Generation of a Human Neuronal Stable Cell Model for Niemann–Pick C Disease by RNA Interference

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Abstract Niemann–Pick type C (NPC) disease is a fatal autosomal recessive neurodegenerative disorder caused, most commonly, by mutations in the *NPC1* gene. At the cellular level, the disease is characterized by the storage of multiple lipids in the endosomal–lysosomal system, including free cholesterol, glycosphingolipids, sphingomyelin and the catabolic product of sphingolipids, sphingosine. Therapeutic options for NPC disease are relatively limited. One drawback for the development of novel therapies is the lack of suitable human neuronal cell models. In this work, a stable SH-SY5Y cell model for NPC disease was generated using short hairpin RNAs. An inhibition of the *NPC1* expression of around 90% was obtained at the RNA level. The *NPC1* knockdown was confirmed at the protein level. To characterize the stable cell line generated, cholesterol

levels were analyzed in the *NPC1*-knockdown SH-SY5Y cells by filipin staining and gas chromatography–mass spectrometry. A characteristic NPC pattern and a twofold increase of the free cholesterol levels, related to intact SH-SY5Y cells, were found. Moreover, sphingolipids were analyzed by liquid chromatography–mass spectrometry and an increase in ganglioside GM2 levels was observed. The stable *NPC1*-knockdown SH-SY5Y cell line generated in the present study provides a human neuronal cell model for this lethal disease that could be a valuable tool for the study of future therapeutic approaches.

Abbreviations

NPC	Niemann–Pick type C
q-PCR	Quantitative real-time PCR
RT	Reverse transcription
shRNA	Short hairpin RNA
siRNA	Small interfering RNA

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Introduction

Niemann–Pick type C (NPC, <http://www.ncbi.nlm.nih.gov/omim/257220>) disease is a fatal autosomal recessive lysosomal storage disorder characterized by hepatosplenomegaly and progressive central nervous system neurodegeneration. The majority of cases (approximately 95%) result from mutations in the *NPC1* gene (http://www.genenames.org/data/hgnc_data.php?hgnc_id=7897; MIM #607623), and mutations in *NPC2* (http://www.genenames.org/data/hgnc_data.php?hgnc_id=14537; MIM #601015) account for the remaining 5% (Patterson et al. 2001). NPC1 is a lysosomal membrane protein containing 13 putative membrane-spanning domains (Carstea et al. 1997) while NPC2 is a soluble protein located in the lumen

of lysosomes (Naureckiene et al. 2000). At the cellular level, the loss of function of either the NPC1 or NPC2 protein leads to the accumulation of cholesterol and other lipids such as sphingomyelin, sphingosine and gangliosides (GM2 and GM3) within the endosomal/lysosomal system in various tissues (Vanier 1999).

Although the functions of the NPC1 and NPC2 proteins remain unclear, current knowledge supports the notion that they function in a coordinated manner and that they are involved in the cellular postlysosomal/late endosomal transport of cholesterol and other molecules (Sleat et al. 2004; Kwon et al. 2009; Wang et al. 2010). However, despite evidence implicating NPC1 and NPC2 in intracellular cholesterol transport, the direct connection between cholesterol accumulation and neurodegeneration in NPC brains, the primary target of the disease, remains controversial. Earlier studies reported that glycolipids were elevated in the brain of NPC patients, whereas there was no significant increase in the cholesterol concentration in disease-affected brains or in NPC animal models (Vanier 1999; Xie et al. 1999). Moreover, the treatment of *Npc1*-deficient mice with *N*-butyldeoxynojirimycin, an inhibitor of the enzyme in the early glycosphingolipid biosynthesis pathway, decreased ganglioside accumulation and the neuropathological changes in their brains (Zervas et al. 2001). These observations seem to indicate that the accumulation of cholesterol in brain cells is not as important as in other tissues and that the disease might be considered a glycolipid-storage disorder rather than a cholesterol-storage disease. However, recent evidence for cholesterol accumulation in the NPC brain has been described. Experiments with filipin or BC theta staining revealed unesterified cholesterol accumulation in the cell bodies of neurons and glia of *Npc1* and *Npc2* mutant mice (Treiber-Held et al. 2003; Reid et al. 2004; Vance et al. 2005). Similarly, other studies have shown that the amount of cholesterol in the brains of *Npc1*-deficient mice immediately after birth, when myelination is incomplete, is higher than in their wild-type counterparts (Xie et al. 2000). The authors concluded that cholesterol accumulates in *Npc1*-deficient neurons and/or glial cells of the central nervous system as in other cell types. Additionally, studies on cultured sympathetic neurons from *Npc1* mutant mice found that cholesterol accumulated in cell bodies, but was reduced in distal axons, suggesting that the transport of endogenously synthesized cholesterol from cell bodies to distal axons is impaired in *NPC1*-deficient neurons (Karten et al. 2002). Despite the increasing number of reports, the importance of cellular cholesterol transport abnormalities in the pathophysiology of the neurodegenerative NPC disease remains unclear, as does the main metabolite responsible for brain injury in this disease.

At present there is no specific treatment for NPC disease. Therapeutic approaches have been developed with two main aims: to reduce the cellular influx of cholesterol or to diminish the accumulation of glycolipids. Unlike other lysosomal diseases, enzyme replacement therapy is not suitable for NPC disease due to the transmembrane nature of the NPC1 protein and the neuropathological features associated with the disease. In contrast, substrate reduction therapy using the imino sugar *N*-butyldeoxynojirimycin (Miglustat) is suitable, since it is a small inhibitor molecule that can cross the blood–brain barrier and diminish glycolipid biosynthesis (Brady 2006). The drug was approved in the European Union for the treatment of progressive neurological manifestations in patients with NPC in January 2009 based on findings from a randomized, controlled clinical trial and a retrospective observational cohort study (Patterson et al. 2007; Pineda et al. 2009). However, even though it seems to partially stabilize some clinical markers, long-term studies are required to prove its effectiveness. Besides, a recent study revealed that *NPC1*-mutant cells have decreased calcium concentrations in lysosomes, initially caused by the storage of sphingosine, with subsequent accumulation of cholesterol, sphingomyelin and glycosphingolipids as downstream effects (Lloyd-Evans et al. 2008). The use of pharmacological agents that increase cytosolic calcium corrected the disease phenotype in those cells and in the mouse model of NPC disease, opening up new possibilities for therapeutic approaches (Lloyd-Evans et al. 2008).

Despite the studies in animal models, the lack of a human neuronal model of the disease is a drawback in the development and trial of novel therapeutic strategies. Human neuroblastoma cells SH-SY5Y have been widely used as a model for neurological diseases. They present many of the biochemical and functional properties of human neurons and can proliferate in culture for long periods. Moreover, SH-SY5Y cells can be differentiated into a more pronounced neuronal phenotype in the presence of agents such as retinoic acid (Xie et al. 2010).

In the current study, a stable cell line model of NPC disease was generated in the SH-SY5Y neuroblastoma cell line using RNA interference mediated by short hairpin RNA (shRNA). Knockdown of the target gene was confirmed at the RNA and protein level. The effect of *NPC1* depletion on the amount of cholesterol was assessed by filipin staining and gas chromatography–mass spectrometry, showing an accumulation of cholesterol in *NPC1*-knockdown SH-SY5Y cells in relation to wild-type cells. Finally, the levels of other lipids were measured by liquid chromatography–mass spectrometry and an increase in GM2-ganglioside was detected. The stable cell line generated provides a human neuronal cell model of the

disease that may be useful for testing and developing new therapeutic strategies for NPC disease.

Materials and Methods

Cell Culture

Human neuroblastoma SH-SY5Y cells were obtained from the American Type Culture Collection and were cultured in a 1:1 mixture of EMEM (LGC Standards, Teddington, UK)/F12 (Invitrogen, Paisley, UK) supplemented with 10% heat-inactivated fetal bovine serum and 1% penicillin–streptomycin (Invitrogen, Paisley, UK). Cells were incubated in a humidified atmosphere with 5% CO₂ at 37°C. For cholesterol measurement, a lipoprotein-deficient fetal bovine serum was used.

Transient NPC1-Knockdown in SH-SY5Y Cells

A small interfering RNA (siRNA) targeting *NPC1* (siNPC1) together with a positive control targeting *GAPDH* (siGAPDH) and a negative control (siC-) were purchased from Applied Biosystems (Life Technologies Corporation, Carlsbad, California). In the initial experiments Lipofectamine™ 2000 (Invitrogen, Paisley, UK) was used as the transfection agent in SH-SY5Y cells with poor results. Consequently, the Amaxa Nucleofector was used in all remaining experiments. Different amounts of siGAPDH were transfected to SH-SY5Y cells using the Amaxa Nucleofector device and the appropriate cell-type-specific solution (Lonza, Basel, Switzerland). The transfection protocol was performed following the manufacturer's instructions. Twenty-four hours after transfection the expression of *GAPDH* was analyzed by quantitative real-time PCR (q-PCR). Once the transfection parameters had been determined, siNPC1 was introduced into the SH-SY5Y cells and *NPC1* expression was measured by q-PCR.

Construction of a Stable Transfected SH-SY5Y Cell Line with shRNA

shRNA expression vectors to knockdown *NPC1* expression were purchased from SABiosciences (Qiagen, Hilden, Germany). Each vector expresses a shRNA under the control of the U1 promoter and either the GFP or neomycin resistance gene. GFP shRNAs were used to estimate the nucleofection efficiency of shRNA in the SH-SY5Y cell line by fluorescence microscopy and FACS. Two different nucleofection programs for SH-SY5Y cells (G-004 and A-023) and different amounts of shRNA (2, 3, 6, and 10 µg) were tested. The G-004 program and 2 µg of shRNA were selected for subsequent experiments. The neomycin-bearing

shRNA constructs (shRNA1 to shRNA4) were then nucleofected and 2 days after transfection selection medium containing 1 mg/ml G418 (Invitrogen, Paisley, UK) was added in order to generate a stable cell line. *NPC1* expression was assessed by q-PCR after 2 weeks of selection. Maximum inhibition was obtained when using shRNA1 and shRNA4. The linearization of the shRNA1 and shRNA4 constructs improved *NPC1* inhibition and was used in subsequent experiments. Two different transfection methodologies were checked for the linearized constructs: Amaxa nucleofection and transfection using Effectene (Qiagen, Hilden, Germany). After 3 weeks of selection, *NPC1* expression was measured by q-PCR. Cell cloning by serial dilution in 96-well plates was performed using intact and linearized shRNA1 constructs. Single cells were picked and grown with antibiotic until clones had grown enough to check *NPC1* expression. No improvement in the percentage of knockdown was obtained using this time-consuming strategy.

Reverse Transcription and Quantitative Real-Time PCR

Total RNA from SH-SY5Y cells was isolated using the QIAshredder™ homogenizer and the RNeasy Mini Kit (Qiagen, Hilden, Germany). Reverse transcription (RT) was performed using the High-Capacity cDNA Reverse Transcription Kit (Life Technologies Corporation, Carlsbad, California) according to the manufacturer's instructions. Real-time PCR samples were prepared using the Roche real-time PCR master mix (Lightcycler 480 Probes Master) and the human-specific Taqman Gene Expression assays for *NPC1* (Hs00264835_m1) and *GAPDH* (Hs99999905_m1). Several reference genes were used to normalize the results: *ACTB* (Hs99999903_m1), *HPRT* (Hs99999909_m1), *PPIA* (Hs99999904_m1), and *SDHA* (Hs00417200_m1). These were selected according to their expression and those that were stably expressed under the experimental conditions were chosen. At least two reference genes were used for each experiment. The Roche Lightcycler 480 software was used to perform advanced relative quantification analysis of gene expression, according to the Lightcycler 480 instrument operator's manual. Triplicate measurements were performed for all samples.

SDS-PAGE and Western Blot Analysis

Protein extracts from SH-SY5Y cells stably transfected with shRNA1 or shRNAC- (100 µg of protein/lane) were subjected to SDS-PAGE (6% polyacrylamide) and electrophoretically transferred onto Immobilon-P transfer membrane (Millipore, Billerica, MA, USA). Membrane was blocked with 5% bovine serum albumin (BSA) in PBS containing 0.2% Tween-20 (PBST) for 2 h. The blotted membranes were probed overnight at 4°C with polyclonal

antihuman NPC1 antibody produced by Eurogentec (Liège, Belgium) diluted 1:100 in 0.1% BSA PBST, and then washed. Antirabbit IgG antibody (Sigma-Aldrich, UK) was used as a secondary antibody, and the immunoreactive bands were detected by incubation of the membrane for 2 min in the following solution: 10 ml of 100 mM Tris-HCl pH 9, 50 ml of 45 mM p-coumaric acid, 50 ml of luminol, and 10 ml of 30% H₂O₂. Anti- α -tubulin monoclonal antibody (Sigma-Aldrich, UK) was used to detect α -tubulin as loading control.

Protein Determination

Protein concentration was determined using the Lowry method.

Filipin test

The filipin test was performed in stable *NPC1*-knockdown SH-SY5Y cells as described by (Kruth et al. 1986).

Cholesterol measurement by gas chromatography–mass spectrometry

SH-SY5Y cells stably transfected with shRNA1 or shRNAC- (around 1×10^6 cells) were washed in PBS, collected by brief trypsinization and transferred to glass vials. An aliquot of the cells was taken for protein quantification. Lipids were extracted with chloroform: methanol (2:1) containing stigmasterol as the internal standard. Samples were derivatized with BSTFA to form the trimethylsilyl derivatives and analyzed by gas chromatography–mass spectrometry. Gas chromatography coupled to electron impact (70 eV) mass spectrometry was carried out using a Fisons gas chromatograph (8,000 series) coupled to a Fisons MD-800 mass-selective detector. The system was equipped with a HP-5-MS capillary column (30 m $\times$ 0.20 mm i.d.), which was programmed to increase from 120°C to 315°C at 5°C/min after an initial delay of 2 min. Analyses were performed in the selected ion-monitoring mode. The ions selected were those at *m/z* 129, 458 and 484.

Sphingolipid Determination

SH-SY5Y cells stably transfected with shRNA1 or shRNAC- (around 1×10^6 cells) were washed in PBS, collected by brief trypsinization and transferred to glass vials. An aliquot of the cells was taken for protein quantification. Sphingolipid extracts, spiked with internal standards, were prepared as described (Shaner et al. 2009) and analyzed by liquid chromatography–mass spectrometry using a Waters Aquity UPLC system connected to a Waters LCT Premier orthogonal accelerated time of flight mass spectrometer (Waters, Millford, MA), operating in positive

electrospray ionization mode in the conditions reported previously (Canals et al. 2009).

Statistical Analysis

Significance was assessed using the Student *t*-test (PASW Statistics 8.0 software, IBM company, Illinois, Chicago). *p* < 0.05 was considered statistically significant.

Results

Transient *NPC1* Knockdown in SH-SY5Y Cells

siRNA transfection into SH-SY5Y cells, which provide a model system for neuronal cells, was optimized using an siRNA targeting *GAPDH* as a positive control. After testing different transfection agents and methodologies, the use of the Amaxa nucleofector proved to be the best method of transfection, achieving approximately 90% knockdown of *GAPDH* expression (data not shown). SH-SY5Y cells were then transfected with an siRNA against the *NPC1* gene (siNPC1) or with a positive or negative control siRNA at three different concentrations by nucleofection. Twenty-four hours after nucleofection *NPC1* expression was assessed by RT-q-PCR. Maximum *NPC1* inhibition (approximately 74%) was obtained when transfecting 300 pmols of siNPC1 (Fig. 1).

Generation of a Stable Transfected SH-SY5Y Cell Line with shRNA

The nucleofection efficiency of shRNA-GFP vectors in the SH-SY5Y cell line was tested by FACS analysis and fluorescence microscopy. A rate of 40–50% of transfection was obtained (not shown). Four different shRNAs (shRNA1 to shRNA4) targeting *NPC1* were introduced into SH-SY5Y cells under the previously optimized conditions. Nucleofection of intact shRNA constructs followed by a 2 week selection with G418 resulted in a maximum inhibition of 50%. Cell cloning by serial dilution in 96-well plates did not improve the percentage of knockdown (data not shown). However, nucleofection of previously linearized shRNA constructs followed by antibiotic selection was substantially more efficient, achieving inhibition of around 90% for shRNA1 and between 60% and 70% for shRNA4 (Fig. 2a). In an attempt to improve these results, single cell clones were generated for the shRNA construct giving the best results (shRNA1). However, similar values of inhibition were obtained (from 70% to 90%). Thus, clones obtained by clonal selection were not used in further experiments. *NPC1* inhibition was

Fig. 1 Transient knockdown of *NPC1* in SH-SY5Y cells. Real-time PCR quantification of *NPC1* RNA levels in SH-SY5Y cells nucleofected with different amounts of siRNA targeting *NPC1* (siNPC1) are shown. Values are expressed as the percentage of *NPC1* expression in SH-SY5Y cells nucleofected with a negative

control siRNA (siC-). Each value is the mean of at least two independent experiments each replicated three times. Error bars correspond to standard deviation (SD). HPRT was used to normalize q-PCR. Similar values were obtained when using actin as an endogenous control

also confirmed at the protein level by Western blotting in stable transfected cells treated with shRNA1 as shown in Fig. 2b.

Accumulation of Cholesterol in Stable *NPC1*-Knockdown SH-SY5Y Cells

Two different methodologies were used to investigate whether *NPC1* knockdown in SH-SY5Y cells results in

free cholesterol accumulation. Firstly, the distribution of unesterified cholesterol was assessed using filipin, an antibiotic that binds specifically to unesterified cholesterol. Fluorescence microscopy of filipin-treated *NPC1*-knockdown cells revealed an intense intracytosolic punctate fluorescence that was not visible in control SH-SY5Y cells (Fig. 3). To confirm the accumulation of unesterified cholesterol in stable *NPC1*-knockdown SH-SY5Y cells, the amount of cholesterol was measured by gas chromatography–mass spectrom-

Fig. 2 Stable knockdown of *NPC1* in SH-SY5Y cells. **(a)** Real-time PCR quantification of *NPC1* RNA levels in SH-SY5Y cells stably transfected with shRNAs against *NPC1*. Values are expressed as the percentage of *NPC1* RNA levels in SH-SY5Y cells transfected with a negative control shRNA (shRNAC-). Data are means $\pm$ SD. HPRT

was used to normalize q-PCR. Similar values were obtained when using SDHA and PPIA as endogenous controls. **(b)** Western blot analysis of SH-SY5Y cells stably transfected with shRNAC- or shRNA1. The same amount of protein was loaded in each lane. Tubulin was used as loading control

Fig. 3 Filipin staining of unesterified cholesterol detected by fluorescence microscopy. (a) SH-SY5Y cells stably transfected with shRNAC- (100 $\times$). (b) Stable *NPC1*-knockdown in SH-SY5Y cells (100 $\times$). (c) Stable *NPC1*-knockdown in SH-SY5Y cells (1,000 $\times$)

etry. The cholesterol content was approximately twofold higher in stable *NPC1*-knockdown SH-SY5Y cells than in control cells (Fig. 4), at 35.3 $\mu\text{g}/\text{mg}$ protein (shRNA1) vs. 16.2 $\mu\text{g}/\text{mg}$ protein (shRNAC-) ($p < 0.001$). Thus, stable *NPC1*-knockdown in SH-SY5Y cells causes an accumulation of unesterified cholesterol.

Sphingolipid Pattern in Stable *NPC1*-Knockdown SH-SY5Y Cells

Different sphingolipids including ceramide, sphingomyelin, glucosylceramide, lactosylceramide, GM1, GM2, and GM3, were measured by liquid chromatography–mass spectrometry in *NPC1*-knockdown cells. The analysis revealed a 1.4-fold increase in GM2 ganglioside with respect to control cells (Fig. 5).

Discussion

At present, there are two murine *NPC1* models with different genetic backgrounds (BALB/c-*npc1*^{nih} and C57BL/KsJ-*npc1*^{spm}) as well as feline and canine *NPC1*

models, all of which are spontaneous (Morris et al. 1982; Miyawaki et al. 1986; Lowenthal et al. 1990; Kuwamura et al. 1993). These animals are useful for studying NPC dysfunction, and for testing compounds with a therapeutic purpose. As far as cell models are concerned, apart from primary neurons from *NPC1* mutant mice, studies have been carried out using mutant Chinese hamster ovary CT cells (Cadigan et al. 1990; Cruz et al. 2000; Pipalia et al. 2006; Rujoi et al. 2010), which display the characteristic NPC trafficking defects, and stable generated murine Neuro-2a cell lines (Klein et al. 2011). However, since these cells are not derived from a human source, their potential as a suitable cellular model is limited. Recently, while the present study was underway, Zampieri et al. (2009) reported the use of human SH-SY5Y neuroblastoma cells, transiently transfected with an siRNA against *NPC1*, as a cell model of NPC to study the role of oxidative stress in this disease. However, despite these various approaches, to our knowledge, no stable human neuronal cell model for NPC disease has been reported to date.

The SH-SY5Y cell line has been widely used as a human cell model of neurons for research on several pathologies, such as Parkinson's disease, since these cells present many

Fig. 4 Unesterified cholesterol content measured by gas chromatography–mass spectrometry. Data are mean $\pm$ SD of two independent experiments, each performed in triplicate. ***, $p < 0.001$

Fig. 5 GM2 relative levels measured by liquid chromatography–mass spectrometry. Data are mean $\pm$ SD of two independent experiments, each performed in triplicate. **, $p < 0.01$. Original values in pmols/mg protein were normalized respect to shRNA C-

of the biochemical and functional features of neurons (Xie et al. 2010). This cell line can proliferate in culture for long periods and can be induced to differentiate into a more pronounced neuronal phenotype in the presence of agents such as retinoic acid.

In this study, SH-SY5Y cells were used to generate a stable human neuronal cell line model for NPC disease. Firstly, SH-SY5Y cells were transfected using an siRNA against the *NPC1* gene (siNPC1) as a proof of concept for the RNA interference strategy using this cell type. Once inhibition by siNPC1 was confirmed, a stable *NPC1*-knockdown SH-SY5Y cell line was created using shRNAs.

Various experimental approaches were tested to determine the optimal conditions. Intact or linearized shRNA constructs were introduced into SH-SY5Y either using the Amaxa nucleofactor system or by standard methods using Effectene as a transfection agent. The level of inhibition obtained using nucleofection was significantly better, showing that the method of transfection plays a decisive role in generating a stable cell line with a high level of inhibition. Linearization was an additional key factor increasing the knockdown efficiency. In contrast, clonal selection by serial dilution in 96-well plates did not improve the results. Finally, differences were observed among the shRNAs tested, shRNA1 being the most efficient. Overall, knockdown levels of around 80–90% were achieved using the optimal conditions. The decrease in NPC protein in SH-SY5Y cells stably transfected with shRNA1 was subsequently confirmed by Western blotting.

A relevant issue was to show that the stable *NPC1*-knockdown SH-SY5Y cell line generated was a suitable model for Niemann–Pick C disease. For this purpose, free cholesterol accumulation, a typical feature of this pathology, was assessed using two different methodologies. First, free cholesterol was determined qualitatively using the filipin test. Unlike control SH-SY5Y cells, *NPC1*-knockdown

SH-SY5Y cells showed significant accumulation of fluorescent perinuclear vesicles similar to those found in *NPC1*-deficient fibroblasts and in mutant CHO cells (Dahl et al. 1992; Coxey et al. 1993). Second, the cholesterol content in *NPC1*-knockdown cells was measured by gas chromatography–mass spectrometry and was found to be twice as high as in control cells, reaching significance. However, the number of experiments should be enlarged to confirm these data. These figures are consistent with previous studies in CHO *NPC1*-null cells (Kosicek et al. 2010) and fibroblasts from NPC patients (Frolov et al. 2003), which showed a twofold increase in the amount of cholesterol. Therefore, these results confirm that *NPC1* knockdown in SH-SY5Y cells results in the accumulation of unesterified cholesterol, a characteristic feature of NPC disease.

The accumulation of ganglioside GM2 observed in the *NPC1*-knockdown SH-SY5Y of the present study is consistent with findings in the brains of NPC patients (reviewed in Vanier 2010) and mouse models (Sleat et al. 2004; Walkley and Vanier 2009). These authors reported a higher increase in GM2 and, in addition, a relevant increase in GM3 that was not observed in our model. Whether these differences are due to technical problems or to particular features of the model remains to be determined. Walkley and Vanier (2009) reported that GM2 accumulation in an *NPC1* mouse model occurs earlier than that of GM3. Thus, our model may correspond to an earlier stage of the disease. Treatment of the *NPC1*-knockdown SH-SY5Y cells with retinoic acid to fully differentiate them to neurons may allow the detection of a pattern of lipid accumulation closer to that reported in patients and mouse models.

In summary, the stable *NPC1*-knockdown SH-SY5Y cell line generated in this study has the main features of NPC disease and provides a useful and accessible human

neuronal cell model in which emerging therapeutic strategies can be assessed to evaluate their benefits in the treatment of this disorder.

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Synopsis

The article describes the generation of a human neuronal cell model for Niemann–Pick C disease, using shRNAs, which will be useful for the assay of novel drugs and therapeutic approaches for this disease with no current treatment.

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