Niemann–Pick C disease: cholesterol handling gone awry

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Niemann–Pick C disease (NPC) is a debilitating, recessive disorder in humans that causes unrelenting neurological deterioration and is complicated by the presence of lipid-laden foamy cells in the major organs of the body. NPC fibroblasts cultured with an excess of low density lipoprotein (LDL) abnormally sequester cholesterol in their lysosomes. Biochemical analyses of NPC cells suggest an impairment in the intracellular transport of cholesterol to post-lysosomal destinations occurs in NPC. The recent identification of the NPC gene, *NPC1*, provides a definitive diagnosis of the disease and a means of studying this key component of intracellular cholesterol transport and homeostasis.

NIEMANN–PICK C disease (NPC) is a devastating, autosomal-recessive, neurovisceral lipid storage disorder that is characterized by a combination of neurodegeneration and hepatosplenomegaly. The cellular phenotype includes a profound accumulation of unesterified lysosomal cholesterol. The gene responsible for NPC, *NPC1*, has been identified by positional cloning and encodes a protein that contains: (1) multiple transmembrane domains; (2) a leucine zipper; (3) a lysosomal/endocytic targeting signal; and (4) a sterol-sensing domain. The putative transmembrane motifs of the NPC1 protein show significant sequence homology to hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase, SREBP (sterol regulatory element binding protein) cleavage-activating protein (SCAP) and Patched (PTCH). Although the mechanism of action of NPC1 has yet to be defined, its identification should lead to a better understanding of the cellular defect underlying NPC and improve our chances of developing effective treatments for this disease.

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Defining the cholesterol pathway: identifying the genetic basis of inherited disease

By identifying the genetic basis of inherited disease information as to how important metabolic proteins function has been gained. Autosomal recessive disorders, such as familial hypercholesterolemia (FH)[1], Wolman disease (WD)[2], cholesteryl ester storage disease (CESD)[2] and NPC (Ref. 3), have taught us a great deal about how cholesterol is internalized and used by a cell. FH is characterized by increased levels of serum low-density lipoprotein (LDL) cholesterol, which causes patients to suffer from coronary artery disease and xanthomas. Heterozygotes for the FH allele typically have heart attacks in the third to fourth decade of life, compared with homozygotes who can have them as early as in childhood. The inability of FH patients to effectively use LDL cholesterol is due to a mutation in the gene encoding the membrane-bound LDL receptor (LDLR), which facilitates the uptake of LDL by receptor-mediated endocytosis[1]. Uptake by LDLR is the first step in the binding and internalization of LDL cholesterol (Fig. 1).

Once inside the cell, LDL is translocated to lysosomes where its cholesteryl ester component is hydrolysed by the enzyme lysosomal acid lipase (LAL), the gene product of *LIPA* (Ref. 2). Mutations in *LIPA* cause at least two distinct disorders, WD and CESD. WD is evident in infancy and causes intense vomiting, abdominal distension, hepatosplenomegaly, diarrhea and anemia; death usually occurs before one year of age. CESD is a milder disease with a variable phenotype in which the most common symptom is hepatomegaly. In both WD and CESD, cholesteryl esters accumulate in the lysosomes. The metabolic step with which NPC is associated is the movement of free cholesterol (i.e. the product of LAL hydrolysis) from the lysosomes to other cellular destinations (Fig. 1)[3–12].

NPC1 encodes a key regulator of the LDL endocytic pathway and modulates levels of intracellular cholesterol[13,14]. Increasing our understanding of the clinical, biochemical and genetic lesions associated with NPC might assist in the development of new strategies to improve the care and treatment of patients and might offer invaluable

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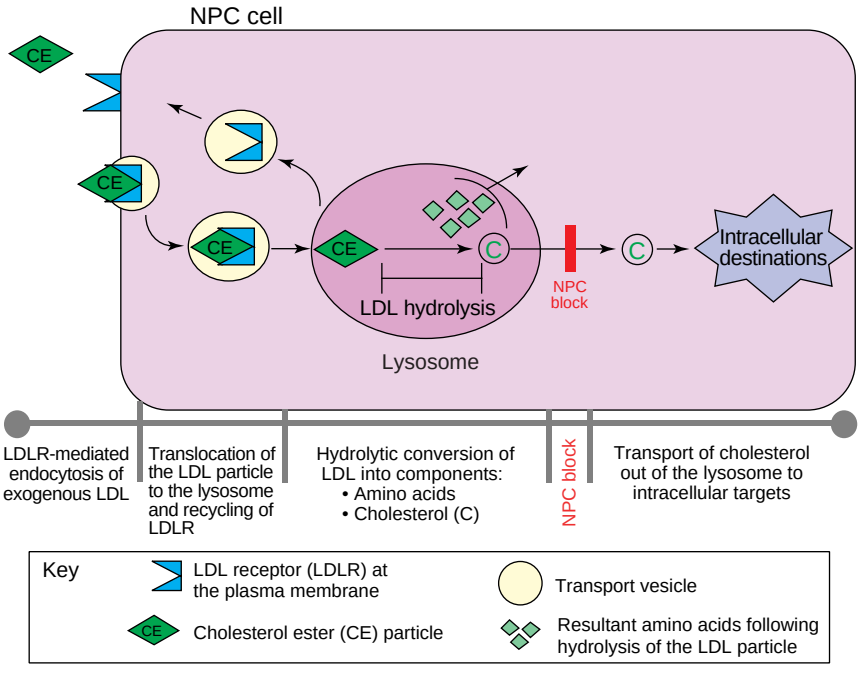

Figure 1. Intracellular processing of low density lipoprotein (LDL)-derived cholesterol in Niemann–Pick C (NPC) cells, adapted from the work of Brown and Goldstein[1]. LDL is internalized by LDL receptor (LDLR)-mediated endocytosis and is translocated to the lysosome where it is hydrolysed into amino acids and free cholesterol. In normal cells, cholesterol is then transported out of the lysosome to be used by the cell to synthesize bile acids, steroids and the plasma membrane. In NPC cells, cholesterol accumulates in the lysosome, affecting the normal homeostatic responses associated with LDL uptake; there is a delay in the downregulation of LDLR levels and *de novo* cholesterol synthesis and in the upregulation of cholesterol esterification.

insight into the cellular mechanisms associated with cholesterol metabolism.

Characteristics of NPC

Clinical background

NPC is a fatal disorder marked by progressive neurological deterioration that is sometimes accompanied by hepatosplenomegaly. The clinical manifestations of NPC vary and have been well documented[3,15,16]. Typically, infants with NPC have neonatal jaundice that resolves, but by early childhood they develop signs of neurological decline. NPC patients are often described as being clumsy and as having slurred speech, and many have behavioral and learning difficulties. As symptoms progress and become more severe, spasticity, cataplexy, seizures and dementia often occur. Although many of the symptoms of NPC vary between patients, one consistent symptom, present in all diagnosed individuals, is that of supranuclear vertical gaze palsy – the impaired ability to move the eye upwards or downwards. As neurological deterioration advances, patients are unable to walk or care for themselves without constant assistance. Death typically occurs during the second decade of life, often because of pulmonary infection[3,15].

The predominance of this disease is estimated to be about one in a million[3,13], suggesting a carrier rate of 1 in 500. However, French Acadians of Nova Scotia and Spanish-Americans of southern Colorado are geographically isolated populations where the prevalence of NPC is much higher[3]. In Yarmouth County, Nova Scotia, the origin of the genetic mutation in *NPC1* can be traced to a couple that immigrated to that region[17]. The Nova Scotia variant, sometimes referred to as Niemann–Pick D (or NPD), has recently been shown to be an allelic variant of NPC (Ref. 18). The carrier rate in Yarmouth County is estimated to be as high as 1 in 10. The identification of *NPC1* provides a basis for a definitive, genetic diagnosis of patients and a means to determine the carrier status of unaffected family members.

Cellular phenotype

NPC is characterized by a profound accumulation of unesterified cholesterol in lysosomes. In cell culture, the introduction of LDL to the fibroblasts of NPC patients causes the cells to accumulate large amounts of lysosomal cholesterol, whereas those of unaffected individuals do not (Fig. 2)[9,10]. The NPC protein could thus act as a 'gatekeeper' in the transport of lysosomal cholesterol to other cellular targets.

Not only does sterol accumulate in the lysosomes of NPC cells but also cholesterol homeostatic responses are affected. In unaffected cells, normal lipid transport from the lysosomes to other locations is marked by three cellular responses: cholesterol esterification by acyl-coA: cholesterol acyl transferase (ACAT), reduced HMG-CoA reductase activity and reduced LDLR levels[11,12]. These collective responses, referred to as mechanisms of cholesterol homeostasis, work together to modulate the levels of post-lysosomal cholesterol[12]. When cholesterol exits the lysosome, it is translocated to the endoplasmic reticulum (ER) and the plasma membrane. The appearance of cholesterol at the ER is reflected in the increased rate of cholesterol esterification by the ER-bound enzyme ACAT, which converts cholesterol to cholesterol esters. The cell also responds to post-lysosomal cholesterol by downregulating *de novo* cholesterol synthesis by reducing HMG-CoA reductase activity and reducing LDL uptake by downregulating LDLR levels. NPC cells show a significant delay in these homeostatic responses. Although it is clear that sequestered cholesterol in NPC cells eventually exits the lysosome, it is at a much slower rate than in unaffected cells[4,19]. The fact that cholesterol homeostatic responses are delayed and not blocked suggests that the NPC protein is necessary but not essential for lysosomal cholesterol efflux.

The diagnosis of NPC patients is based on a clinical evaluation that is supported by both cytological and biochemical assessments. Skin-punch biopsies are performed on patients to isolate fibroblasts. These fibroblasts are cultured with LDL and are stained with filipin to reveal the presence of accumulated, unesterified cholesterol in the lysosomes (Fig. 2). The addition of [^{3}H]oleate to the culture medium also permits the assessment of cholesterol esterification; low rates of cholesterol esterification are indicated by low levels of cholesteryl-[^{3}H]oleate[11,12].

The NPC mutation has been used as a tool to define the metabolic steps associated with intracellular cholesterol trafficking. In addition to the impaired translocation of endocytosed cholesterol out of the lysosomes[3–12], it has been shown that the movement of cholesterol from the plasma membrane to the ER is diminished[8,19–21]. Measurement of cholesterol released from the plasma membrane of NPC cells by sphingomyelinase shows that lower levels of ACAT-derived cholesterol esters are present in NPC cells than in normal controls. These data indicate that the NPC protein plays a role in the translocation of cholesterol from the plasma membrane to the ER. An abnormal accumulation of cholesterol in the Golgi of NPC cells indicates that this organelle plays a role in sterol transport[10]. The normal increase in filipin–cholesterol deformations in the membranes of *cis*/*medial*-cisternae and *trans*-Golgi vacuoles is not observed in NPC; cholesterol levels only increase in the membranes of *trans*-Golgi cisternae[22]. Furthermore, the treatment of NPC cells with sphingomyelinase and brefeldin A, which disrupts the Golgi and fuses it with the ER, results in increased levels of cholesterol esterification by ACAT, further indicating the presence of cholesterol in the Golgi[19].

Results of a recent study monitoring the intracellular trafficking of LDL-derived cholesterol within human subjects have confirmed the findings of *in vitro* studies[23]. In this elegant experiment, the fates of *de novo* and exogenously derived LDL were compared. Both [^{3}H]LDL and [^{14}C]mevalonate, a precursor of *de novo* cholesterol synthesis, were introduced into the serum of NPC patients and unaffected individuals. The metabolism of these tagged substrates was monitored in the plasma and bile of the subjects. As predicted, the intracellular processing of the [^{3}H]LDL was abnormal in the NPC group, as indicated by a lower level of [^{3}H]cholesterol in the plasma and bile. The amount of [^{14}C]cholesterol in the plasma and bile of NPC and control group subjects was virtually identical, suggesting that the *de novo* synthesis of cholesterol remains unaffected in NPC patients.

In an attempt to slow, stop or reverse the progressive decline of NPC patients, both therapeutic and dietary strategies have been assessed[3,24–27]. Neither the administration of cholesterol-lowering agents nor limiting dietary cholesterol has altered the neurological decline of NPC patients. Standard care is currently used to treat patients for seizures, cataplexy, dystonia and other major symptoms of the disease. To date, there are no other treatments with which to halt a patient's progressive neurological decline[3].

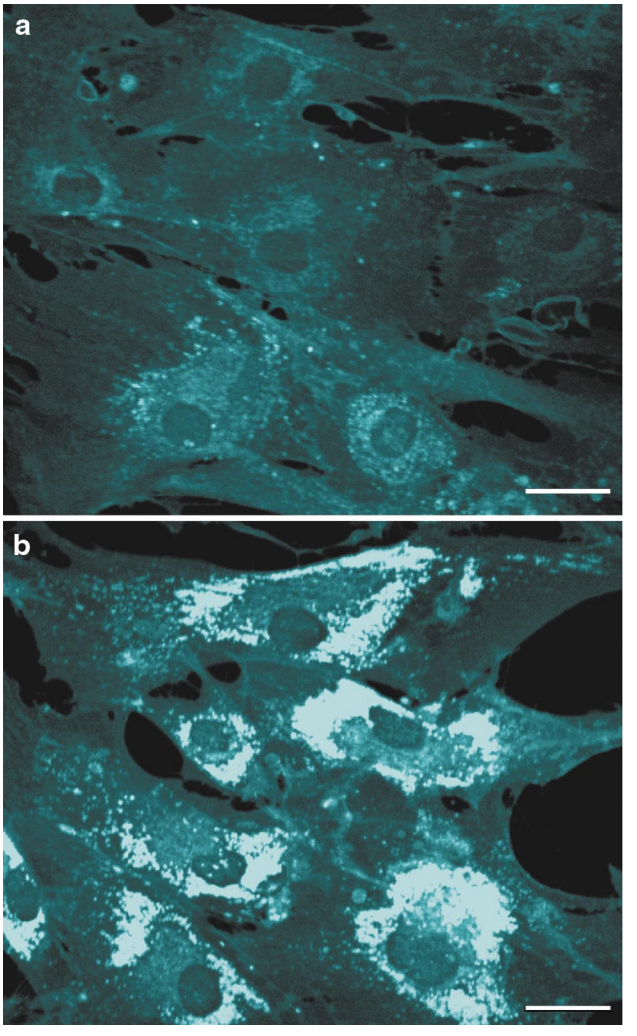

Figure 2. A fibroblast from (**a**) an unaffected individual and (**b**) an individual with Niemann–Pick C (NPC). Both are stained with filipin, a fluorescent cytochemical marker that recognizes unesterified cholesterol within the cell. Control cells show the general staining of unesterified cholesterol in the plasma membrane and perinuclear region, whereas NPC cells show profound staining of unesterified cholesterol within the lysosomes. Scale bars, 25 μm. Kindly provided by E.J. Blanchette-Mackie and N. Dwyer, National Institutes of Health, Bethesda, MD, USA.

Methods of studying the NPC defect

Animal models

Many naturally occurring animal models of NPC have been studied, including two mouse models of NPC on BALB/c and C57BL/6 genetic backgrounds, and a feline model[5,6,28,29]. The clinical and cytopathological course of these three models shows considerable similarity to the human disorder. Cell lines derived from these animals are impaired in their ability to esterify exogenously derived cholesterol[7,29,30]. The two mouse models have independent disruptions of the same gene, *npc1*, which is the murine ortholog of the human *NPC1* gene[14,31,32]. It has yet to be shown that the cat model has an orthologous defect.

The lysosomal storage disorder (LSD) mouse on a BALB/c strain (called BALB/c *npc*^{nih}) was the first animal model of NPC and has been the focus of extensive studies since 1977 (Ref. 5). These mice are born with lower body weights than their unaffected littermates and exhibit a progressive neurological condition that is accompanied by hepatosplenomegaly and wasting[5]. Hind-limb tremors, leading to eventual paralysis, cripple the mouse. Life expectancy is one-third of that of unaffected mice. In the feline model, affected cats are also of lower body weight than their littermates[29]. By eight weeks of age, they show neurological decline in the form of whole body tremors. An important reason for studying these animals is to understand the effects of the NPC mutation on biological systems, primarily that of the nervous system.

Infiltration by foamy macrophages of the liver, lung, spleen and lymph nodes is observed in all three animal models of NPC, and is a feature of the human disease[3,5,29,33]. Because neurodegenerative decline is the most debilitating aspect of NPC, an important goal of

Glossary

Cholesterol – A steroid alcohol that is important as a precursor of steroid hormones, bile, plasma and intracellular membranes.

Cholesterol homeostasis – The steady state of intracellular cholesterol that is maintained by different, inducible responses within the cell.

Filipin–cholesterol deformations – Pits and protuberances in the membranes of a cell that are caused by the interaction of a sterol-specific stain with free cholesterol.

Hepatosplenomegaly – Abnormally enlarged liver and spleen.

Leucine zipper – A motif within a protein that is ~30 amino acids long and that contains leucine repeats; the motif is believed to form homo- or heterodimers with other leucine-zipper-containing proteins.

Lipid storage cells – Any cell that abnormally accumulates lipids.

Lysosome – An organelle within the cell that has hydrolytic enzymes that break down proteins and certain carbohydrates.

Microsatellite markers – Polymorphic, simple repeat sequences that are amplified by PCR to follow the inheritance of specific chromosome alleles.

Ortholog – A gene that has evolved directly from an ancestral locus.

Pericentromeric – A subchromosomal region, either on the long or short arm, that is in proximity to the centromere.

Phenotype – An outward characteristic of an organism resulting from genetic and environmental interaction.

Positional cloning – The identification of a gene based on its chromosomal location.

research has been to understand the mechanisms associated with neurological deterioration in the central nervous system (CNS) and peripheral nervous system (PNS). Neuropathological studies of the CNS in animal models of NPC have detected defects in the brain that include the neuronal storage of lipids, neuroaxonal dystrophy and Purkinje cell loss[3,29,33–35]. The lysosomal contents of brain tissue indicate that, although a significant accumulation of glycolipids occurs, the levels of lysosomal cholesterol are very low[3]. However, studies of the NPC feline model have shown that a fatty compound, the ganglioside GM2, does accumulate in neuronal cells[36], and recent investigations have attempted to address the sequestration of gangliosides in NPC cells[37,38]. The accumulation of lysosomal GM2 in normal fibroblasts grown in the presence of progesterone[37] mimicks that seen in NPC fibroblasts[38]. Because it is a fatty compound that accumulates in the nervous system of NPC patients, the presence of GM2 might contribute to the neurological deterioration of NPC individuals.

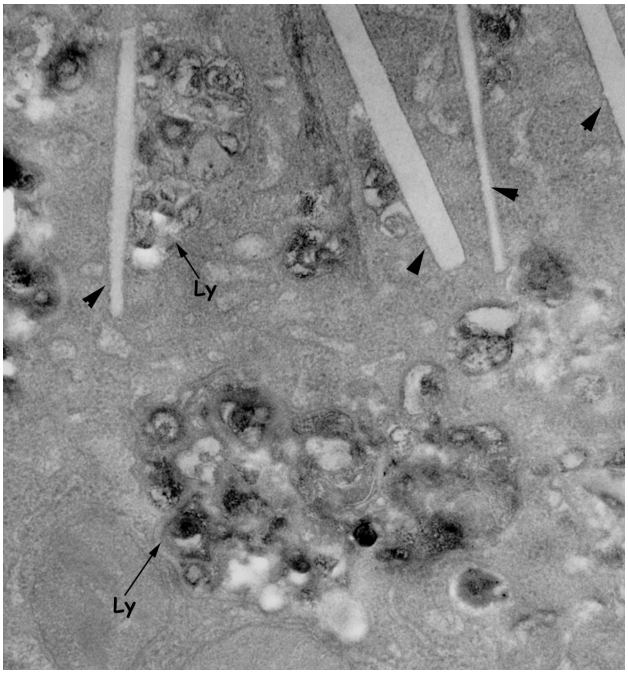

Figure 3. A hepatocyte from the liver of a mutant BALB/c *npc*^{nih} mouse that has been maintained on a 2% cholesterol diet, showing cholesterol lipidosis. Lysosomes (Ly), which proliferate in hepatocytes, are heterogeneous in structure and contain cholesterol-enriched membranous inclusions, as well as cholesterol crystals (arrowheads), which appear lucent in this electron micrograph. Kindly provided by E.J. Blanchette-Mackie and N. Dwyer, National Institutes of Health, Bethesda, MD, USA.

Goodrum and Pentchev[39] have examined the ability of the NPC mouse to use cholesterol to regenerate myelin in the PNS following local trauma. In response to a crushed nerve, newly regenerated myelin sheaths in BALB/c *npc*^{nih} mice were thinner and contained less cholesterol compared with those of controls.

Animal models allow novel treatments intended to slow, halt or reverse NPC-induced damage to the nervous system to be assessed. Nutritional studies have been conducted to assess the role that dietary cholesterol plays in disease progression. After four weeks on a 1% cholesterol diet, livers from BALB/c *npc*^{nih} mice and wild-type controls were examined. The BALB/c *npc*^{nih} mice had hepatomegaly and an accumulation of unesterified cholesterol within their liver cells, whereas unaffected mice did not[40]. Electron micrographs from hepatocytes isolated from BALB/c *npc*^{nih} mice that were fed a 2% cholesterol diet revealed cholesterol lipidosis. Lysosomes within these hepatocytes contained cholesterol-enriched membranous inclusions in addition to cholesterol crystals (Fig. 3).

Compounds that induce the NPC phenotype in normal cells

One approach to characterizing the nature of the NPC lesion is to evaluate drugs that induce similar cholesterol transport blocks in normal (non-NPC) cells. Liscum and Faust[41] treated Chinese hamster ovary (CHO) cells with a hydrophobic amine, 3-b-[2-(diethylamino)ethoxy]androst-5-en-17-one (also called U18666A), resulting in the accumulation of lysosomal cholesterol and diminished rates of cholesterol esterification. Similar to the effects induced by the NPC mutation, the U18666A-induced block of intracellular cholesterol movement was accomplished without inhibiting lipoprotein

uptake and cholesterol ester hydrolysis[41]. Other hydrophobic amines, including imipramine[42], stearylamine, RV-538 and sphinganine[43], induce the NPC-like cellular phenotype (Fig. 4).

Progesterone also disrupts the efflux of lysosomal cholesterol. If progesterone is added to the medium of cultured fibroblasts, lysosomal cholesterol accumulates and cholesterol ester synthesis is diminished[44]. When the progesterone is removed from the cells, there is a reversal of the NPC-like phenotype that is demonstrated by a rapid increase in cholesterol esterification as cholesterol leaves the lysosomes[44].

Although their specific molecular interactions remain unknown, the effects of the compounds depicted in Fig. 4 are all reversible. Attempts have been made to identify the genes that confer U18666A- and progesterone-resistance in CHO cell mutants[41]. This approach might eventually explain the metabolic step that is associated with the movement of cholesterol from the lysosome to other cellular targets, leading to new treatment strategies for this illness.

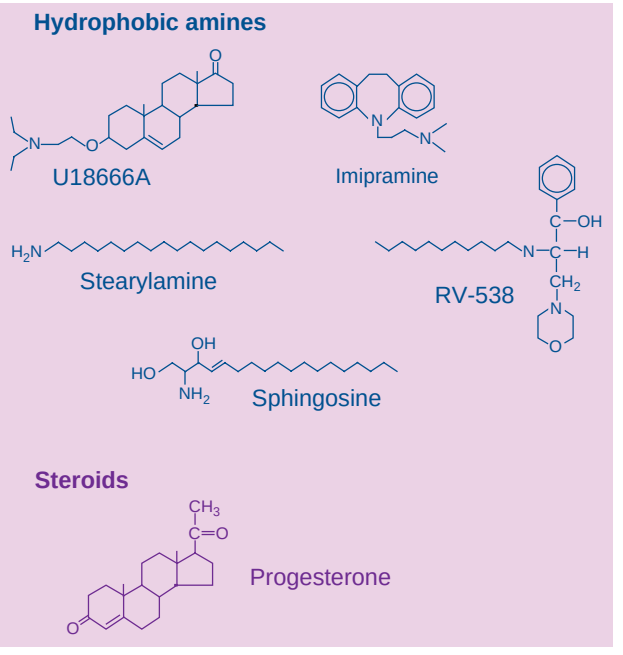

Figure 4. Hydrophobic amines and steroids that block the movement of lysosomal cholesterol in normal cells. Hydrophobic amines are distinguished by the nitrogen-containing chain or side chain. Progesterone is the only example of a non-nitrogen-containing steroid that is able to induce a Niemann–Pick C (NPC)-like block.

The molecular genetics of NPC

Identification of the NPC1 gene

The clinical, biochemical and cytological criteria used to diagnose individuals with NPC have provided sufficient confidence to conclude that 12 unrelated families, each with at least two affected offspring, have all been affected by a defect in the same gene. This diagnostic confidence allowed a positional cloning approach to be used to identify the NPC gene based on the pattern of its inheritance in these families. Linkage analysis confirmed the localization of the gene on the pericentromeric region of human chromosome 18, a region with conserved synteny to the murine *spm* locus[13,31]. Refinement of the genetic and physical interval placed the NPC gene in a 1 cM region of the proximal long arm of chromosome 18, in band 18q11 (Refs 13,31,45).

The gene responsible for NPC, *NPC1*, has now been identified[13]. Confirmation of its identity came with the discovery of several *NPC1* mutations in patients with NPC. Additionally, *NPC1* cDNA expression constructs introduced into cultured human NPC fibroblasts are capable of reversing the impaired transport of lysosomal cholesterol. Furthermore, the molecular defect in the BALB/c *npc*^{nih} mouse is a homoallelic deletion in the *npc1* murine ortholog[14]. The mouse mutation is caused by the insertion of a 824-bp retrotransposon-like sequence that deletes 703 bp of genomic *npc1* sequence, resulting in a premature stop codon approximately one-third of the way into the putative open reading frame. This important finding suggests that the BALB/c *npc*^{nih} mouse carries a null mutation resulting in a truncated and nonfunctional mouse npc1 protein.

Structure of the NPC1 protein

The 142-kDa NPC1 protein is mainly hydrophobic. Several sequence motifs that share significant homology to other known proteins are indicative of the function of NPC1 (Fig. 5)[13]. NPC1 contains 13–16 potential transmembrane domains, suggesting that it could be an integral membrane protein. It also has a putative lysosomal targeting sequence (comprising the four amino acids, Leu, Leu, Asn, Phe) at its C-terminus, which is identical to that of the lysosomal integral membrane protein II (LIMP II), linking NPC1 to the intracellular site where cholesterol accumulates[46]. At the N-terminus, the 'NPC1 domain', comprising 112 amino acids, appears to be unique to NPC1 and is highly conserved among all of the known NPC1 orthologs, including those in mouse, worm (*Caenorhabditis elegans*) and yeast (*Saccharomyces cerevisiae*). The NPC1 domain contains a putative leucine zipper sequence, indicating that the protein forms dimers with other leucine-zipper-containing

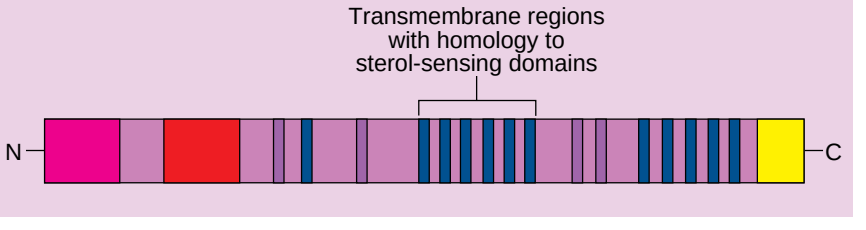

Figure 5. A schematic representation of the human Niemann–Pick C (NPC1) protein highlighting its putative domains. The NPC1 protein is 1278 amino acids long. A 24 amino acid motif (shown in pink) at the N-terminus probably targets NPC1 to the endoplasmic reticulum. The 'unique NPC1 region' (shown in red), from amino acids 55–165, is highly conserved among the NPC1 orthologs. It contains a leucine zipper that allows it to interact with other leucine-zipper-containing proteins. There are 13–16 suggested transmembrane domains (shown in blue and purple), 12 of which have significant homology to Patched, a tumor suppressor protein (shown in blue). Five transmembrane regions have sequence homology to the sterol-sensing regions of SREBP (sterol regulatory element binding protein) cleavage-activating protein (SCAP) and hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase. A putative lysosomal targeting sequence, comprising the amino acids Leu, Leu, Asn, Phe, occurs at the C-terminus (shown in yellow).

The outstanding questions

- Where is the NPC1 protein located in the cell?
- What role does NPC1 play in transporting cholesterol out of lysosomes?
- Do other proteins associate with NPC1 to enable its metabolic role?
- Does NPC1 exclusively transport cholesterol?
- What is the identity of the *NPC2* gene, and what role does it play in cholesterol transport?
- Do NPC1 and NPC2 interact?
- Is cholesterol the 'offending' metabolite that results in NPC-associated neurological damage or is it just an indicator of the disease process?
- In NPC cells, how does the sequestered cholesterol eventually exit the lysosome?
- Are there any other transport 'mechanisms' that translocate cholesterol out of the lysosome?

proteins. Understanding the activity associated with this sequence might help identify other proteins involved in NPC1 function and could explain its intracellular role.

Five of the putative transmembrane domains within NPC1 share a high degree of homology with the proposed sterol-sensing domains of proteins, such as SCAP and HMG-CoA reductase, that are involved in cholesterol homeostasis[48]. The sterol-sensing domain of HMG-CoA reductase is the molecular target for the degradation of this protein in response to intracellular sterol levels. The SCAP sterol-sensing domains regulate the ability of SCAP to release (by the proteolytic cleavage of SREBPs) transcription factors that, in turn, regulate the expression of HMG-CoA reductase in response to lowered intracellular sterol levels[47]. NPC1 shares the most extensive homology with PTCH, a Hedgehog signaling protein and putative tumor-suppressor. NPC1 and PTCH share homology in 12 of the putative transmembrane domains of NPC1. The significance of this homology is not clear, but continued investigation into the functions of both NPC1 and PTC might uncover mechanistic similarities that are shared among these proteins.

The NPC2 gene

Through cellular complementation and linkage analysis, a second gene has been discovered that is capable of causing NPC[13,48,49]. Mutations in this gene, called *NPC2*, cause a clinical phenotype that is indistinguishable from that caused by mutations in *NPC1*. Although its relationship and possible interaction with *NPC1* is unclear, identification of the *NPC2* gene might provide further insights into the mechanisms of intracellular cholesterol homeostasis.

Concluding remarks

The identification of NPC1 brings us a step closer to understanding the complex role of this protein. Years of research have shown that NPC1 facilitates the translocation of exogenously derived cholesterol from the lysosome to the ER and plasma membrane. Moreover, NPC1 modulates the movement of cholesterol that is recruited from the plasma membrane to the ER. In addition to its role as a 'gateway' for the translocation of cholesterol from varied cellular loci, it also regulates intracellular levels of cholesterol, thus maintaining homeostasis. It is not clear how all of these important functions can be attributed to this protein. Twenty years of intensive research, since the discovery of the NPC murine model in 1977, have brought the field to a point where studies can begin to answer specific questions.

To begin unravelling the functions of NPC1, the identification of its subcellular location should first be addressed; antibodies against NPC1 should help identify its intracellular localization. It is also important to identify other proteins that might have a role in the metabolic step attributed to NPC1. The amino acid motifs discovered on NPC1 suggest that this protein might interact with other proteins. These interactions might be essential for the normal functioning of NPC1. Immunoprecipitation of NPC1 with its associated proteins, or the yeast two-hybrid system, might help identify proteins that interact with NPC1. Another method of identifying proteins that are important for cholesterol trafficking is to identify disease genes that produce disorders with similar cytochemical and clinical consequences, such as *NPC2*.

Understanding the role of cholesterol and its mechanism of intracellular translocation might explain the neurological deterioration that is associated with all NPC patients. Is the presence of accumulated cholesterol detrimental to the health of a cell, or is there a separate toxic lysosomal resident that plays this role? It is possible that cholesterol acts as a marker that signifies the presence of another problem. In the brain, the lysosomal accumulation of other compounds, such as GM2, suggests that the altered transport of essential components also causes neurological breakdown, thereby reducing the potential pathogenicity of cholesterol accumulation.

Understanding the function of NPC1 and its associated proteins could increase our understanding of its mechanism of action. The potential exists to develop novel diagnostics and therapeutics for NPC1 based on this information. Mutation analysis can be used to definitively diagnose NPC1 patients, to determine the carrier status of individuals and in prenatal screening. In the development of novel therapeutic strategies, the animal models of NPC1 can be used to evaluate potential treatments. In understanding this system, it might become possible to manipulate the rate of cholesterol use; this could be an important breakthrough for those affected by other cholesterol-related abnormalities, such as atherosclerosis.

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