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The pathogenesis of Niemann-Pick type C disease: a role for autophagy?

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Abstract

Niemann-Pick type C disease (NPC) is a sphingolipid storage disorder that results from inherited deficiencies of intracellular lipid trafficking proteins, and is characterized by an accumulation of cholesterol and glycosphingolipids in late endosomes and lysosomes. Patients with this disorder develop progressive neurological impairment that often begins in childhood, is ultimately fatal and is currently untreatable. How impaired lipid trafficking leads to neurodegeneration is largely unknown. Here we review NPC clinical features and biochemical defects, and discuss model systems used to study this disorder. Recent studies have established that NPC is associated with an induction of autophagy, a regulated and evolutionarily conserved process by which cytoplasmic proteins are sequestered within autophagosomes and targeted for degradation. This pathway enables recycling of limited or damaged macromolecules to promote cell survival. However, in other instances, robust activation of autophagy leads to cell stress and programmed cell death. We summarize evidence showing that autophagy induction and flux are increased in NPC by signaling through the class III-PI3K/Beclin-1 complex. We propose that an imbalance between induction and flux through the autophagic pathway contributes to cell stress and neuronal loss in NPC and related sphingolipid storage disorders, and discuss potential therapeutic strategies for modulating activity of this pathway.

Keywords

Autophagy; Beclin-1; central nervous system; lysosome; mouse model; mTOR; neurodegeneration; Niemann-Pick type C disease

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Further reading, resources and contacts

Publications

There are several excellent, recent reviews on autophagy and disease, including:

Levine B, Kroemer G. Autophagy in the pathogenesis of disease. *Cell*, 2008; 132:27-42.

Mizushima N, Levine B, Cuervo AM, and Klionsky DJ. Autophagy fights disease through cellular self-digestion. *Nature*, 2008, 451:1069-1075.

Websites

The Ara Parseghian Medical Research Foundation supports scientific research on Niemann-Pick type C disease and related disorders. Their website includes information about the Foundation, the diseases they target with research support, and links to other sources of information. www.parseghian.org

The National Niemann-Pick Disease Foundation provides support for patients and their families, and promotes scientific research on the Niemann-Pick disease group. www.nnpdf.org

I Niemann-Pick type C disease

The sphingolipid storage diseases are a collection of ~40 genetically distinct disorders caused by inherited deficiencies of lysosomal hydrolytic activities or lipid transport which result in intracellular accumulations of cholesterol and lipids in the endosomal/lysosomal network. These disorders have an aggregate prevalence of approximately 1 in 8000 births worldwide (1). Among this group is Niemann-Pick type C disease (NPC, MIM 257220), an autosomal recessive, progressive neurovisceral disorder characterized by abnormal cholesterol trafficking and by intracellular accumulations of unesterified cholesterol and glycosphingolipids in late endosomes and lysosomes (2–8). NPC is caused by loss of function mutations in either *Npc1* (9) or *Npc2* (10), genes whose protein products mediate proper intracellular lipid transport through pathways that are incompletely understood.

NPC patients develop symptoms over a wide range of ages (11), and there is no strict correlation between disease-causing mutations and the severity of the clinical phenotype (4,12). While patients may initially exhibit systemic findings such as hepatosplenomegaly or obstructive jaundice, all eventually develop neurological and/or psychiatric symptoms, the severity of which is inversely associated with lifespan (13,14). Patients presenting with neurological symptoms during the first two years of life, classified as the most extreme, infantile cases of NPC, demonstrate delayed motor development and hypotonia, and typically die within their first 5 years (15). The classical form of NPC, which encompasses ~60–70% of cases, presents between 3 and 15 years of life. Parents of these patients are often the first to notice troublesome signs of the disease, such as loss of speech or increasing clumsiness. The disorder progresses to cause a constellation of neurological symptoms that may include cerebellar ataxia, dysarthria, dysphagia, cataplexy, seizures, dystonia, vertical gaze palsy, progressive dementia and death by 8 – 25 years (11). Adult onset cases of NPC are well documented although much less common, and their unusual clinical presentation often initially leads to misdiagnosis. Most adults with NPC develop symptoms in their second or third decades of life, though onset can occur as late as the mid-sixth decade (16). These adult patients develop symptoms similar to the classical form of juvenile NPC and usually die when in their late 30's or 40's. At the current time, there are no effective treatments available to patients with this devastating disorder.

II NPC pathology and biochemical defects

While heterogeneity characterizes the clinical presentation of NPC, there is less pathological heterogeneity at the biochemical level. The vast majority of cases show prominent accumulations of unesterified cholesterol, sphingolipids and complex gangliosides in late endosomes and lysosomes of most cells types of the body (2–4,11). Notably, a subset of patients with certain mutations demonstrate much less lipid storage (17), and central nervous system (CNS) pathology is accompanied by particularly prominent storage of sphingolipids and complex gangliosides. These lipid accumulations result from impairments of cholesterol and sphingolipid trafficking that cause a multifaceted toxic response in the liver, spleen and brain.

A. Histopathology

The progression of NPC in the periphery is characterized by an enlargement of the liver and spleen that results from the presence of lipid-laden macrophages, termed foam cells. Kupffer cells in the liver and splenic macrophages, predominantly in the red pulp, exhibit marked cytoplasmic vacuolization due to an accumulation of cholesterol, phospholipids and glycolipids.

Impairment of lipid trafficking also affects the CNS and results in neuron loss throughout the brain (18). The occurrence of swollen neuronal cell bodies in many brain regions, including cortex, basal ganglia, thalamus, cerebellum and brainstem is also characteristic of NPC and

reflects lipid accumulation within late endosomes and lysosomes. The cell body of these neurons and their axonal processes also contain granular material, which by electron microscopy appears as membrane-bound polymorphous bodies containing loosely packed lamellae or as dense osmiophilic inclusions. Additional neuronal pathology includes the formation of ectopic dendrites, swelling of proximal axons to so-called meganeurites, and the presence of axonal spheroids, changes indicative of a neuroaxonal dystrophy (18). Like several other neurodegenerative diseases, NPC is classified as a tauopathy due to the presence of intracellular aggregates of the microtubule binding protein tau, which are biochemically identical to aggregates in Alzheimer disease (19). These changes in the NPC brain are accompanied by gliosis in grey and white matter, microglial activation and cerebral atrophy.

B. Biochemistry

Lipid trafficking defects within the NPC brain reflect defects in the pathway by which cholesterol and other lipids arrive in neurons and are sorted intracellularly. Studies in normal mice have demonstrated that cholesterol accumulation in the CNS markedly increases during the first three weeks a life, a period of late development and myelination (20,21). Lipoprotein cholesterol from the periphery does not cross the blood brain barrier to enter the CNS, even during this early phase of brain development, and therefore *de novo* synthesis is required to meet the needs of both the developing and mature nervous system (22–24). Neurons and other CNS cell types obtain the cholesterol they require through endogenous synthesis or by uptake of lipoprotein cholesterol particles produced and released within the nervous system, possibly by astrocytes (25). Once target cells internalize these particles, unesterified cholesterol and companion lipids are trafficked from the endosomal/lysosomal system to compartments such as the Golgi and endoplasmic reticulum for processing and to serve as substrates for further reactions (26). Derivatives of cholesterol include oxysterols, which signal a decrease in the *de novo* synthesis of cholesterol and therefore provide negative feedback (27,28).

In NPC fibroblasts, lipoprotein cholesterol particles are internalized without disruption but become entrapped within trafficking vesicles, particularly late endosomes and lysosomes (Fig. 1) (29–31). The insufficient efflux of cholesterol and lipids out of these structures causes a two-fold problem: unesterified cholesterol, sphingolipids and complex gangliosides accumulate within vesicles in the cell's cytoplasm, while there is a simultaneous paucity of cholesterol and other essential lipids in organelles to which these compounds were originally destined. Decreased cholesterol in the endoplasmic reticulum, Golgi apparatus and other internal organelles causes deleterious effects on a multitude of processes dependent on proper membrane composition (32). Disrupted trafficking also results in a paucity of substrate for further synthetic reactions. For example, decreased availability of unesterified cholesterol impairs production of oxysterols, which may lead to increased cholesterol synthesis and an exacerbation of cholesterol accumulation in NPC cells (33).

III Genetics of NPC

Almost a century has passed since the original reports by Albert Niemann and Ludwick Pick described patients with hepatosplenomegaly and rapidly progressive brain damage. Since then, biochemical and genetic studies have helped define the classification of the Niemann-Pick disease group. Niemann-Pick types A and B disease (MIM 257200 and 607616), distinct from NPC, are caused by loss of function mutations in the acid sphingomyelinase gene, encoding an enzyme that metabolizes sphingomyelin to ceramide and phosphocholine. While fibroblasts from NPC patients may show reduced sphingomyelinase activity in culture, this reduction is due to an imbalanced lipid and cholesterol environment, and removal of cholesterol from the media recovers enzymatic activity (34). In contrast, NPC fibroblasts exhibit an accumulation of unesterified cholesterol indicative of a defect in cholesterol trafficking. The major complementation group for NPC (11) was mapped to the proximal long arm of chromosome

18, and mutations in the *Npc1* gene were found in ~95% of patients with this disease (9). The remaining 5% of NPC patients were subsequently found to have loss of function mutations in a separate gene, *Npc2* (10).

The *Npc1* gene encodes a multipass transmembrane protein, NPC1, which is localized to late endosomes and lysosomes (35,36). The encoded protein is predicted to contain a sterol-sensing domain, similar to that found in regulators of cholesterol metabolism and in Patched, a receptor for the secreted signaling protein Hedgehog (9,37). Experimental evidence indicates that NPC1 is involved in the efflux of LDL-derived unesterified cholesterol from late endosomes and lysosomes to other intracellular compartments (30,31). Multiple disease-causing mutations within the *Npc1* coding region have been identified which lead to its loss of function (38). The NPC1 protein is conserved within the animal kingdom, and loss of function mutations in orthologous genes in yeast (39), worms (40), flies (41), mice (42), cats (43) and dogs (44) disrupt lipid trafficking. Introduction of the yeast *Npc1* ortholog, *Ncrp1*, into Chinese hamster ovary cells deficient in NPC1 is sufficient to reverse lipid trafficking defects associated with *Npc1* deficiency, demonstrating that this protein is functionally conserved from yeast to mammals (39).

The *Npc2* gene encodes a small, soluble protein that is localized to lysosomes, but is also secreted (10,45). Loss of function mutations in this gene result in a disease phenotype that is clinically and biochemically indistinguishable from the disease caused by *Npc1* mutations. The NPC2 protein binds cholesterol, and structural analysis has identified a hydrophobic pocket that serves as a putative cholesterol-binding domain (46). Disease causing mutations in *Npc2* that affect amino acid residues surrounding this pocket impair cholesterol binding (47). Analysis of *Npc1/Npc2* double mutant mice demonstrates that the disease phenotype is similar to that occurring in *Npc1* or *Npc2* single mutants, providing genetic evidence that the NPC1 and NPC2 proteins function in the same pathway to regulate intracellular lipid transport (48).

IV NPC1 deficient mice

A. Phenotype

The most commonly used mammalian model of NPC, BALB/cNctr-*Npc1*^{mln}/J mice (Jackson Laboratory #003092), contains a transposon insertion into *Npc1* exon 9 (49). Animals homozygous for this insertional mutation fail to express functional NPC1 protein and exhibit a degenerative phenotype that begins around 7 weeks (49,50). The severity of the phenotype progresses with age and includes weight loss, tremors, ataxia, and death by 10–12 weeks. Liver and spleen contain an abundance of foam cells, reminiscent of the histopathological changes seen in humans with this disorder (Fig. 2). *In vivo* knockdown of *Npc1* only in the liver of normal mice recapitulates the hepatic pathology associated with NPC, generating a novel model to examine liver dysfunction associated with disease independent of CNS involvement (51).

Global loss of NPC1 expression leads to CNS pathology including vacuolated neurons that accumulate glucosylceramide, lactosylceramide, complex gangliosides and cholesterol (52–54), abnormally swollen axons, and decreased myelination in the cerebral white matter (55). A prominent feature of CNS pathology is loss of Purkinje cells, the major neuronal cell type transmitting output from the cerebellum (56). These highly specialized neurons are responsible for timing and smoothness of movements, and dysfunction of or lesions within the cerebellum can lead to ataxia. Loss of Purkinje cells is initially apparent in the cerebellar vermis by 7 weeks. Progressive Purkinje cell loss proceeds from the anterior to posterior vermis, yet largely spares lobules IX and X, even in mice with end-stage disease (56). Interestingly, 2-year-old *Npc1* +/- mice also exhibit mild Purkinje cell loss suggesting that haploinsufficiency for NPC1 may have adverse consequences in the aging brain (57).

B. Lipid trafficking defects

NPC1 deficient mice have been used to explore how lipid trafficking defects lead to progressive neurodegeneration. Attention has focused on the role of glycosphingolipids, including complex gangliosides such as GM2, which are not commonly found in the adult brain but accumulate in NPC neurons (54). These gangliosides are involved in normal brain development, and their presence in the NPC brain has been implicated in pathogenesis, including the formation of ectopic dendrites that branch from swollen axons (18). These abnormally long and inappropriately located processes may contribute to neuronal dysfunction. Based on this notion, on indications that some glycosphingolipids are toxic, and on findings that cholesterol accumulation in the NPC brain is ganglioside-dependent (58), glycosphingolipid synthesis has emerged as a therapeutic target in NPC and related sphingolipid storage disorders (59–61).

Trafficking defects also lead to diminished production of cholesterol derivatives including neurosteroids, which normally help mediate brain development, growth and differentiation (62). A single injection of the neurosteroid allopregnanolone, whose production is significantly decreased in NPC mice, is sufficient to promote Purkinje cell survival and delay disease phenotype (63). Although initial evidence suggested that this beneficial effect was mediated through GABA_A receptor signaling, more recent studies indicate that it occurs by activation of the pregnane X receptor (PXR) or liver X receptor (LXR) (64). These steroid receptors are ligand activated transcription factors that bind the oxysterols 25-hydroxycholesterol and 27-hydroxycholesterol (27,28). Pathways activated by the receptors promote efflux of cholesterol from late endosomes and lysosomes, and suppress endogenous cholesterol synthesis. Exogenous application of either 25-hydroxycholesterol or 27-hydroxycholesterol is sufficient to correct sterol defects in NPC1 deficient human fibroblasts and to mobilize cholesterol from the late endosomal/lysosomal compartment (65). LXR agonists also promote cholesterol efflux from the NPC mouse brain, slow neurodegeneration and prolong life (66), suggesting that activation of these pathways is a promising therapeutic strategy.

C. Cell autonomous versus non-cell autonomous cell death

Defects in lipid trafficking that occur in NPC lead to cell autonomous neuronal death. This was established by an analysis of chimeric mice that express functional NPC1 protein in only a subset of cells (67). In this model, NPC1 deficient Purkinje cells died, even when neighboring cells harbored normal levels of NPC1 protein. Conversely, Purkinje cells with functional NPC1 were spared, even in an NPC1-deficient environment. Examination of NPC1 deficient Purkinje cells in mutant mice revealed evidence of autophagy, suggesting that this pathway is activated in the NPC brain and may play a role in pathogenesis.

V Autophagy

Autophagy is a conserved cellular pathway that mediates the turnover of damaged or aged macromolecules and organelles. Three types of autophagy have been described, including microautophagy, chaperone mediated autophagy and macroautophagy. For the purposes of this review, we will discuss only macroautophagy and refer to it simply as “autophagy”.

At basal levels, autophagy is a house keeping mechanism for the constitutive degradation of long-lived proteins and other macromolecules inaccessible to the ubiquitin-proteasome pathway. Regulated degradation is governed by activation of autophagy related genes, or *atg*'s (68). Autophagy begins with the generation of an isolation membrane, a crescent shaped double membrane that originates from either smooth ER or another subcellular location that has yet to be defined (Fig. 3) (69). This isolation membrane surrounds cargo targeted for degradation. During the engulfment process, two ubiquitin-like conjugation reactions are required for progression through autophagy: the formation of an Atg12-Atg5-Atg16 complex

and the conjugation of Atg8 (also known as microtubule-associated protein 1 light chain 3 protein, LC3) to phosphatidylethanolamine (70). This latter step is commonly used as a biochemical marker for the induction of autophagy (71,72). Following engulfment, mature autophagosomes fuse with lysosomes to enable degradation of contents and recycling of their components, a process indicative of flux through the autophagic pathway (73).

Autophagy can be induced above basal levels by several stimuli, including amino acid starvation or cellular stress. This induction is mediated by signaling through the mammalian target of rapamycin (mTOR) or the Class III-PI3K/Beclin-1 complex (74). mTOR is a negative regulator of autophagy and its chemical inhibition by rapamycin relieves this blockade (74, 75). The Class III-PI3K/Beclin-1 complex is necessary for the induction of autophagy and can activate autophagy independent of mTOR inhibition (76).

Autophagy is necessary for normal CNS development and function, and has been implicated in the pathogenesis of a diverse array of neurodegenerative diseases, including protein aggregation disorders such as Alzheimer (77), Parkinson (78), and Huntington disease (79), and the sphingolipid storage disorders (80). For age-related diseases associated with protein misfolding, inducing autophagy in model systems enhances the clearance of misfolded or mutant proteins that are not efficiently degraded by the proteasome. This enhancement of protein clearance abrogates disease phenotypes (81), indicating that autophagy may have beneficial effects in some disease processes. Conversely, genetic deletion in mouse CNS of either of two autophagy related genes, *atg5* (82) or *atg7* (83), leads to neurodegeneration and the accumulation of ubiquitinated protein aggregates, demonstrating that basal autophagy contributes significantly to protein quality control and cell survival in the nervous system.

Although the induction of autophagy may alleviate disease in some instances, excessive autophagy can have deleterious effects in others. In mouse embryonic fibroblasts deficient in apoptosis (*bax/bak* $-/-$ cells), the classic programmed cell death pathway, application of death stimuli such as staurosporin or etoposide triggers an apoptosis-independent cell death pathway (84). Further genetic manipulation of these cells through RNAi knockdown of two autophagy regulators, *atg5* and *beclin-1*, inhibited cell death, demonstrating the occurrence of a death program mediated by autophagy. Excessive activation or failure to complete autophagy has since been implicated in numerous disease processes, including some neurodegenerative diseases.

VI Autophagy in NPC and other lysosomal storage disorders

Induction of autophagy in NPC1 deficient mice was first reported by Ko et al, who observed frequent autophagic vacuoles in Purkinje cells of mutant mice accompanied by increased accumulation of lipidated LC3 in cerebellar lysates (67). Similar findings have since been reported by our lab and others (85,86), confirming autophagy induction in the NPC brain, and extending this observation to additional pathologically affected organs, such as liver, and to primary human fibroblasts from NPC patients. Flux through the autophagic pathway is also increased in NPC1 deficient cells, as confirmed by monitoring the degradation of long-lived proteins and by establishing that inhibition of lysosomal proteases increases lipidated LC3 levels in mutant cells (86); in contrast, inhibition of lysosome function does not alter LC3 levels in pathological situations characterized by impaired autophagosome-lysosome fusion (73). The induction of autophagy in NPC1 deficiency is not mediated by signaling through the Akt-mTOR-p70 S6K pathway, but rather by the class III-PI3K/Beclin-1 complex (86). Beclin-1 expression is mildly elevated in NPC1 deficient mouse tissue and human fibroblasts, and Beclin-1 knock-down by siRNA decreases long-lived protein degradation (86). Induction of autophagy and increased Beclin-1 expression are similarly observed in NPC2 deficient primary human fibroblasts and in control cells treated with U18666A (86–88), a compound known to induce

an accumulation of unesterified cholesterol. In contrast, Gaucher disease fibroblasts, which traffic sphingolipids normally (26), show wild type levels of Beclin-1 and LC3-II, suggesting that autophagy induction is sensitive to alterations in these lipids (86). We envision that the class III-PI3K/Beclin-1 complex senses changes due to the dysregulation of lipid trafficking in NPC and other disorders and activates autophagy.

While NPC1 deficiency is characterized by elevated induction and flux through the autophagic pathway, ubiquitinated proteins accumulate in the mutant mouse brain. These ubiquitinated proteins can be visualized in cerebellar tissue of NPC1 deficient mice by immunohistochemistry (56). Fractionation studies indicate that much of this material is in the endosomal/lysosomal compartment (85). The paradoxical accumulation of ubiquitinated proteins in the setting of increased autophagic degradation suggests that flux through this pathway is not sufficient to handle the quantity of proteins targeted for degradation (Fig. 4). Situations where induction and flux are disproportionate to each other may lead to autophagic stress, a possible mediator of neuronal dysfunction and a precursor to cell death (89).

Impaired flux through the autophagic pathway has also been observed in lysosomal storage disorders characterized by deficiencies of specific lysosomal hydrolases, including Pompe disease (90), mucopolipidosis type IV (91), multiple sulfatase deficiency and mucopolysaccharidosis type IIIA (92). Mouse models of many of these disorders exhibit increased lipidated LC3, an accumulation of enlarged autophagosomes, and impaired autophagosome-lysosome fusion resulting in decreased substrate degradation. A similar block of autophagosome-lysosome fusion has been documented in a mouse model of juvenile neuronal ceroid lipofuscinosis, a degenerative disorder caused by mutations in a novel, multipass transmembrane protein localized to late endosomes and lysosomes (93). These data suggest that impaired flux through the autophagic pathway is common to many lysosomal storage diseases, and that an imbalance between induction and flux may contribute to the pathology of this diverse array of disorders by causing autophagic stress or triggering autophagic cell death.

VII Clinical implications and outstanding research questions

Current therapeutic options for patients with the sphingolipid storage disorders are limited (see Table 1 for a summary of NPC therapeutic strategies). For diseases caused by deficiencies of lysosomal hydrolases, ideal therapies would replace the deficient enzymes, however administration presents a significant challenge. Intravenous infusion of active enzymes has yielded some success in Gaucher disease, Fabry disease, mucopolysaccharidosis I and glycogen storage disease type II (94). Yet, this approach treats only the systemic and not CNS disease manifestations because of limited blood brain barrier permeability. Similarly, bone marrow transplantation is effective at reducing systemic but not neurological symptoms in NPC (95), as is the case for most, but not all sphingolipid storage disorders (96). An alternative treatment strategy is substrate reduction therapy, which aims to reduce accumulating material in endosomes and lysosomes based on the notion that excess substrate contributes to pathogenesis. In the case of NPC, reduction of intracellular cholesterol by a combination of cholestyramine, lovastatin and nicotinic acid was able to lower liver and blood levels of cholesterol, but had little effect on disease progression (97,98). For disorders characterized by neurodegeneration and the accumulation of glycosphingolipids in the CNS, small molecule inhibitors of glycosphingolipid synthesis have attracted considerable attention. Imino-sugars such as miglustat (*N*-butyl-deoxynojirimycin) are reported to act through this mechanism, delay symptom onset and prolong survival in mouse models of NPC (60) and Sandhoff disease (99). A recently completed clinical trial of miglustat in a small number of NPC patients similarly yielded a mild, encouraging effect after one year of treatment (61).

Despite these various treatment strategies, only modest clinical improvements have been achieved and new therapeutic approaches are needed. The recently described relationship between autophagy and NPC may lead to novel therapeutic targets. A critical, unanswered question is whether activation of this pathway in NPC patients is detrimental and promotes cell death, or is compensatory and promotes cell survival. To resolve this issue, autophagy will need to be modulated in NPC model systems and effects on disease phenotype established. If it is determined that increasing autophagy is beneficial, then administration of compounds like rapamycin would be an interesting therapeutic strategy. Rapamycin, in the form of Rapamune® (also termed sirolimus), has been tested in humans as an immune suppressant for organ transplant patients (100). Alternatively, if autophagy is found to enhance the NPC phenotype, small molecule inhibitors of this pathway may provide therapeutic benefit. A search for compounds that specifically modulate the activity of the autophagic pathway in the CNS will likely yield important reagents for investigational studies of disease mechanisms, and may provide lead compounds for the development of new treatments.

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Figure 1. Lipid trafficking defects in Niemann-Pick type C disease

Left panel. Under normal conditions, lipoprotein cholesterol particles enter the endosomal network after binding to cell surface receptors. Unesterified cholesterol and companion lipids are trafficked from the endosomal/lysosomal system to the Golgi, endoplasmic reticulum and other intracellular compartments.

Right panel. NPC1 or NPC2 loss of function inhibits the egress of unesterified cholesterol and sphingolipids from late endosomes and lysosomes, resulting in their accumulation at these sites and a paucity of these lipids at the intracellular compartments to which they were destined. Abbreviation: ER, endoplasmic reticulum.

Figure 2. NPC histology

Foamy macrophages are abundant in the liver and spleen of NPC1 deficient mice (examples at arrowheads). In the brain, loss of cerebellar Purkinje cells is a prominent pathologic feature. These large, pyramidal-shaped neurons normally reside at the interface of the molecular layer and granular cell layer of the cerebellar folia (examples at arrowheads). These cells die in NPC1 deficient mice and are replaced by reactive astrocytes.

Figure 3. The autophagic pathway

Autophagy occurs at low, basal levels and can be induced by multiple stimuli, including amino acid starvation or mTOR inhibition. It begins with the formation of an isolation membrane, a crescent-shaped sequestering membrane also termed a phagophore, and the induction of a ubiquitin-like conjugation reaction that couples soluble LC3-I with phosphatidylethanolamine. Lipidated LC3-II studs the phagophore membrane as it engulfs macromolecules, organelles and other cargo destined for degradation. Mature autophagosomes fuse with lysosomes to form autolysosomes, thereby degrading cargo and enabling components to be recycled. Not shown is the fusion of some autophagosomes with endosomes to form so-called amphisomes, another vesicle that fuses with lysosomes to facilitate cargo degradation.

Abbreviations: mTOR, mammalian target of rapamycin; LC3, microtubule-associated protein 1 light chain 3 protein

Activity of the autophagic pathway

Figure 4. Autophagic induction and flux in NPC and other lysosomal storage disorders

In healthy cells, macromolecules are efficiently targeted for degradation by autophagy in a process characterized by proportionate induction and flux through the pathway. This steady-state equilibrium is disrupted in NPC and other lysosomal storage diseases. In NPC, our working model holds that sphingolipid trafficking defects alter membrane lipid composition and induce autophagy through the activity of the class III-PI3K/Beclin-1 complex. This induction can be measured experimentally by an accumulation of LC3-II in NCP1- or NPC2-deficient protein lysates. The enhanced induction of autophagy occurs coordinately with a more modest increase in autophagic flux, resulting in the accumulation of ubiquitinated proteins in tissues from NPC patients and animal models. Disproportionate induction and flux through the autophagic pathway can lead to cell stress (so-called “autophagic stress”) and may trigger cell death, with dire consequences for NPC patients.

Abbreviation: LC3, microtubule-associated protein 1 light chain 3 protein

Table 1

NPC therapeutic targets and strategies

Mechanism of Action	Therapeutic	Refs
<i>I. Current and former strategies</i>		
A. Introduction of functional <i>Npc1</i> or <i>Npc2</i>	Bone Marrow transplantaion	#95
B. Substrate reduction therapy		
i. Reduce intracellular cholesterol	Cholestyramine, lovastatin and nicotinic acid	#97,98
ii. Reduce glycosphingolipid accumulation	Miglustat (N-butyl-deoxynojirimycin)	#61
<i>II. Possible future strategies</i>		
A. Activate endogenous pathways that promote cholesterol efflux	LXR or PXR agonist	#63–66
B. Chemical modulation of autophagy	Rapamycin derivatives or novel autophagy inhibitors	#67,85,86