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Review

Niemann–Pick C1-Like 1 and cholesterol uptake

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ABSTRACT

Niemann–Pick C1-Like 1 (NPC1L1) is a polytopic transmembrane protein responsible for dietary cholesterol and biliary cholesterol absorption. Consistent with its functions, NPC1L1 distributes on the brush border membrane of enterocytes and the canalicular membrane of hepatocytes in humans. As the molecular target of ezetimibe, a hypocholesterolemic drug, its physiological and pathological significance has been recognized and intensively studied for years. Recently, plenty of new findings reveal the molecular mechanism of NPC1L1's role in cholesterol uptake, which may provide new insights on our understanding of cholesterol absorption. In this review, we summarized recent progress in these studies and proposed a working model, hoping to provide new perspectives on the regulation of cholesterol transport and metabolism.

1. Introduction

Cholesterol is an essential structural component of mammalian cell membranes and plays crucial roles in intracellular transport, cell signaling, nerve conduction, etc. In mammals, it serves as the precursor for bile acids, vitamin D and steroid hormones in liver, skin and steroidogenic tissues (*e.g.*, adrenal gland, testis and ovary), respectively. Proper regulation of cholesterol homeostasis in the body is important for human health. Clinical and animal studies have established a direct correlation between plasma cholesterol level and the risk of coronary artery disease, the leading cause of death in developed societies [1–3].

Cholesterol homeostasis in the body is maintained mainly by *de novo* synthesis, intestinal absorption, and biliary and fecal excretion. The cholesterol biosynthesis is a well-defined energy-consuming and feedback-regulated process, which involves at least 20 enzymatic reactions [4]. All the genes that involved cholesterol biosynthesis are regulated by a family of membrane-bound transcription factors, designated sterol regulatory element-binding proteins (SREBPs) [5–7]. However, the molecular mechanism and regulation of intestinal cholesterol absorption were poorly understood until the discovery of the NPC1L1 protein. Recent studies have demonstrated that NPC1L1 is the key player in both dietary cholesterol absorption and biliary cholesterol re-absorption. It is also the molecular target of ezetimibe, an inhibitor of cholesterol absorption that has been approved for the treatment of hypercholesterolemia [8–10]. This review focuses on the molecular characterization of NPC1L1, discusses its role in small intestine and liver, and highlights the mechanisms of NPC1L1-mediated cholesterol uptake.

2. Discovery and characterization

NPC1L1 was first identified as a homolog of Niemann–Pick C1 (NPC1) protein [11], the deficiency of which causes Niemann–Pick disease type C1, a genetic disorder characterized by intracellular accumulation of unesterified cholesterol in the endosomal/lysosomal system of neurons that causes neurodegeneration and premature death [12,13]. However, the function of NPC1L1 remained unclear until 2004, when Altmann and colleagues rediscovered it using a genomic–bioinformatics screening approach in a search for critical intestinal cholesterol transporters [14].

Human *NPC1L1* gene maps to chromosome 7p13, spans 29 kb, encodes a 5 kb mRNA and predominantly produces a protein of 1332 amino acids [11,14,15] (Fig. 1). The amino acid sequence of NPC1L1 shares 51% similarity and 42% identity with that of NPC1 [11]. Like its homolog NPC1, NPC1L1 also has a typical signal peptide, 13 transmembrane regions, 3 large loops that protrude into the extracellular space (or the endosomal lumen), several smaller cytoplasmic loops, and a less conserved C-terminal cytoplasmic tail [16,17].

A conserved sterol-sensing domain (SSD) has been identified in NPC1L1, which is also found in several other polytopic transmembrane proteins [18], including NPC1, HMG-CoA reductase (the rate-limiting

Abbreviations: NPC1L1, Niemann–Pick C1-Like 1; LDL, low-density lipoprotein; PM, plasma membrane; ERC, endocytic recycling compartment; NTD, N-terminal domain; NFC, NPC1L1–Flotillin–Cholesterol

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enzyme in the cholesterol biosynthetic pathway [4,5]), SREBP cleavage-activating protein (an escort protein for SREBPs), and Patched (a membrane receptor for the cholesterol-linked signaling peptide Hedgehog [19]).

In 2009, a sterol-binding pocket was identified through studying the crystal structure of the N-terminal domain (NTD) of NPC1 [20]. Like NPC1, NPC1L1 also contains a conserved cysteine-rich N-terminal "NPC1" domain [11]. Consistently, recent studies proved the existence of a similar but larger sterol-binding pocket in the NPC1L1-NTD [21,22]. Unlike the pocket in NPC1-NTD, which is open to solvent, the pocket in NPC1L1-NTD is closed from solvent.

Multiple N-glycosylation sites have been identified throughout the three large extracellular/luminal loops of NPC1L1 [23] (Fig. 1). N-Glycosidase digestive experiments confirmed that the mature NPC1L1 protein undergoes heavy glycosylation [15,23,24]. This kind of modification may protect NPC1L1 from solubilization by bile salt and proteolytic degradation by intestinal digestive enzymes. Mutations at or around these N-glycosylation sequence would significantly affect the maturation and function of NPC1L1, leading to its rapid degradation through the ER-associated degradation (ERAD) pathway [24,25].

NPC1L1 is proved to be the molecular target of ezetimibe, a cholesterol absorption inhibitor [14,26]. Studies using *in vitro* ezetimibe binding assays indicated that ezetimibe directly bind to the second extracellular loop of NPC1L1 [27]. The glucuronide-derivative of ezetimibe (its metabolized form *in vivo*) also specifically binds to NPC1L1 with a high affinity [26].

3. Functions of NPC1L1 protein in small intestine and liver

Since cholesterol *de novo* biosynthesis is an energy-consuming process requiring substantial energy input, the body has evolved to take up readily available cholesterol molecules from the gut lumen [28]. NPC1L1, a transmembrane protein highly expressed in the intestinal epithelial cell (the enterocyte) of small intestine in mammals, has been shown to play a crucial role in cholesterol absorption. In addition, significant expression of NPC1L1 is also observed in human liver, but not in mouse liver [29]. The function of NPC1L1 protein in small intestine and in liver has been intensively studied.

NPC1L1, primarily expressed in the jejunum and proximal ileum, locates to the brush border membrane of the enterocyte, which is the interface between the intestinal lumen and the intracellular compartments [14,30] (Fig. 2). As cholesterol is minimally soluble in aqueous environment, it needs to be partitioned into bile salt micelles before being delivered to the brush border membranes, where it is transported by NPC1L1 into the enterocyte [31]. NPC1L1 null mice are found to be phenotypically normal, with no obvious abnormalities except a significant (>70%) reduction in intestinal cholesterol absorption, and insensitive to ezetimibe treatment [14]. The mice are resistant to diet-induced hypercholesterolemia, similar to the ezetimibe-treated wild-type mice [14,29]. These data provide compelling evidence that NPC1L1 is the key player in the ezetimibe-sensitive cholesterol uptake process in small intestine.

In humans and nonhuman primates, NPC1L1 also localizes to the canalicular membrane of the hepatocyte (Fig. 2), facilitating biliary cholesterol re-absorption. Liver-specific NPC1L1 transgenic mice displayed hypercholesterolemia, a dramatic decrease of biliary cholesterol and a modest increase of liver cholesterol [32]. Adenovirus-mediated exogenous expression of NPC1L1 in mice liver also caused similar phenotypes [24,33], indicating that human hepatic NPC1L1 facilitates the re-absorption of cholesterol from bile and prevents the loss of cholesterol. A recent study reported that ezetimibe treatment restored biliary cholesterol excretion in mice expressing NPC1L1 only in liver [34], suggesting that hepatic NPC1L1 is also a target of ezetimibe.

4. Mechanism of NPC1L1-mediated cholesterol uptake

4.1. NPC1L1 facilitates cholesterol uptake through vesicular endocytosis

When expressed in McArdle-RH7777 rat hepatoma cell (also called CRL-1601 cell), NPC1L1 was found on both plasma membrane (PM) and intracellular endocytic recycling compartment (ERC) [15]. Acute cholesterol depletion induced with methyl-β-cyclodextrin (CDX) can stimulate the relocation of NPC1L1 from ERC to PM. Intriguingly, only the PM-localized NPC1L1 can increase cholesterol uptake [15]. According to these studies, one can think logically that there are at least two possible mechanisms for NPC1L1 to mediate cholesterol uptake: 1) NPC1L1 may facilitate cholesterol influx as a classic transporter similar to the ATP-independent glucose transporters (GLUTs), which enhance glucose absorption according to a model of alternate conformation [35,36]; 2) NPC1L1 may mediate cholesterol inward transportation through vesicular endocytosis, a mechanism similar to LDL receptor-mediated internalization of LDL [37,38]. Thus, the key difference between the two models is whether the endocytosis of NPC1L1 itself is required for cholesterol uptake.

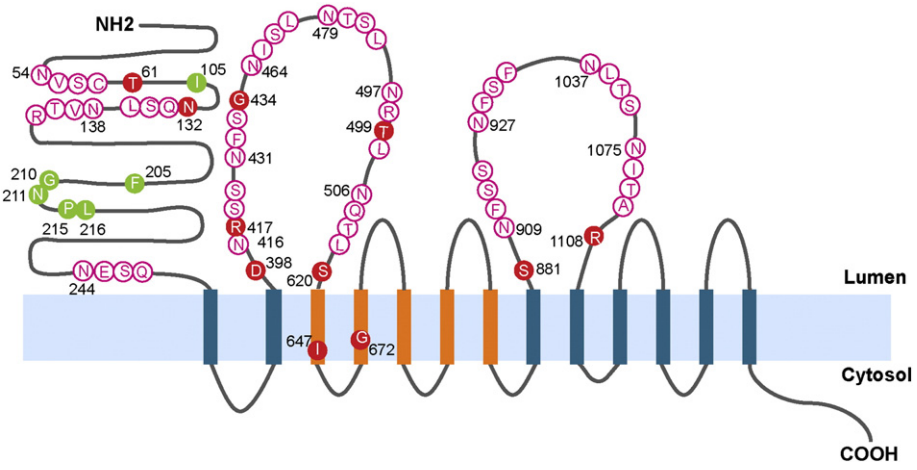

Fig. 1. Membrane topology of human NPC1L1. The blue and orange strips indicate the transmembrane domains of NPC1L1, and the orange ones denote the sterol-sensing domain (SSD). Putative N-linked glycosylation sequences are colored in pink. Residues highlighted in red indicate the identified mutations responsible for very low cholesterol absorption in populations [24,78,79]. Residues that influence the cholesterol-binding affinity are highlighted in green circles [22].