Niemann-Pick Type C Disease and Intracellular Cholesterol Trafficking*

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The human NPC1 encodes a 1278-amino acid (170–190 kDa) glycoprotein with 13 putative transmembrane domains, including a conserved “sterol-sensing domain” (SSD) located between the third and seventh transmembrane domains. SSDs consist of ~180 amino acids organized in five consecutive transmembrane domains. The SSD is found in several other polytopic membrane proteins that are involved in cellular cholesterol homeostasis (2), cell-cell signaling (3), and the dietary uptake of cholesterol (4). SSDs are needed for NPC1 protein to function in intact cells (5). Binding occurs between NPC1 and a photo-activatable analog of cholesterol (azocholestanol); the binding is partially blocked by cholesterol and is much diminished in NPC1 proteins that contain mutations within the SSD (6). Thus, one function of the SSD in NPC1 protein is to mediate sterol binding. NPC1 may work as a lipid permease (7); however, the substrate specificity and the role of the SSD in mediating permease activity have not yet been determined. In addition to the SSD, a cysteine-rich luminal loop between TMD 8 and 9 (8) and the region between amino acids 1038 and 1253 are also important for NPC1 function (9).

NPC1 protein is predominantly located within the late endosomal membrane but is also transiently associated with lysosomes and the trans-Golgi network (10). Multiple peptide sequences within the protein are responsible for targeting to the endosomal compartment (11). Late endosomes are comprised of limiting membranes and internal membranes (12). The exact location of the NPC1 protein in the late endosomal membranes is not known.

NPC2 is a soluble lysosomal protein that can be secreted from cells. It uses mannose 6-phosphate marker for targeting to the late endosome (13) and is a high affinity cholesterol-binding protein (14). NPC2 also binds fatty acids in vitro but with lower affinity (14). A crystal structure in the ligand free state shows that the protein has three small hydrophobic cavities that form a “gate,” which may represent the incipient cholesterol-binding site that dilates to accommodate the cholesterol molecule; the gate involves tyrosine 100 and phenylalanine 66 (15).

Roles of NPC1 and NPC2 in Endosomal/Lysosomal Lipid Trafficking

Low Density Lipoprotein (LDL)-derived Cholesterol—In mammalian cells, LDL, the principal cholesterol carrier in the blood, binds to the LDL receptor, internalizes, and enters the endocytotic compartment. There, its main cargo, comprised of cholesteryl esters, is dissimilated by hydrolysis to cholesterol and fatty acids. Hydrolysis of cholesteryl esters requires the enzyme acid lipase. In tissue culture cells, most of the lipase is located in endocytic compartments that are distinct from the late endosomes/lysosomes; after lipase action, the liberated cholesterol appears in the late endosomes/lysosomes (16). In NPC1 cells (i.e. cells affected by the NPC1 mutation), the transport of cholesterol from the late endosomes to various destinations, including the plasma membrane, is defective (17). At present, it is not clear how NPC1 and NPC2 work in concert to transport cholesterol.

Oxysterols play an important role in mediating cellular cholesterol homeostasis. Cells produce more oxysterols when cultured in the presence of LDL, and this production is decreased in cells overexpressing NPC1 and NPC2 (18). These results suggest that NPC1 and NPC2 may participate in delivering LDL-derived cholesterol to proper cellular site(s) for conversion to oxysterols.

Sterols Synthesized from Acetate—In mammals, extra-hepatic tissues synthesize as much cholesterol as the liver (19). In Chinese hamster ovary cells and human fibroblast cells, biosynthesis of sterols takes place at the endoplasmic reticulum (ER). After synthesis, most sterols are rapidly transported from the ER to the caveola/leaflet raft domain of the plasma membrane (PM) in an energy-dependent manner. This process does not require NPC1 (20). After reaching the PM, the newly synthesized sterol may recycle rapidly (within minutes) between the PM and the recycling endosome (21). After 8 or more hours, the endogenously synthesized sterols accumulate in the late endosomal/lysosomal compartment of NPC1 cells but not in normal cells. The recycling of these sterols from the late endosomes to the PM, and the esterification of these molecules within the ER are also partially defective in NPC1 cells (22–24). The effect of NPC1 on trafficking of endogenously synthe-
sized sterols is cell-type dependent; macrophages and glial cells are prominently affected by the NPC1 mutation, whereas embryonic fibroblasts are less affected (25, 26).

Sterol Synthesis, Transport, and Secretion in Brain Cells—In mammals, the brain contains more unesterified cholesterol, most of which is acquired by endogenous synthesis, than any other organ in the body (27). Both neurons and astrocytes isolated from the NPC1/−/− mouse exhibit trafficking defects in exogenously provided cholesterol and endogenously synthesized sterol (28–30, 25). Despite these defects, NPC1/−/− mouse astrocytes synthesize and secrete the NPC2 and apolipoprotein E proteins (31, 32).

Glycosphingolipids and Other Lipids—In addition to cholesterol, various other lipids, such as sphingomyelin, glucosylceramide, certain gangliosides (especially GM2 and GM3), and lysobisphosphatic acid, also accumulate in NPC1 cells (1). Gangliosides are acidic glycosphingolipids that are normally present in cell membranes at high levels. Mutations in genes encoding enzymes or proteins involved in the catabolism of glycosphingolipids cause various glycosphingolipids to accumulate within lysosomes, leading to secondary cholesterol accumulation (33, 34). N-Butyl deoxynojirimycin (NB-DNJ) is an inhibitor of the enzyme glucosylceramide synthetase, a key enzyme involved in the biosynthesis of gangliosides in animal cells. In NPC1 cells, some of the endosomal malfunction can be corrected by treating cells with NB-DNJ (35); however, the drug has little effect on reversing the cholesterol trafficking defect (22, 35). Thus, it is unlikely that the cholesterol trafficking defects observed in NPC1 cells are due to secondary consequence of glycosphingolipid accumulation.

The accumulation of glycosphingolipid in NPC1 cells may be explained by the high affinity between cholesterol and sphingolipids, which are the major components of lipid microdomains or “rafts.” Accumulation of one raft lipid in late endosomes/lysosomes may lead to the trapping and accumulation of another raft lipid (36). In addition, it has been shown that in NPC1 cells endosomal/lysosomal cholesterol accumulation causes inhibition of lysosomal sphingomyelinase (37) and lysosomal glucosylceramidase (the enzymes responsible for degrading sphingomyelin and glucosylceramides) (38). The lower glucosylceramidase activity in NPC1 cells has been attributed to mislocalization of the enzyme due to cholesterol loading. It is also possible that, in addition to cholesterol trafficking, NPC1 may also be involved in sphingolipid recycling. Studies in yeast show that a mutation in the sterol-sensing domain of NPC1 results in defective recycling, localization, and increased quantities of complex glycosphingolipids, without obvious changes in sterol metabolism (39).

Endosomal Cholesterol and Rab Proteins—Various abnormalities can cause endosomal cholesterol to accumulate and perturb the functions of Rab7 and Rab4 proteins. Late endosomes and lysosomes exhibit bidirectional motility, moving back and forth between the periphery and the pericentriolar region of cells. Endosomal motility is controlled in part by Rab proteins, small GTPases that are intimately involved in various membrane trafficking events. Rab7 and the related Rab9 are located in the late endosomes. Rab7 interacts more with earlier endosomes and lysosomes, whereas Rab9 interacts more with the trans-Golgi (40). Rab4 is located in early endosomes. Mammalian cells treated with the hydrophobic amine “U-drug” or cells doubly deficient in the major late endosomal/lysosomal membrane proteins Lamp1/Lamp2, exhibit significantly reduced motility of the late endosomes, accumulate endosomal cholesterol, and exhibit NPC-like phenotypes (41, 42). The above observations may be explained by cholesterol accumulation due to various endosomal abnormalities, leading in turn to the inhibition of Rab7 and Rab4 (42, 43). The inhibition of Rab7 reduces the motility of the late endosomes (42). Strikingly, overexpressing Rab9 corrects the lipid trafficking defects in NPC1 cells (44, 45). Despite the predicted pleiotropic effect of

**Fig. 1.** Cholesterol accumulation in Purkinje neurons of NPC1 mice at postnatal day 9. Cerebellar brain sections from PND 9 NPC1 (BALB/c NPC1/H11002) mice and WT mice were stained with the cholesterol binding agent BC-theta (red) and anti-Calbindin antibodies (a Purkinje cell marker protein; blue). Main panel, NPC1 Purkinje dendrite, cholesterol accumulation indicated by arrows, scale bar is 5 μm; lower left panel, NPC1 Purkinje cell body, indicated by asterisk; lower right panel, WT Purkinje dendrite, scale bar is 10 μm. Reproduced with permission from Reid et al. (53).
overexpressing certain Rab proteins, this procedure may provide novel therapeutic treatment of NPC disease, which is fatal and currently has no cure.

**Mutations in Other Proteins That Produce an NPC-like Phenotype**—Several other proteins residing in late endosomes/lysosomes, including MLN64 and MENTHO (46), may also be involved in endosomal cholesterol movement. The role of MLN64 in sterol trafficking is not clear because mice with targeted mutation of MLN64 are healthy and display only minimal disturbances in sterol dynamics (47). A novel CHO cell mutant without the NPC1 mutation but with defects in late endosomal cholesterol trafficking has been isolated (48). ABCA1 is a key protein that mediates apoA-I-dependent sterol efflux. In cells lacking ABCA1, cholesterol and sphingomyelin accumulate in abnormally structured late endocytic vesicles, and these lipids exhibit impaired intracellular movement (49). Several other proteins residing in late endosomes/lysosomes, especially in the brain, future NPC-related research will provide insight into cellular lipid trafficking and the etiology of the disease.

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**References**

1. Patterson, M. C., Vanier, M. T., Suzuki, K., Morris, J. A., Carstea, E., Neufeld, E. E., Blanche-Mackie, J. E., and Pentchev, P. G. (2001) in *The Metabolic and Molecular Bases of Inherited Disease* (Scriver, C. R., Beaudet, A. L., Sly, W. S., Valle, D., eds) 8th ed., Vol. 3, pp. 3611–3633, McGraw-Hill, New York.

2. Radakrishnan, A., Sun, L. P., Kwon, H. J., Brown, M. S., and Goldstein, J. L. (2004) *Mol. Cell* **15**, 259–268.

3. Kobayashi, T., Beuchat, M.-H., Chevallier, J., Makino, A., Mayran, N., Escola, J. M., Lebrand, C., Cousin, F., Kobayashi, T., and Graupner, G. (2002) *J. Biol. Chem.* **277**, 32157–32164.

4. Naureckiene, S., Sleat, D. E., Lackland, H., Fensom, A., Vanier, M. T., Watti, B., Witters, M., Ladoux, R., Jadot, M., and Lebel, P. (2000) *Science* **290**, 2296–2301.

5. Ko, D. C., Binkley, J., Sisow, A., and Scott, M. P. (2003) *Proc. Natl. Acad. Sci. U. S. A.* **100**, 2518–2525.

6. Friedland, N., Liu, H. L., Lobel, P., and Stock, A. M. (2003) *Proc. Natl. Acad. Sci. U. S. A.* **100**, 2512–2517.

7. Sugii, N., Reid, P. C., Sugii, N., Du, H., and Chang, T. Y. (2003) *J. Biol. Chem.* **278**, 27180–27189.

8. Hofman, K. M., and Liscum, L. (2003) *J. Biol. Chem.* **278**, 14850–14856.

9. Frolov, A., Zielinski, S. E., Crowley, J. R., Vockley, C. W., Marks, D. L., Platt, F. M., van Blitterswijk, W. J., and Sillence, D. J. (2004) *J. Biol. Chem.* **279**, 48214–48223.

10. Karten, B., Vanc, D. E., Raman, R. M., and Faust, J. R. (1989) *J. Cell. Biol.* **108**, 1625–1636.

11. Scott, C., Higginson, M. E., Davies, J. P., and Ioannou, Y. A. (2004) *J. Biol. Chem.* **279**, 45324–45334.

12. Kuwabara, P. E., and Labouesse, M. (2002) *Trends Genet.* **18**, 193–201.

13. Okada, M., Nishimura, T., and Takahashi, K. (2002) *J. Biol. Chem.* **277**, 19520–19527.

14. Kuwabara, P. E., and Labouesse, M. (2002) *Trends Genet.* **18**, 193–201.

15. Scott, C., Higgins, M. E., and Snow, K. (2003) *Hum. Mutat.* **22**, 313–325.

16. Zhang, M., Dwyer, N. K., Nakanishi, S., Sawata, Y., Muramatsu, H., and Kato, T. (2003) *Science* **300**, 2296–2298.

17. Davies, J. P., Chen, F. W., and Ioannou, Y. A. (2003) *J. Biol. Chem.* **278**, 347–356.

18. Frolov, A., Zielinski, S. E., Crowley, J. R., Vockley, C. W., Marks, D. L., Platt, F. M., van Blitterswijk, W. J., and Sillence, D. J. (2004) *J. Biol. Chem.* **279**, 48214–48223.

19. Kuwabara, P. E., and Labouesse, M. (2002) *Trends Genet.* **18**, 193–201.

20. Scott, C., Higgins, M. E., and Snow, K. (2003) *Hum. Mutat.* **22**, 313–325.

21. Zhang, M., Dwyer, N. K., Nakanishi, S., Sawata, Y., Muramatsu, H., and Kato, T. (2003) *Science* **300**, 2296–2298.

22. Davies, J. P., Chen, F. W., and Ioannou, Y. A. (2003) *J. Biol. Chem.* **279**, 48214–48223.

23. Kuwabara, P. E., and Labouesse, M. (2002) *Trends Genet.* **18**, 193–201.

24. Kuwabara, P. E., and Labouesse, M. (2002) *Trends Genet.* **18**, 193–201.

25. Kuwabara, P. E., and Labouesse, M. (2002) *Trends Genet.* **18**, 193–201.

26. Kuwabara, P. E., and Labouesse, M. (2002) *Trends Genet.* **18**, 193–201.

27. Kuwabara, P. E., and Labouesse, M. (2002) *Trends Genet.* **18**, 193–201.

28. Kuwabara, P. E., and Labouesse, M. (2002) *Trends Genet.* **18**, 193–201.

29. Kuwabara, P. E., and Labouesse, M. (2002) *Trends Genet.* **18**, 193–201.

30. Karten, B., Vance, D. E., Campbell, R. B., and Vance, J. E. (2002) *Proc. Natl. Acad. Sci. U. S. A.* **100**, 13473–13478.

31. Mutka, A. L., Lusa, S., Linder, M. D., Jokitalo, E., Kopra, O., Jauhiainen, M., and clan, M. T., and Dash, R. M. (2002) *Nat. Rev. Mol. Cell Biol.* **3**, 274–285.

32. Kuwabara, P. E., and Labouesse, M. (2002) *Trends Genet.* **18**, 193–201.

33. Kuwabara, P. E., and Labouesse, M. (2002) *Trends Genet.* **18**, 193–201.

34. Puri, V., Jefferson, J. R., Singh, R. D., Wheatley, C. L., Marks, D. L., Platt, F. M., van Blitterswijk, W. J., and Sillence, D. J. (2004) *J. Biol. Chem.* **279**, 1637–1659.

35. te Vruchte, D., Lloyd-Evans, E., Veldman, R. J., Neville, D. C., Dwek, R. A., Platt, P. M., van Blitterswijk, W. J., and Sillence, D. J. (2004) *J. Biol. Chem.* **279**, 48214–48223.
Simons, K., and Gruenberg, J. (2000) Trends Cell Biol. 10, 459–462.

Reagan, J. W., Jr., Hubbert, M. L., and Sheldes, G. S. (2000) J. Biol. Chem. 275, 38104–38110.

Salvini, R., Scarpa, S., Ciaffoni, F., Tatti, M., Ramoni, C., Vanier, M. T., and Vaccaro, A. M. (2004) J. Biol. Chem. 279, 17674–17680.

Krishnamurthy, M., Higaki, K., Tinkelenberg, A. H., Almanzar-Paramio, D., Wilcox, L., Erdeniz, N., Redican, F., Padamsee, M., Liu, Y., Khan, S., Alcantara, F., Ciaffoni, F., Carstea, E. D., Morris, J. A., and Sturley, S. L. (2004) J. Cell Biol. 164, 547–556.

Pfeffer, S., and Aivazian, D. (2004) Nat. Rev. Mol. Cell. Biol. 5, 886–896.

Eskelinen, E. L., Schmidt, C. K., Neu, S., Willenborg, M., Fuertes, G., Salvadore, N., Tanaka, Y., Lullmann-Rauch, R., Hartmann, D., Heeren, J., von Figura, K., Knecht, E., and Säffig, P. (2004) Mol. Biol. Cell 15, 3132–3145.

Lebrad, C., Corti, M., Goodson, H., Cosson, P., Cavalli, V., Mayran, N., Faure, J., and Gruenberg, J. (2002) EMBO J. 21, 1289–1300.

Choudhury, A., Sharma, D. K., Marks, D. L., and Pagano, R. E. (2004) Mol. Biol. Cell 15, 4500–4511.

Choudhury, A., Dominguez, M., Puri, V., Sharma, D. K., Narita, K., Wheatley, C. L., Marks, D. L., and Pagano, R. E. (2002) J. Clin. Invest. 109, 1541–1550.

Walker, M., Davies, J. P., and Ioannou, Y. A. (2003) J. Lipid Res. 44, 243–253.

Alpy, F., Wendling, C., Rio, M. C., and Tomasetto, C. (2002) J. Biol. Chem. 277, 50780–50787.

Kishida, T., Kostetskii, I., Zhang, Z., Martinez, F., Liu, P., Walkley, S. U., Dwyer, N. K., Blanchette-Mackie, E. J., Radice, G. L., and Strauss, J. F., III (2004) J. Biol. Chem. 279, 19276–19285.

Frolov, A., Srivastava, K., Daphna-Iken, D., Traub, L. M., Schaffer, J. E., and Ory, D. S. (2001) J. Biol. Chem. 276, 46414–46421.

Neufeld, E. B., Stonik, J. A., Demosky, S. J., Jr., Knapper, C., Combs, C. A., Cooney, A., Comly, M., Dwyer, N., Blanchette-Mackie, J., Remaley, A. T., Santamarina-Fojo, S., and Brewer, H. B., Jr. (2004) J. Biol. Chem. 279, 15571–15578.

White, N. M., Corey, D. A., and Kelley, T. J. (2004) Am. J. Respir. Cell Mol. Biol. 31, 538–543.

Wassif, C. A., Vied, D., Tsokos, M., Connor, W. E., Steiner, R. D., and Porter, F. D. (2002) Mol. Genet. Metab. 75, 325–334.

Ong, W.-Y., Kumar, U., Switzer, R. C., Siddhu, A., Suressh, G., Hu, C.-Y., and Patel, S. C. (2001) Exp. Brain Res. 141, 218–231.

Reid, P. C., Nakashita, N., Sugii, S., Ohno-Iwashita, Y., Shimada, Y., Hickey, W. F., and Chang, T. Y. (2004) J. Lipid Res. 45, 582–591.

Takikita, S., Fukuda, T., Meth, I., Yagi, T., and Suzuki, K. (2004) J. Neuropathol. Exp. Neurol. 63, 660–673.

Zervas, M., Dobrenis, K., and Walkley, S. U. (2001) J. Neuropathol. Exp. Neurol. 60, 49–64.

German, D. C., Liang, C. L., Song, T., Yazdani, U., Xie, C., and Dietschy, J. M. (2002) Neuroscience 109, 437–450.

Xie, C., Burns, D. K., Turley, S. D., and Dietschy, J. M. (2000) J. Neuropathol. Exp. Neurol. 59, 1106–1117.

Lofts, S. R., Krocken, R. P., Walkley, S. U., Bryant, M. A., Incao, A., Heidenreich, R. A., and Pavan, W. J. (2002) Hum. Mol. Genet. 11, 3107–3114.

Sleat, D. E., Wiseman, J. A., El-Banna, M., Price, S. M., Verot, L., Shen, M. M., Tint, G. S., Vanier, M. T., Walkley, S. U., and Lobel, P. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 5886–5891.

Zervas, M., Somers, K. L., Thrall, M. A., and Walkley, S. U. (2001) Curr. Biol. 11, 1283–1287.

Compagnone, N. A., and Mellon, S. H. (2000) Front Neuroendocrinol. 21, 1–56.

Griffin, L. D., Gong, W., Verot, L., and Mellon, S. H. (2004) Nat. Med. 10, 704–711.