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NPC1, intracellular cholesterol trafficking and atherosclerosis

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ABSTRACT

Post-lysosomal cholesterol trafficking is an important, but poorly understood process that is essential to maintain lipid homeostasis. Niemann-Pick type C1 (NPC1), an integral membrane protein on the limiting membrane of late endosome/lysosome (LE/LY), is known to accept cholesterol from NPC2 and then mediate cholesterol transport from LE/LY to endoplasmic reticulum (ER) and plasma membrane in a vesicle- or oxysterol-binding protein (OSBP)-related protein 5 (ORP5)-dependent manner. Mutations in the *NPC1* gene can be found in the majority of NPC patients, who accumulate massive amounts of cholesterol and other lipids in the LE/LY due to a defect in intracellular lipid trafficking. Liver X receptor (LXR) is the major positive regulator of NPC1 expression. Atherosclerosis is the pathological basis of coronary heart disease, one of the major causes of death worldwide. NPC1 has been shown to play a critical role in the atherosclerotic progression. In this review, we have summarized the role of NPC1 in regulating intracellular cholesterol trafficking and atherosclerosis.

Contents

1. Introduction

Cholesterol is an abundant metabolite in mammalian tissues that plays important roles in normal embryonic development, cell differentiation, and nerve conduction. Membrane fluidity of all cells is tightly regulated by the ordered packing of cholesterol between phospholipid molecules. It is also the precursor of a variety of biologically active molecules such as bile acids, vitamin D and steroid hormones. Thus, an insufficient supply of cholesterol will have detrimental effects on

Abbreviations: NPC1, Niemann-Pick type C1; LE/LY, late endosome/lysosome; LDL, low-density lipoprotein; LDL-C, LDL-cholesterol; ER, endoplasmic reticulum; SSD, sterol-sensing domain; NTD, N-terminal domain; apoE, apolipoprotein E; LXR, liver X receptor; OSBP, oxysterol-binding protein; ORP5, OSBP-related protein 5; TGN, trans-Golgi network; oxLDL, oxidized low-density lipoprotein; ERK1/2, extracellular signal-regulated kinase 1/2; COX-2, cyclooxygenase-2; PPARα, peroxisome proliferator-activated receptor α; SREBP, sterol regulatory element-binding protein; LDLR, low-density lipoprotein receptor; ABCA1, ATP-binding cassette transporter A1; DHC, dihydrocapsaicin; apoA-I, apolipoprotein A-I.

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cell function, tissue development as well as whole-body physiology. Too much cholesterol, however, can also result in pathological consequences. Both clinical and animal studies have established a direct link between plasma cholesterol levels and the risks of coronary heart disease, one of the major causes of death worldwide [1,2].

The control of intracellular cholesterol levels is a complicated process, involving cholesterol uptake, biosynthesis, transport, metabolism and secretion. Interestingly, cholesterol uptake and secretion have been well described in several recent reviews [3,4]. Nevertheless, intracellular cholesterol trafficking remains elusive. Growing evidence shows that Niemann-Pick type C1 (NPC1) plays a key role in the process [5]. NPC1 is a transmembrane glycoprotein located in the limiting membrane of late endosome/lysosome (LE/LY). It can transfer low-density lipoprotein (LDL)-cholesterol (LDL-C) from LE/LY to endoplasmic reticulum (ER) for esterification or to plasma membrane for efflux, a crucial process governing the balance between macrophage cholesterol import and export, with potential consequences in atherogenesis [6]. This review focuses on the molecular characterization, cholesterol transport function and involved mechanisms of NPC1, and explores its emerging pathogenic significance and therapeutic potential in atherosclerosis.

2. Structure and characterization

The *NPC1* gene maps to chromosome 18q11–12, spans more than 47 kb, and consists of 25 exons that range in size from 74 to 788 nucleotides, and introns that range from 0.097 to 7 kb in length [7]. The human NPC1 protein is composed of 1278 amino acid residues, with 13 putative transmembrane helices, three large loops projecting into the lumen of LE/LY, four small luminal loops, six small cytoplasmic loops and a cytoplasmic tail (Fig. 1).

Five segments (residues 615–797) from the third to seventh transmembrane helix form the so-called sterol-sensing domain (SSD), which shares high sequence homology with those of several other cholesterol metabolism-related membrane proteins, such as Patched (a regulatory protein in the Hedgehog signaling pathway), sterol regulatory element binding protein cleavage activating protein (SCAP), 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) and NPC1-like protein 1 (a protein responsible for dietary cholesterol and biliary cholesterol absorption). SSD is an important binding site between cholesterol and NPC1. It can bind azocholestanol, a photoactivatable analog of cholesterol, in cells, and two loss-of-function mutations (P692S and Y635C) in the region severely block this effect [8]. In contrast, two gain-of-function mutations (L657F and D787N) in the SSD lead to a nearly 2-fold increase in the rates of moving LDL-C out of LE/LY [9]. NPC1 is synthesized in ER, transited through the Golgi and finally targeted to LE/LY. The dileucine motif present at the C-terminus has been reported to promote NPC1 trafficking to the endosomal compartment by direct interaction with the clathrin adaptor protein complex 1 [10]. A motif located in the SSD also contributes to this process [11]. Therefore, although the precise function of NPC1 SSD *in vivo* is still not fully understood, it is essential for normal cholesterol efflux from LE/LY.

NPC1 has three large luminal loops. The first loop (residues 25–264) at the N-terminus is called the N-terminal domain (NTD) or NPC1 domain, which is highly conserved in vertebrate and yeast orthologs of NPC1. It is composed of 18 conserved cysteine residues involved in intra-domain disulfide links, and a leucine zipper motif. When NTD was secreted as a soluble dimer in cultured cells, high affinity binding to cholesterol and 25-hydroxycholesterol was observed, suggesting NTD as a possible sterol binding site for NPC1 [12]. Nevertheless, the full-length NPC1 protein with a Q79A mutation, which disrupts the sterol binding by NTD, can still restore normal cholesterol transport out of LE/LY in NPC1-deficient Chinese hamster ovary cells, indicating that other cholesterol binding sites such as SSD except NTD may be present in the NPC1 protein [12]. The second loop, namely the region between transmembrane helices 2 and 3, can interact with NPC2 [13]. The third loop (residues 855–1098) locates between membrane-spanning domains 8 and 9. It is rich in conserved cysteine residues and consists of a ring finger motif. To date, of the over 200 NPC disease-causing mutations identified in the *NPC1* gene [14], approximately half of these mutations are localized to this region, including the most common mutation in Western Europeans, I1061T, which accounts for about 20% of the total number of NPC disease cases [15].

3. Cholesterol trafficking function of NPC1 and NPC disease

Cells can obtain cholesterol by two ways. One is *de novo* synthesis, a well-defined energy-consuming and feedback-regulated process which is involved in a variety of enzymatic reactions [16]. The other is exogenous uptake mainly from circulating LDL through receptor-mediated endocytosis [3]. After delivery from the early endosome to LE/LY, cholesterol esters carried by LDL are hydrolyzed by lysosomal acid lipase to release free cholesterol. Then, NPC1 mediates the trafficking of free cholesterol from LE/LY to other cellular compartments, including the plasma membrane and ER. In NPC1-absent cells, although the endocytic uptake of LDL-C and subsequent hydrolysis of cholesterol esters are normal, the transfer of unesterified cholesterol from LE/LY is impaired. Consequently, normal amounts of cholesterol fail to reach the plasma membrane and ER to modulate cholesterol balance [17]. In agreement, overexpression of human NPC1 protein greatly promotes LDL-C influx into plasma membrane and ER in Chinese hamster ovary cell lines [18].

NPC disease is a rare, fatal autosomal recessive neurovisceral condition characterized by progressive neurological deterioration and premature death, with an estimated incidence of 1:150,000 live births. Approximately 95% of cases are caused by mutations in the *NPC1* gene, while the other 5% is related to mutations in the *NPC2* gene [19]. Loss of function of either of these proteins leads to impaired trafficking

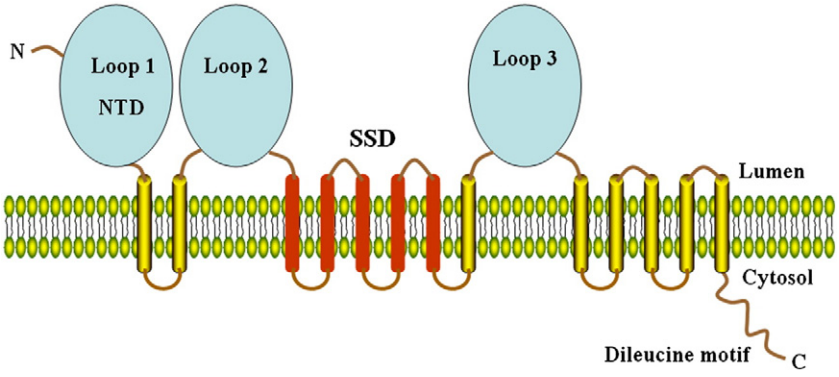

Fig. 1. Schematic of NPC1 protein structure. NPC1 protein has 13 transmembrane domains, three large luminal loops and a cytoplasmic tail. Loop 1 or NTD containing a leucine zipper motif can bind cholesterol. SSD is also a binding site for cholesterol and serves as an LE/LY targeting motif. A dileucine motif at the C-terminus is important for NPC1 localization in LE/LY. Loop 2 is able to directly bind NPC2. Loop 3 is composed of a ring finger motif.