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REVIEW

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The role of vesicular transport in ABCA1-dependent lipid efflux and its connection with NPC pathways

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Abstract The membrane transporter ATP-binding cassette transporter A1 (ABCA1) has been shown to be the rate-limiting step in the initial formation of plasma high-density lipoprotein (HDL) particles. The mechanisms of action of ABCA1, including its role in the vesicular transport of lipids to the cell surface for the lipidation of HDL apolipoproteins, are not fully understood. Niemann–Pick type C (NPC) disease is most often caused by mutations in the NPC1 gene, whose protein product is believed to facilitate the egress of cholesterol and other lipids from late endosomes and lysosomes to other cellular compartments. This report reviews current knowledge regarding the role of ABCA1 in vesicular lipid transport mechanisms required for HDL particle formation, and the relationship between ABCA1 and NPC1 in this process.

Keywords ABCA1 · NPC · Apolipoprotein A-I · Vesicular transport · Cholesterol · HDL · Cholesterol transport · Cholesterol efflux · Atherosclerosis

Abbreviations HDL: high-density lipoprotein · ABCA1: ATP-binding cassette transporter A1 · ApoA-I: apolipoprotein A-I · NPC: Niemann–Pick type C · PL: phospholipids · C: cholesterol · ER: endoplasmic reticulum · TGN: *trans*-Golgi network

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Introduction

Accumulation of excess cholesterol in the artery wall is the biochemical hallmark of atherosclerosis. The removal of cholesterol from arterial wall cells and other tissues is primarily carried out by the high-density class of lipoproteins (HDL) in a process referred to as reverse cholesterol transport. In recent years it has become clear that the initial lipidation of apolipoprotein A-I (apoA-I, the main protein of HDL) and other HDL apolipoproteins by the membrane lipid transporter ATP-binding cassette transporter A1 (ABCA1) is the rate-limiting step in plasma HDL particle formation. The mechanisms by which ABCA1 facilitates the initial lipidation of HDL apolipoproteins, and whether this involves mainly cell surface or also intracellular vesicular trafficking events, are incompletely understood, but are of major scientific and potential therapeutic importance. Another protein facilitating intracellular lipid

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transport, the Niemann–Pick type C1 protein (NPC1), is mutated in the majority of patients with the fatal neurodegenerative disorder Niemann–Pick type C disease. The mechanism of action of this protein is also not yet known, but is thought to involve the mobilization of cholesterol from late endosomes and lysosomes for transport to other cell compartments. Recent findings including the impaired regulation of ABCA1 and mobilization of lipids to apoA-I in NPC disease cells have suggested an interaction of ABCA1 and NPC1 in cholesterol transport and in maintaining cell cholesterol balance. The purpose of this report is to review evidence regarding the role of vesicular transport of ABCA1 in the formation of HDL particles, and the potential interactions between ABCA1 and NPC1 in this process.

Functions of ABCA1

ABCA1, first known as ABC1, was cloned in 1994 by Luciani and colleagues [1] as a homolog of yeast ced-7, and is a member of the large superfamily of ABC transporters that use ATP as an energy source to transport lipids and other molecules across membranes [2]. At approximately the same time, defective cholesterol [3, 4] and phospholipid [4] efflux to apoA-I from fibroblasts derived from patients with the extreme HDL deficiency syndrome Tangier disease were described, which suggested that the removal of cellular lipids to apoA-I is a major predictor of plasma HDL-C levels. In 1999, the mutation in Tangier disease was mapped to chromosome 9q31 in the ABCA1 gene [5–8]. These discoveries have set off an enormous amount of research on the regulation, mechanism of action, and suitability of ABCA1 as a target to increase HDL formation therapeutically for the treatment and prevention of atherosclerosis.

ABCA1 is a full ATP-binding cassette transporter consisting of two similar halves, each with a transmembrane domain and a nucleotide-binding domain, that are linked covalently [9]. The nucleotide-binding domains consist of two conserved peptide motifs known as Walker A and Walker B, which are present in most proteins that utilize ATP in energizing their transport activity [10]. ABCA1 is expressed ubiquitously, but in highest concentrations in the liver, brain, adrenal glands, and macrophage foam cells [11–13]. ABCA1 expression is increased upon cholesterol loading of cultured cells including macrophages and fibroblasts [8, 11], consistent with its role as a mediator of removal of excess cell cholesterol. Its expression is regulated at multiple levels, including liver X receptor (LXR) and retinoid X receptor (RXR) binding to the promoter region of the ABCA1 gene, with LXR being activated by elevated levels of oxysterols in cholesterolloaded cells [14, 15]. Several additional modes of regulation of ABCA1 by retinoids, cyclic AMP, peroxisome proliferator-activated receptor agonists, and polyunsaturated fatty acids have been described (for a review, see [9]). ABCA1 is stabilized by apoA-I binding to cells [16], through apoA-I-dependent inhibition of calpain-mediated proteolysis of ABCA1 [17, 18]. Multiple constitutive and apoA-I-stimulated phosphorylation events have been described that both enhance [18–22] or inhibit [23] ABCA1 activity.

The exact mechanism of action of ABCA1 is still unknown. It was initially proposed to mediate translocation of phosphatidylserine (PS) to the outer leaflet of the plasma membrane in its role in engulfment of apoptotic cells [1, 24]. With respect to HDL formation, it was proposed that the redistribution of PS in the outer leaflet by ABCA1 creates a favorable lipid environment for apoA-I docking and removal of membrane phospholipids and cholesterol, and that this does not require a direct ABCA1–apoA-I interaction [24]. It was suggested in several subsequent cross-linking studies that apoA-I binds directly to ABCA1 or to a molecular complex containing ABCA1, and that this binding is necessary to stimulate cholesterol and phospholipid efflux [25–28]. Whether ABCA1 mediates phospholipid and cholesterol delivery directly (one-step model), phospholipid delivery directly and cholesterol delivery indirectly but essentially simultaneously (a variation of the one-step model), or only phospholipid delivery, with cholesterol delivery occurring in a separate second step (two-step model), to apoA-I has yet to be resolved.

In contrast to cholesterol delivery from cells to preformed HDL particles, which may be mediated by several mechanisms physiologically, it appears that no other membrane transporter can substitute for the role of ABCA1 in mediating significant lipidation of HDL apolipoproteins, and any discussion of apoA-I-dependent lipid efflux from cells therefore refers to ABCA1-mediated lipid efflux. Although ABCA7 has also been found to mediate phospholipid [29] or phospholipid and cholesterol efflux [30] to apoA-I, ABCA7 apparently cannot substitute for ABCA1 under conditions of ABCA1 deficiency, such as Tangier disease, to raise HDL levels. While apoA-I is the most abundant HDL protein in plasma and the main protein used in cholesterol efflux studies, other exchangeable alpha-helical proteins including apoA-II, apoE, and the apo C's also interact with ABCA1 [31] and are likely involved and activate similar pathways of vesicular lipid transport as apoA-I.

Vesicular transport and ABCA1

The importance of vesicular transport of intracellular cholesterol and phospholipids in ABCA1-dependent lipidation of apoA-I has been suggested by cell localization and studies using inhibitors of vesicular transport. Precise localization of ABCA1 within cells has been difficult due to a lack of highly specific ABCA1 antibodies suitable for immunofluorescence studies. Experiments using biotinylation [8], anti-FLAG antibody in ABCA1-FLAG transfected cells [25], green fluorescent protein (GFP)-ABCA1 transfected cells [24, 32], and a polyclonal antibody [33] suggest ABCA1 localizes to the plasma membrane, and to intracellular compartments including the Golgi complex [24, 33]. GFP-ABCA1 fusion proteins were found to lo-

calize to the plasma membrane, and traffic in vesicles between intracellular compartments including early and late endosomes and lysosomes and the plasma membrane [34]. More recent studies using FLAG- or hemagglutinin-tagged ABCA1 also suggest the presence of ABCA1 in the late endosome/lysosome compartment [35].

Smith and colleagues have reported the uptake and resecretion of labeled apoA-I [36] and colocalization of ABCA1-GFP and apoA-I in intracellular compartments [37]. In addition to mediating lipid efflux at the cell surface, these results suggest ABCA1 may be internalized along with apoA-I in vesicles to intracellular compartments where ABCA1 pumps lipids into the vesicles for association with apoA-I, and subsequent release of nascent HDL particles upon fusion with the plasma membrane (retroendocytosis) [9, 38]. It was suggested in a recent study that trafficking of ABCA1, and possibly apoA-I, to the late endosome/lysosome compartment is responsible for a quantitatively significant percentage of total ABCA1-dependent cholesterol efflux [35]. Chen et al. [35] found that internalization of ABCA1 was necessary to effectively mobilize cholesterol derived from acetylated LDL in cells coexpressing scavenger receptor A, where the cholesterol would be expected to be largely in the late endosome/lysosome pool during the time course of efflux used. Cells expressing a mutant form of ABCA1 (ABCA1delPEST) showed impaired ABCA1 internalization and considerably less ability to efflux this pool of late endosome/lysosomal cholesterol to apoA-I. These results suggest that internalization of ABCA1 is necessary for the mobilization of LDL-derived and other pools of cell cholesterol, at least during their transit through the late endosome/lysosome compartment.

A role for Golgi complex-derived lipids in the lipidation of apoA-I by ABCA1 was suggested by a number of experiments using control and Tangier disease cells [39–41]. A markedly hypertrophic Golgi complex was described in Tangier disease fibroblasts [39, 42], suggesting a direct or indirect role for ABCA1 in offloading lipids or proteins from this organelle. Mendez and Uint [40] reported that disruption of the Golgi apparatus using brefeldin A inhibited phospholipid and cholesterol efflux to lipid-free apoA-I by more than 80%, but that efflux of these lipids to protein-depleted lipid acceptors was unaffected. Remaley and colleagues [41] similarly found that brefeldin A almost completely blocked cholesterol and phospholipid efflux to apoA-I plus several other amphipathic alpha-helical apolipoproteins, including apoA-II, A-IV, C-I, C-II, and C-III. Besides impaired trafficking of Golgi-derived lipids to the plasma membrane, an additional or alternate explanation of these findings is the reduction in transport of newly synthesized ABCA1 to the cell surface seen in brefeldin A-treated cells [34]. Zha et al. [43] reported that transport of Golgi-derived vesicles to the plasma membrane increased twofold in response to incubation with apoA-I in normal, but not in Tangier disease fibroblasts. Altogether, these results suggest a major role for the Golgi complex in the delivery of lipids as well as ABCA1 to the plasma membrane during ABCA1-mediated lipid efflux to apoA-I.

Possible mechanisms by which apoA-I stimulates the Golgi-dependent transport of lipids to the plasma membrane for removal include an immediate signaling cascade induced by the interaction of apoA-I with ABCA1 at the cell surface, or following the depletion of plasma membrane lipids by apoA-I [21, 44]. The signaling induced by apoA-I may require a direct interaction between apoA-I and ABCA1, with the signals transmitted either upon binding of apoA-I or only following ABCA1-dependent efflux of lipids to apoA-I. Another scenario is that apoA-I binding to the cell surface stimulates a signaling cascade regulating ABCA1 activity in the Golgi itself [33]. The most likely situation may be that induction of Golgi-dependent vesicular transport of lipids to the plasma membrane follows depletion of cell surface lipids by the apoA-I–ABCA1 interaction at the plasma membrane.

Vesicle fusion proteins associated with ABCA1 were recently examined [45]. Syntaxin-13 was found to coprecipitate and colocalize with ABCA1, and silencing rRNA directed against syntaxin-13 markedly reduced apoA-I-mediated phospholipid efflux [45]. Expression of ADP-ribosylation factor (ARF)-like 7 (ARL), a regulatory GTPase involved in vesicle budding, was found to positively affect apoA-I-dependent cholesterol efflux and localize in perinuclear and plasma membrane compartments, suggesting a potential linkage to ABCA1-mediated lipid mobilization [46].

A model can be generated in which ABCA1 selectively mediates depletion of the pool of cholesterol derived from endosomal/lysosomal degradation and hydrolysis of stored cholesteryl esters, that would otherwise enter the regulatory pool of cholesterol to suppress endogenous cholesterol synthesis and LDL receptor expression, and stimulate storage of excess cholesterol as cholesteryl esters by acyl coenzymeA:cholesterol acyltransferase (ACAT). The ABCA1–apoA-I interaction appears to preferentially deplete this regulatory pool of cholesterol that would otherwise be esterified by ACAT, as shown by the complete absence of depletion by apoA-I of ACAT-accessible cholesterol in Tangier disease cells [4], and consistent with increased HDL-C in ACAT-1-deficient mice [47]. The flow of intracellular cholesterol transport mediated by the apoA-I–ABCA1 interaction is proposed to be from the late endosomal/lysosomal compartment and through the *trans*-Golgi network, from which cholesterol and phospho/sphingolipidrich vesicles move to the plasma membrane (Fig. 1). Whether or not this process absolutely requires the function of the Niemann–Pick type C1 protein is discussed below.

NPC disease

NPC disease is a severe autosomal recessive lipidosis characterized by the accumulation of unesterified cholesterol in the endosomal/lysosomal system [48]. Affected individuals show progressive neurodegeneration and he-

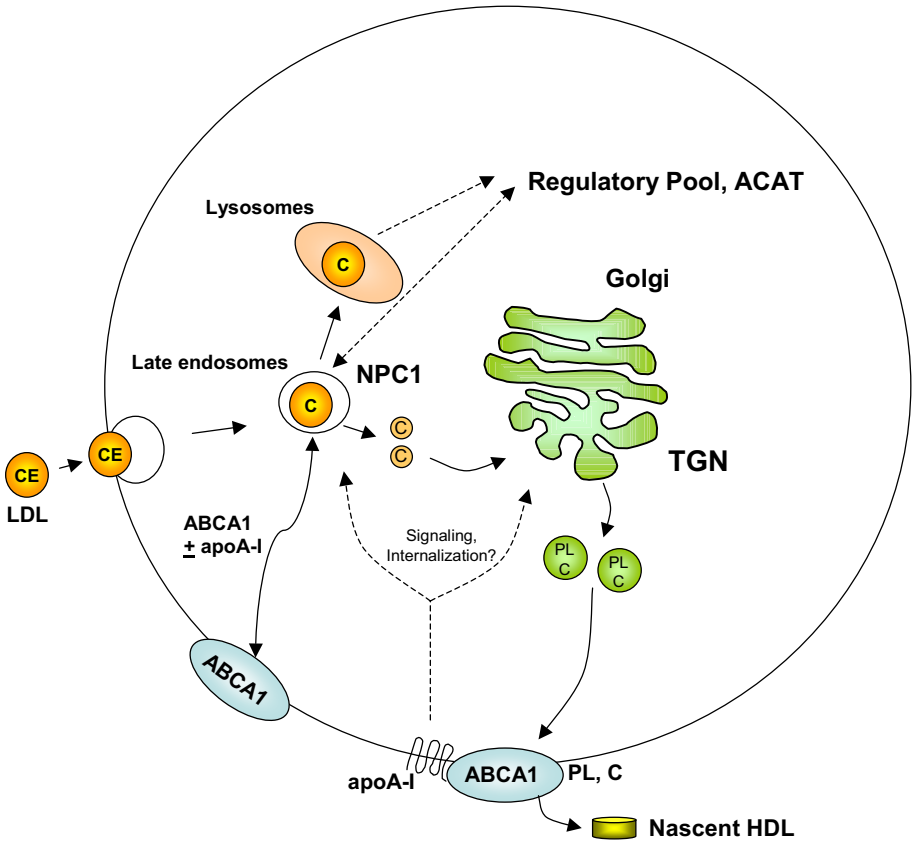

Fig. 1 Model of ATP-binding cassette transporter A1 (ABCA1)-mediated lipid efflux from cells. ABCA1 mobilizes phospholipids and cholesterol to apoA-I and other apolipoproteins at the cell surface to generate nascent HDL. Depletion of cell surface lipids induces a signaling cascade to mobilize lipids from late endosomes/lysosomes, via the *trans*-Golgi network (TGN) to the plasma membrane, a process facilitated by the Niemann–Pick type CI protein (NPC1). ABCA1 is rapidly recycled between the plasma membrane and intracellular compartments including late endosomes/lysosomes, possibly with some apoA-I, and this process also facilitates delivery of lipids to the cell surface for HDL genesis

patosplenomegaly, and the disease is frequently fatal in the first or second decade [49]. The most prominent cellular feature is the accumulation of unesterified cholesterol and glycosphingolipids in the late endosome/lysosome compartment, as well as the *trans*-Golgi cisternae [50], and a delay in the transfer of unesterified cholesterol to other intracellular destinations [51]. NPC disease is caused by mutations in two genes: *NPC1*, which accounts for 95% of NPC patients, and *NPC2* (*HE-1*), which accounts for the remaining 5% of patients. The *NPC1* gene was identified in both humans and mice, and was subsequently cloned [52, 53]. There is no clinical distinction between patients with NPC1 mutations and those with NPC2 mutations, and their cultured fibroblasts are biochemically and phenotypically identical [51].

NPC1 and NPC2 function

NPC1 is a large glycoprotein (170–190 kDa) with 13 transmembrane domains, a small cytoplasmic C-terminal tail, and a putative sterol-sensing domain (SSD) homologous to that found in HMG-CoA reductase, SREBP cleavage-activating protein (SCAP), Patched, and the putative intestinal cholesterol transporter NPC1L1 [54, 55]. NPC1 is structurally similar to the resistance-nodulation-cell division (RND) family of prokaryotic permeases [54, 56]. RND permeases are efflux pumps that utilize proton-motive force to extrude compounds including hydrophobic drugs, heavy metals, antibiotics, and lipooligosaccharides [56].

At steady state, NPC1 has been shown to reside in the membranes of late endosomes, which confirmed earlier findings by subcellular fractionation of mouse liver [57] and immunocytochemical studies on cultured cells [58, 59]. Adenovirus-mediated gene transfer to overexpress NPC1 in mouse liver followed by immunofluorescence has also shown NPC1 protein in a vesicular compartment [60]. In normal fibroblasts the NPC1 protein is localized in the late endosomal/lysosomal compartment, and to a lesser extent, the *trans*-Golgi network (TGN) [57, 61–63]. NPC1 in the TGN might be newly synthesized proteins or NPC1 interacting transiently with the TGN. Some investigators have also shown that the vesicular transport proteins Rab7 and Rab9 colocalize with NPC1, and are involved in mediating the fission–fusion of vesicles transported between early and late endosomes/lysosomes and the TGN [57, 64, 65].

NPC1 is involved in the internalization of GM2 gangliosides into the late endocytic pathway, and in LDL cholesterol-loaded normal fibroblasts NPC1 is localized in late endosomes [66]. Although the specific function of NPC1 is not yet known, its putative sterol-sensing domain and its dysfunctional link to lysosomal cholesterol sequestration underscores its importance in the mobilization of membrane cholesterol within the late endocytic pathway. In an attempt to unravel the function of NPC1, Ohgami et al. [67] used a photoactivatable cholesterol analog, [^{3}H]7,7-

azocholestanol, to label fluorescent-tagged NPC1 (NPC1-GFP), and showed that NPC1 interacts directly with the analog and that the interaction requires a functional sterol-sensing domain. NPC2 also interacted with the analog via its cholesterol binding domain. Although it has been proposed that NPC2 may be necessary to mediate the interaction between NPC1 and cholesterol [68], the interaction between NPC1 and [^{3}H]7,7-azocholestanol did not require NPC2 [67].

NPC2 is a small, soluble glycoprotein (18 kDa) that at steady state localizes in the lumen of lysosomes. It was originally characterized as an important secreted protein from human epididymis (HE1) [69], but in subsequent studies it was shown that the protein is ubiquitously expressed in tissues. As a soluble lysosomal protein, NPC2 is sorted in the Golgi apparatus as a mannose 6-phosphate-tagged protein, and subcellular fractionation of rat liver identified endogenous hepatic NPC2 in the lysosomal fraction [59]. Chikh et al. [70] reported that NPC2 is indeed localized in lysosomes, and *N*-glycosylation of the asparagine residue at position 58 (Asn-58) is essential for lysosomal targeting and NPC2 function.

Emerging evidence suggests that as a putative RND permease, NPC1 may function as a sterol-modulated transmembrane efflux pump for different lipids and proteins from late endosomes to the TGN. Although NPC1 protein resides in late endosomes, it has been shown to interact with the TGN and ER. Several investigators have reported that NPC1 and NPC2 proteins work in concert and play a major role in intracellular cholesterol trafficking by moving unesterified cholesterol from late endosomes/lysosomes [59, 71–74]. Hence, NPC cells are defective in delivering cholesterol, proteins, and other cargo from late endosomes to TGN, supporting a model that cholesterol moves from late endosomes/lysosomes to the TGN in a process requiring NPC1 activity [57, 74, 75].

ABCA1 and NPC: lessons from Tangier disease

Whether or not the delivery of late endosomal/lysosomal cholesterol to ABCA1 for HDL particle formation requires the activity of NPC1, however, remains controversial. Studies indicating the localization of ABCA1 in late endosomes [34, 35] suggest the possibility that ABCA1 and NPC1 work together in intracellular compartments to mobilize cholesterol and phospholipids for lipidation of apoA-I. Neufeld and colleagues [42] have recently reported that Tangier disease fibroblasts accumulate cholesterol and sphingomyelin as well as large amounts of NPC1 protein in late endocytic vesicles, and that exogenous apoA-I abrogated the cholesterol-induced retention of NPC1 in wild-type, but not in Tangier disease cell late endosomes. This result suggests NPC1 in Tangier disease cells accumulates in these endosomes in an attempt to mobilize cholesterol normally mobilized by the actions of ABCA1 and apoA-I, but cannot do so in the absence of functional ABCA1. Expression of ABCA1-GFP in Tangier disease cells resulted in the correction of accumulation of cholesterol and NPC1 in late endosomes, in concert with restoration of apoA-I-mediated cholesterol efflux [42]. These results suggest ABCA1 indirectly mediates the trafficking of NPC1 in cells by converting late endocytic lipids that retain NPC1 to pools that can associate with apoA-I in the generation of nascent HDL particles [42]. ABCA1 function is therefore required for appropriate trafficking of NPC1 in cells, but appears to influence NPC1 trafficking indirectly rather than directly.

ABCA1 and NPC: lessons from NPC disease

Additional insights into the relationship between ABCA1 and NPC1 functions come from HDL formation studies using human and mouse NPC disease cell culture models.

Sequestration of cholesterol in late endosomes/lysosomes has been identified as the reason for impaired down-regulation of HMG-CoA reductase and LDL receptor expression in response to LDL loading, as well as decreased delivery of cholesterol to the ER for esterification by ACAT, in NPC disease [72, 73, 76]. Consistent with impaired egress of late endosomal/lysosomal cholesterol and therefore impaired regulation of other sterol-responsive genes in NPC disease, we postulated there would also be a failure to up-regulate ABCA1 appropriately in the face of rising cell cholesterol in this disorder. ApoA-I-mediated efflux of phosphatidylcholine, sphingomyelin and cholesterol was found to be diminished from NPC disease human fibroblasts, as were basal and both LDL- and nonlipoprotein cholesterol-stimulated levels of ABCA1 mRNA and protein [77]. Cholesterol efflux to apoA-I was diminished from NPC disease fibroblasts using cells labeled in LDL-derived, plasma membrane, newly synthesized, or total cell cholesterol pools, suggesting all of these pools supply cholesterol to ABCA1 for efflux [77]. Surprisingly, despite the much lower expression of ABCA1, binding of apoA-I to *NPC1*$^{-/-}$ human fibroblasts was similar to normal fibroblasts, indicating factors other than or in addition to ABCA1 predict apoA-I binding to cells [77]. Consistent with impaired ABCA1 regulation and diminished apolipoprotein-mediated lipid efflux, we also showed for the first time that plasma HDL-C levels were low (below 1 mmol/l or 40 mg/dl) in 17 of 21 (81%) NPC disease patient fasting plasma samples studied [77]. These results are consistent with cellular lipid efflux to apoproteins, and therefore ABCA1 activity at the tissue culture level being a major predictor of plasma HDL-C levels [78]. They also identify NPC disease as a new cause of low HDL-C, and the first known cause of low HDL-C as a consequence of impaired ABCA1 *regulation*, rather than *mutation*. These results are summarized in Fig. 2.

Interestingly, HDL-C levels in plasma of Npc1-deficient mice are not diminished compared to normal mice [60, 79], suggesting variable effects of NPC1 protein deficiency on HDL metabolism in humans compared to mice. In a study using cultured *Npc*$^{-/-}$ mouse peritoneal macrophages, Chen et al. [80] found impaired apoA-I-mediated efflux of LDL-derived (endosomal/lysosomal) cholesterol, but no

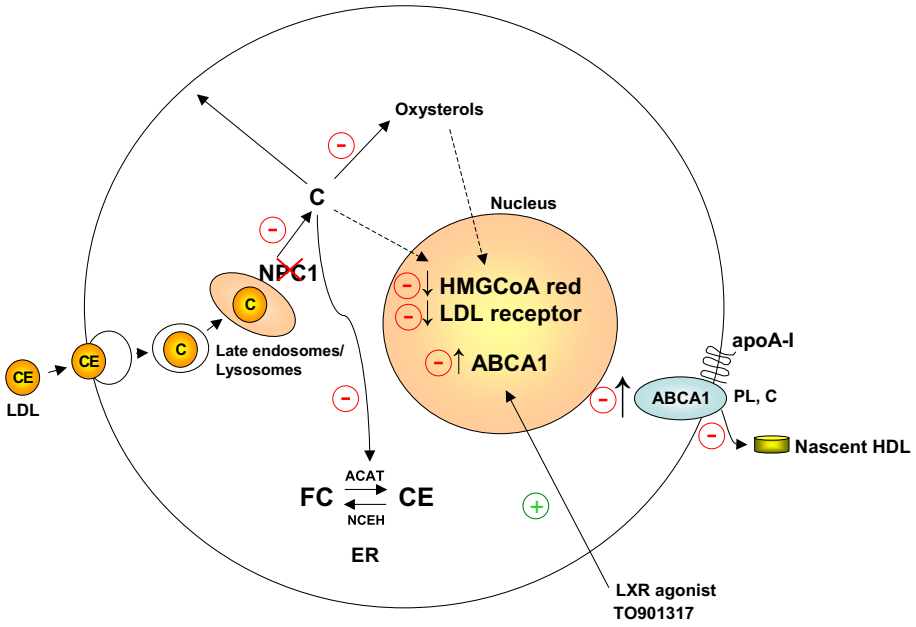

Fig. 2 Model of impaired ABCA1 regulation and HDL genesis in Niemann–Pick type C disease. Cholesterol derived from LDL or endogenous production normally leaves late endosomes/lysosomes by the actions of NPC1 to regulate cholesterol homeostasis at the endoplasmic reticulum (ER) and nuclear levels. Defects in NPC disease are indicated by *red symbols*. In the presence of NPC1 mutations, cholesterol is retained in the endosomal/lysosomal compartment and fails to downregulate cholesterol synthesis and LDL uptake appropriately as well as to deliver cholesterol to the ER for esterification by ACAT. ABCA1 is also not appropriately upregulated in the face of increased cell cholesterol content, leading to impaired efflux of lipids to apoA-I for nascent HDL formation

impairment of phospholipid efflux, suggesting the initial formation of apoA-I-phospholipid complexes is normal in *Npc1*$^{-/-}$ mice. The authors concluded that late endosomal/lysosomal cholesterol trafficked through the TGN serves as a preferential source of cholesterol for lipidation of apoA-I by ABCA1, and that Npc1 has a role in facilitating this trafficking.

In their studies, Tabas and colleagues have shown that elevated free cholesterol in macrophages leads to impaired apoA-I-mediated cholesterol efflux due to activation of proteasomal degradation of ABCA1 [81]. They also made the interesting observations that macrophages heterozygous for NPC deficiency or treated with U18666A to induce a mild NPC phenotype were protected against this loss of ABCA1 function and protein [81]. Further studies from their laboratory indicate that elevated free cholesterol in the endoplasmic reticulum induces apoptosis, and that partial NPC1 deficiency protects against apoptosis in cultured [82] as well as arterial wall macrophages [83]. These results suggest that partial NPC1 deficiency might protect against acute ischemic events by preventing cellular apoptosis and necrosis in atherosclerotic plaques as well as degradation of ABCA1 caused by accumulation of free cholesterol in the endoplasmic reticulum. This conclusion needs to be weighed against the partial down-regulation of ABCA1 and defect in cholesterol and phospholipid mobilization to apoA-I seen in *NPC1*$^{+/-}$ human fibroblasts [77], which could translate into decreased plasma HDL-C levels and an increased risk for development of atherosclerosis in NPC disease heterozygotes.

Bypassing the NPC mutation

Earlier studies had shown that incubation of NPC disease human fibroblasts with the oxysterol 25OH-cholesterol restores regulation of cholesterol synthesis and LDL receptor expression [76], and decreases the accumulation of lysosomal cholesterol [84]. In a recent study, Frolov et al. [85] found that production of the oxysterols 25OH- and 27OH-cholesterol is impaired in *NPC1*$^{-/-}$ human fibroblasts, and confirmed that addition of these oxysterols to cultured fibroblasts reduces cholesterol accumulation in late endosomes/lysosomes. These studies indicate the removal of excess cholesterol in the endosomal/lysosomal compartment in NPC disease can be achieved even in the absence of NPC1 protein activity. We have recently completed studies showing that addition of the LXR agonist TO901317 to human *NPC1*$^{-/-}$ fibroblasts increases ABCA1 expression, phospholipid and LDL-derived cholesterol efflux to apoA-I, and HDL particle formation as assessed by two-dimensional gel electrophoresis, to similar levels seen in *NPC1*$^{+/+}$ cells (Boadu et al., unpublished data). These results further indicate that depletion of the excess cholesterol accumulated in NPC disease cells can be achieved in the absence of NPC1 protein activity, and that the NPC1 protein is not required for the delivery of late endosomal/lysosomal cholesterol to ABCA1 (Fig. 1). Upregulation of ABCA1 expression by oxysterol- or TO901317-induced activation of LXR may provide the mechanism by which exogenous oxysterols [84, 85] or other LXR agonists deplete excess cholesterol from late endosomes and

lysosomes in *NPC1*$^{-/-}$ cells. Whether or not this increased ABCA1 mobilizes cholesterol from the plasma membrane and indirectly from late endosomes/lysosomes, or directly from the late endosomes/lysosome compartment [42], remains to be determined. The recent studies by Chen and colleagues [35] suggest that at least part of this would represent direct mobilization of lipids by ABCA1 in the endosomal/lysosomal compartment. Although functional NPC1 certainly facilitates the egress of late endosomal and lysosomal cholesterol in normal cells, these results suggest NPC1 may not be an absolute requirement for the mobilization of cell cholesterol for HDL particle formation by ABCA1.

Future directions

Further research is required to resolve the relative roles of direct versus indirect removal of intracellular pools of lipids by ABCA1 in total ABCA1-dependent lipid efflux. The overall role of vesicular versus nonvesicular transfer of lipids between closely apposed membranes or by diffusible carrier proteins in HDL formation, not discussed in this review, also remains to be determined [86, 87]. The relationships between ABCA1 and NPC1, and whether an NPC1-independent increase in ABCA1 activity can correct the lipid storage defect and neuropathology in NPC disease, are intriguing and important questions for further investigation.

Conclusions

ABCA1 is the rate-limiting protein in the initial formation of HDL particles, by delivering lipids to HDL apolipoproteins. The NPC1 protein facilitates intracellular trafficking of cholesterol and other lipid molecules. A deeper understanding of the mechanisms of lipid transport induced by these proteins, and their interactions, will provide new insights into potential therapies for atherosclerosis and NPC disease.

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