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Review

Defective cholesterol trafficking in Niemann-Pick C-deficient cells

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ABSTRACT

Pathways of intracellular cholesterol trafficking are poorly understood at the molecular level. Mutations in Niemann-Pick C (NPC) proteins, NPC1 and NPC2, however, have led to insights into the mechanism by which endocytosed cholesterol is exported from late endosomes/lysosomes (LE/L). Mutations in NPC1, a multi-spanning membrane protein of LE/L, or mutations in NPC2, a soluble luminal protein of LE/L, cause the neurodegenerative disorder NPC disease. This review focuses on data supporting a model in which movement of cholesterol out of LE/L is mediated by the sequential action of the two NPC proteins. We also discuss potential therapies for NPC disease, including evidence that treatment of NPC-deficient mice with the cholesterol-binding compound, cyclodextrin, markedly attenuates neurodegeneration, and increases life-span, of NPC1-deficient mice.

1. Introduction

Niemann-Pick type C (NPC) disease is a fatal, autosomal recessive disorder that affects approximately 1 in 150,000 live births [1]. The majority of cases result from mutations in the *NPC1* gene (95%) while the remainder (5%) are caused by mutations in the *NPC2* gene. Loss of function of either of these proteins results in an accumulation of cholesterol and other lipids, including sphingomyelin, sphingosine and gangliosides (GM2 and GM3), within the late endosomes/lysosomes (LE/L). In mice and humans, this impairment leads to progressive neurodegeneration, hepatosplenomegaly and, ultimately, premature death. Typically, clinical manifestations become evident in early childhood, although age of onset can range from the perinatal period to adulthood. Disease progression is often more rapid when onset of symptoms occurs in early life [2].

Cells acquire cholesterol through endogenous synthesis, as well as through the uptake of exogenous sources of cholesterol, particularly low density lipoproteins (LDLs). LDLs are brought into the cell via receptor-mediated endocytosis and are delivered to the LE/L, where cholesterol esters (CE) within the core of the LDL particle are hydrolyzed by acid lipase [3]. Unesterified cholesterol exits the LE/L, apparently through an NPC1/NPC2-dependent mechanism, and is distributed to the plasma membrane as well as the endoplasmic reticulum (ER). The ER serves as a cholesterol sensor, allowing the cell to regulate cholesterol synthesis and uptake via the sterol regulatory element-binding protein pathway [4]. Additionally, cholesterol at the ER can be re-esterified by acyl-CoA:cholesterol acyltransferase (ACAT) [3].

Initial studies by Pentchev et al. found that fibroblasts from NPC1-deficient patients have a defect in cholesterol esterification despite having normal ACAT activity [5]. Staining of these cells with filipin, which labels unesterified cholesterol, suggested that although LDL-cholesterol is internalized and hydrolyzed normally in these fibroblasts, its movement to the ER is blunted [5]. Consequently, LDL is unable to decrease cholesterol biosynthesis and LDL-receptor activity, both of which are regulated at the ER [6]. Cytochemical techniques confirmed that there is a massive storage of cholesterol within the lysosomes of NPC-deficient fibroblasts [7]. More recent studies have begun to unravel the molecular and cell biological basis of these findings.

2. NPC1 and NPC2 proteins

Positional cloning techniques have identified the human *NPC1* gene (on chromosome 18q11) and its orthologs in mice, the yeast *Saccharomyces cerevisiae*, the nematode *Caenorhabditis elegans* [8,9]

Abbreviations: ACAT, acyl-CoA:cholesterol acyltransferase; ALLO, allopregnanolone; CD, cyclodextrin; CE, cholesterol esters; CNS, central nervous system; ER, endoplasmic reticulum; GSL, glycosphingolipid; LDL, low density lipoprotein; LE/L, late endosomes/lysosomes; NPC, Niemann-Pick type C

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and *Drosophila* [10]. Expression of NPC1 in cultured fibroblasts from patients with NPC disease abolished the lysosomal accumulation of cholesterol [8]. NPC1 is an integral membrane protein that is primarily localized to the LE/L, although this protein also appears to cycle through the *trans*-Golgi network [11,12]. Human NPC1 consists of 1278 amino acids and contains 13 putative transmembrane domains that are separated by three luminal glycosylated loops [8,13]. Moreover, NPC1 contains a sterol-sensing domain that has a similar sequence to that in 3-hydroxy-3-methylglutaryl-CoA reductase, sterol regulatory element-binding protein cleavage activating protein and Patched [8].

Through studies that were designed to characterize the lysosomal proteome, the gene responsible for NPC2 disease was subsequently identified [14]. NPC2 (previously called HE1) is a ubiquitously expressed 151-amino acid, soluble lysosomal protein [14]. Originally, NPC2 was identified as a cholesterol-binding protein that is a major secretory component of epididymal fluid [15]. Fluorescence quenching assays showed that NPC2 can transfer cholesterol between phospholipid vesicles. In addition, the transfer of cholesterol from phospholipid vesicles to NPC2 is dramatically increased by the presence of bis(monoacylglycero)phosphate (a LE/L phospholipid that accumulates in NPC-deficient cells) in donor vesicles [16].

Homozygous mutations in either NPC1 or NPC2 result in virtually identical cellular and clinical phenotypes [17]. Likewise, when NPC1-deficient mice are crossed with *Npc2* hypomorphic mice, which retain 0–4% residual NPC2 protein, the phenotype is indistinguishable from that of either NPC1- or NPC2-deficient mice [18], suggesting that these proteins function in tandem, or sequentially, in the same pathway. Binding studies and crystal structures of NPC1 and NPC2 have provided valuable insights into the functions of these proteins. NPC2 binds cholesterol with high affinity in a process that is sensitive to modification of the hydrophobic side-chain of cholesterol [15,19]. A crystal structure of bovine NPC2 bound to cholesterol sulfate shows that cholesterol binds in a deep hydrophobic pocket, with only the sulfate (substituted for the 3β-OH moiety) of the sterol exposed to solvent [20]. NPC1 also binds cholesterol and fluorescent sterol analogs, as well as oxysterols [19,21,22]. More recently, Kwon et al. reported the crystal structure of the N-terminal loop of NPC1 and revealed that this loop binds to cholesterol so that the 3β-OH group is buried within the binding pocket and the iso-octyl side-chain is exposed on the surface [23]. Since NPC1 and NPC2 appear to bind cholesterol in opposite orientations, a model for the functions of the two NPC proteins was proposed. In this model, unesterified cholesterol in the interior of the LE/L is initially bound by NPC2 which subsequently transfers the cholesterol to the N-terminal cholesterol-binding domain of NPC1 in the limiting membrane of the LE/L (Fig. 1) [23]. This model predicts that NPC1 inserts the iso-octyl side-chain of cholesterol into the lysosomal outer membrane, although this feature remains to be confirmed [23]. In support of this model, NPC2 greatly accelerates the bi-directional transfer of cholesterol from NPC1 to liposome acceptors, particularly those containing the LE/L-specific phospholipid, bis(monoacylglycero)phosphate [24]. The role that the sterol-sensing domain of NPC1 plays in this process remains unclear since mutations in this domain prevent the binding of a photoactivatable analog of cholesterol to NPC1 in intact cells [25]. However, direct binding of this cholesterol analog to the sterol-sensing domain was not demonstrated [25], leaving open the possibility that this domain modulates cholesterol binding to the N-terminal loop of NPC1. An alternative model that has been proposed, and not yet ruled out, for the sequential action of NPC1 and NPC2 is that NPC1 transfers cholesterol to NPC2. No direct interaction between the NPC1 and NPC2 proteins has been detected.

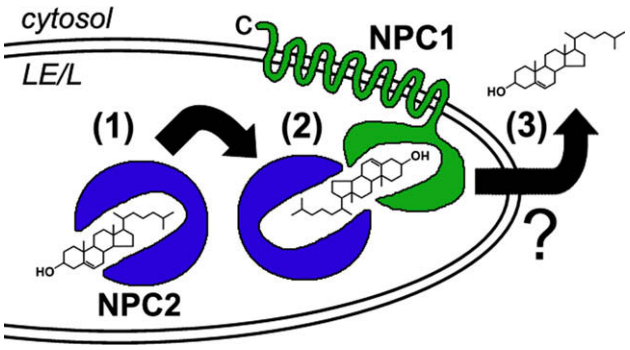

Fig. 1. Potential mechanism for NPC1/NPC2-mediated cholesterol export from LE/L. (1) NPC2 (soluble protein) binds unesterified cholesterol in the LE/L with the iso-octyl chain in the binding pocket, an event that may be enhanced by bis(monoacylglycero)phosphate. (2) NPC2 transfers cholesterol to the N-terminal loop of NPC1 (a multi-pass membrane protein) which binds cholesterol with the hydroxyl group in the binding pocket. (3) Cholesterol is exported from LE/L by an unknown mechanism.

Other experiments, in which NPC1 was expressed in *Escherichia coli*, indicated that NPC1 might be a fatty acid transporter [26]. In these studies, NPC1 was able to transport oleic acid, but not cholesterol or CE, across the *E. coli* membrane [26]. Nevertheless, subsequent work showed that the flux of fatty acids through the NPC1-deficient LE/L is normal, suggesting that fatty acid export from the LE/L does not require NPC1 in mammalian cells [27]. The finding that NPC1 that is expressed in *E. coli* cannot mediate the transbilayer movement of cholesterol might indicate that NPC1 requires the presence of functional NPC2 in order to transport cholesterol.

3. Abnormal lipid trafficking in NPC-deficient cells

The mechanisms responsible for the various pathways of inter-organelle trafficking of cholesterol within cells are largely undefined. The endocytic uptake of LDLs, and the subsequent hydrolysis of CE, are normal in NPC1-deficient cells [5]. However, the export of cholesterol and other lipids from the LE/L is defective. Consequently, normal amounts of cholesterol fail to reach the plasma membrane and the ER for regulation of cholesterol homeostasis [5,6]. Since cholesterol trafficking to the ER is impaired in NPC-deficient cells, the cells sense a lack of cholesterol. Thus, cholesterol synthesis and LDL-receptor expression are increased despite abnormally elevated cholesterol levels in the LE/L [6]. The mechanism by which cholesterol reaches the ER from the LE/L remains to be defined. Recent findings suggest that cholesterol is first trafficked from the LE/L to the *trans*-Golgi network prior to reaching the ER in a process that is dependent upon a SNARE complex, although other possibilities exist [28]. Endogenously-synthesized cholesterol can also accumulate within the LE/L, albeit much more slowly than does LDL-derived cholesterol [29]. Unlike LDL-derived cholesterol, the transport of endogenously-synthesized cholesterol to the plasma membrane is not impaired in NPC1-deficient fibroblasts [29,30]. Presumably through formation of endocytic vesicles and membrane recycling, this source of cholesterol can, however, also become trapped in the LE/L. Furthermore, cholesterol that is taken up through bulk-phase endocytosis also enters the LE/L and, as a consequence, becomes trapped within the LE/L compartment of NPC1-deficient cells [31]. Interestingly, the intracellular transport of cholesterol derived from high density lipoproteins appears to be unaffected by NPC1 deficiency, suggesting that cholesterol derived from these lipoproteins is processed in the cell through a pathway different from that used by LDL-cholesterol [32].

In addition to cholesterol, a variety of other lipids, including sphingomyelin, sphingosine, bis(monoacylglycero)phosphate, and