal absorption of sterol lipids, especially cholesterol. Inhibition of NPC1L1 transport can reduce the level of blood lipids. NPC1L1-knockout ($NPC1L1^{-/-}$) mice did not develop hyperlipidemia or atherosclerosis when fed a high-cholesterol diet.[12,13] In addition, the decreased cholesterol absorption of NPC1L1-deficient mice was similar to that of the lipid-lowering effect of ezetimibe. Moreover, ezetimibe could not further reduce the amount of cholesterol absorption in $NPC1L1^{-/-}$ mice.[14]

MOLECULAR MECHANISM OF NPC1L1 CHOLESTEROL TRANSPORT

NPC1L1 works with the clathrin/adaptor protein complex 2 (clathrin/AP2) to transport cholesterol. NPC1L1 binds to cholesterol through the extracellular N-terminal domain, causing it to aggregate and bind to the sterol-sensing domain (SSD) of NPC1L1. And then, it endoannexes and interacts with the clathrin/AP2 complex to form endosomes, which are subsequently transported to late endosomes and lysosomes for digestion.[15] Most of the remaining NPC1L1 is transported to the endocytic recycling compartment (ERC), where it is separated from the cholesterol and returned to the cell surface to restart cholesterol transport[16] (Figure 1). Studies have

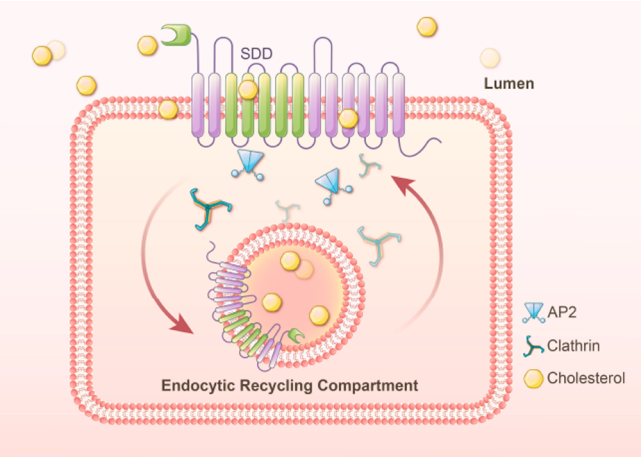

Figure 1. NPC1L1 mediates the cholesterol absorption pathway.

shown that transcription factors closely related to cholesterol metabolism regulate the expression of NPC1L1, such as sterol regulatory element-binding protein 2 (SREBP2), peroxisome proliferator-activated receptor α(PPARα) and peroxisome proliferator-activated receptor δ(PPARδ).[17] Hepatocyte nuclear factor 4α(HNF4α) can directly bind to the promoter of NPC1L1 and synergistically increase the activity of the NPC1L1 promoter with SREBP2. Heterodimer ABCG5/G8, a member of the ATP binding cassette transport (ABC) family, is an ATP-dependent transmembrane transporter.[18] It is mainly distributed on the cell membrane of hepatocytes and intestinal epithelial cell. ABCG5/G8 and NPC1L1 have the same organizational distributions but perform opposite functions. Both of them regulate cholesterol outflow into cells; therefore, ABCG5/G8 and NPC1L1 work together to regulate cholesterol transport in the small intestine. NPC1L1 determines the rate of regulation, and the range of regulation is calculated by the amount of cholesterol absorbed by NPC1L1 minus the amount of cholesterol excreted by ABCG5/G8. The study showed that the cholesterol concentration in the bile of constructed transgenic mice with liver-specific expression of human NPC1L1 was significantly reduced but had no effect on the expression of ABCG5/G8 on the timid duct. In addition, the bile cholesterol levels returned to normal after the transgenic mice were treated with ezetimibe. This suggests that NPC1L1 absorbs cholesterol from the bile back into the liver so as to prevent too much cholesterol from being transported into the bile by ABCG5/G8 to maintain cholesterol homeostasis.[19]

NPC1L1 AND DISEASES

High levels of blood cholesterol can lead to atherosclerosis. Inhibiting the expression of NPC1L1 can reduce the blood cholesterol level. Studies have shown that in mice with both *NPC1L1* and *ApoE* genes knocked out, cholesterol absorption and blood cholesterol level were significantly reduced, almost completely preventing further atherosclerosis.[20] These results indicated that the deficiency of NPC1L1 expression could effectively inhibit the absorption of cholesterol in mice fed a high cholesterol diet.

NPC1L1 also plays an important role in liver diseases such as NAFLD. The pathological changes of nonalcoholic fatty liver are similar to those of alcoholic fatty liver, including simple fatty liver, steatohepatitis, fatty liver fibrosis, and fatty cirrhosis. Excessive cholesterol in the diet can cause the accumulation of cholesterol and triglycerides in the liver; therefore, reducing the absorption of intestinal cholesterol can reduce the accumulation of triglycerides in the liver. Studies have shown that NPC1L1 plays an important role in metabolic disease, and the inactivation of NPC1L1 prevented the development of fatty liver in mice fed high cholesterol. Ezetimibe could effectively treat hepatic steatosis in wild-type mice fed high fat and high cholesterol. In addition, $NPC1L1^{-/-}$ mice could not form fatty liver even if fed high-fat diet. In recent years, when researchers used ezetimibe to treat fatty liver, it was found that the fat content in the liver of some obese people was significantly reduced, and related enzyme and lipid were effectively improved. Liver steatosis is closely related to insulin resistance. Insulin in patients could not inhibit liver gluconeogenesis but promoted liver fat biosynthesis. Both ezetimibe and $NPC1L1^{-/-}$ could improve insulin sensitivity and inhibit hepatic steatosis.

By constructing the Bama miniature pigs with liver-specific expression of human NPC1L1, our research group showed that NPC1L1 could regulate the transcription of genes related to lipid synthesis and activate the activity of fatty acid synthase, ultimately leading to the increase of free fatty acids.[6] Excessive free fatty acids could cause mitochondrial swelling, increased membrane permeability, and inflammatory cell infiltration. In particular, a high concentration of free fatty acid could also cause lipid peroxidation leading to liver damage, which could develop into steatohepatitis and liver fibrosis and eventually cirrhosis. As a marker of lipid peroxidation, the concentration of malondialdehyde (MDA) increased significantly, indicating that severe lipid peroxidation occurred in the liver tissue of TghNPC1L1 pigs. The findings presented above suggest that NPC1L1 plays an important role in lipid metabolism disorders and liver diseases. Inhibiting the expression of NPC1L1 or cutting off the NPC1L1 cholesterol transport pathway can

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effectively prevent and treat metabolic diseases such as nonalcoholic fatty liver disease and type 2 diabetes mellitus. NPC1L1 is expected to become a new therapeutic target for liver diseases.

CONCLUSIONS

NPC1L1 is a key transporter of intestinal cholesterol absorption and regulates the balance of the cholesterol metabolism in the body. However, the molecular mechanism of NPC1L1 in lipid metabolism disorders leading to liver disease has not been fully elucidated. It is necessary to elucidate the molecular pathological mechanism of NPC1L1 in the regulation of fatty liver, inflammatory response, and insulin resistance, especially in the development of NASH. It is also necessary to conduct a preliminary study on the pathogenesis of NASH caused by the involvement of NPC1L1 in high-fat diets, so as to lay a theoretical foundation for revealing the important biological functions and regulatory mechanisms of NPC1L1 in the regulation of cholesterol and lipid metabolism. Thus, it lays a theoretical foundation for revealing the important biological functions and regulatory mechanisms of NPC1L1 in the regulation of cholesterol and lipid metabolism. It also lays an important foundation for revealing the pathogenesis, early diagnosis, and treatment of major metabolic diseases such as NASH, cardiovascular and cerebrovascular diseases, fatty liver, diabetes, and obesity. Therefore, it is of great significance to deeply reveal the function of NPC1L1 in the liver and the molecular mechanism in lipid metabolism for the prevention and treatment of metabolic diseases, and it is expected to become a new target for the treatment of liver diseases.

ASSOCIATED CONTENT

Supporting Information

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Author biographies (PDF)

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Notes

The authors declare no competing financial interest.

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