Neutral Lipid Trafficking Differentiates Niemann-Pick C (NPC) 1 from NPC2 Mutant Fibroblasts

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Introduction

Niemann Pick C disease (NPC) is an inherited human metabolic disease that results in neuron degeneration and low density lipoprotein derived (LDL) cholesterol accumulation in cells. Although mutations of two separate genes, NPC1 and NPC2 result in similar pathology, 95% of the patients have the defective NPC1 gene. Both NPC1 and NPC2 proteins reside in the late endocytic compartment of cells; NPC1 is in late endosomes while NPC2 resides in lysosomes. The classic phenotype in both the NPC1 and NPC2 cells is accumulation of LDL derived free cholesterol in lysosomes due to the inability of mutant cells to traffic cholesterol to other cellular sites. However, the question remains whether these two proteins have differential roles in post-endocytic trafficking of endocytosed cholesterol as well as other lipids. Recently, our laboratory has found a difference in the trafficking of a glycolipid (GM2) between these two mutant genotypes. Now, this present study shows that intracellular neutral lipid (cholesterol ester) accumulation in response to LDL uptake is different between NPC1 and NPC2 mutant fibroblasts. This suggests that the two proteins may play separate roles in intracellular cholesterol trafficking.

Figure 3 – Unesterified cholesterol accumulation in a mutant NPC fibroblast visualized with filipin fluorescence.

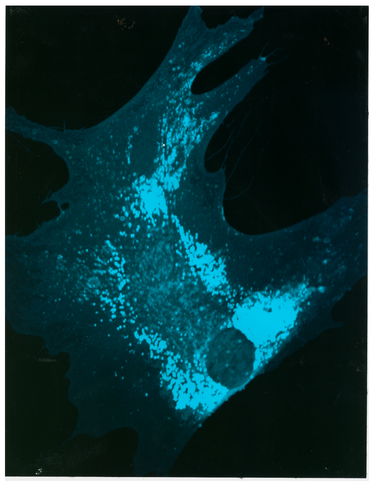


Figure 1 – Neutral lipid droplets do not accumulate in NPC2 fibroblasts after endocytic uptake of LDL in contrast to Normal, NPC1, and Tay-Sachs (TS) fibroblasts.

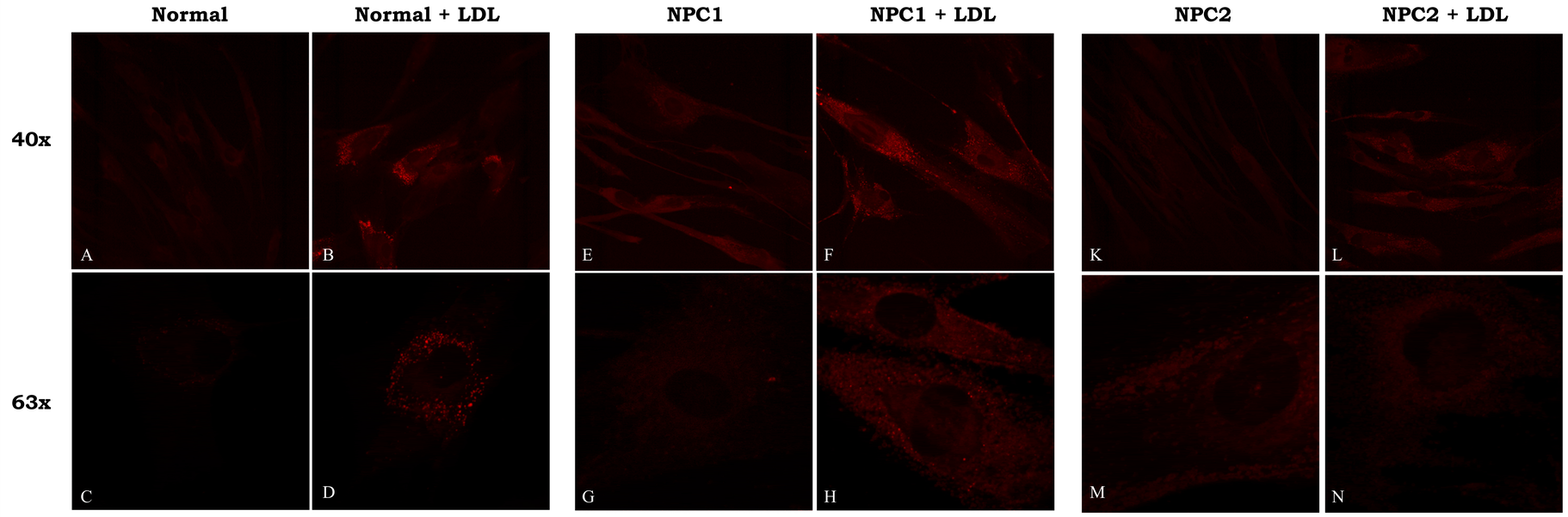
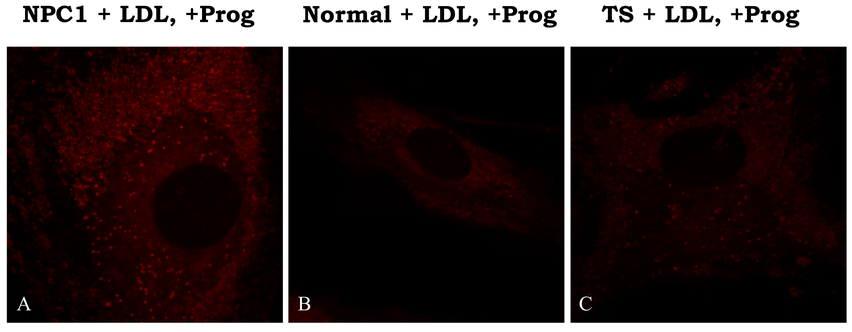


Figure 2 – Progesterone (Prog) prevents the accumulation of LDL derived neutral lipid droplets in normal and Tay-Sachs fibroblasts, but not in NPC1 fibroblasts. (63x)



- Normal fibroblasts: Nile Red positive lipid droplets accumulate after LDL uptake (Fig. 1B, 1D).
- Tay-Sachs fibroblasts: Cells from patients with an inherited metabolic disease that results in accumulation of GM2 in lysosomes. Similar to normal cells, Nile Red positive lipid droplets accumulate after LDL uptake (Fig. 1J).
- NPC1 fibroblasts: Similar to normal cells, Nile Red positive lipid droplets accumulate after LDL uptake (Fig. 1F and 1H).
- **NPC2 fibroblasts: Nile Red positive lipid droplets do not accumulate after LDL uptake (Fig. 1L and 1N).**

Progesterone Study:

Progesterone blocks cholesterol transport from lysosomes to the ER, preventing cholesterol ester accumulation and formation of Nile Red positive droplets in normal (Fig. 2B) and Tay-Sachs fibroblasts (Fig. 2C). However, Nile Red positive lipid droplets do accumulate in NPC1 fibroblasts (Fig. 2A).

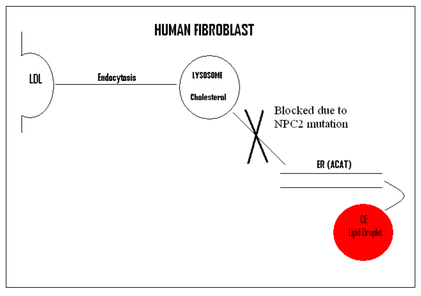
Methods

- Four cell lines were cultured: Normal, NPC1 null, NPC2 null, and Tay-Sachs (TS).
- 72 hours before experiments, cells were placed in delipidated media to upregulate LDL receptors.
- 24 hours before the experiment, selected cells were incubated with one of two treatments: +LDL or +LDL/+Progesterone.
  - +LDL is fed a bolus of low density lipoprotein.
  - +LDL/+Progesterone is fed a bolus of low density lipoprotein and a treatment of progesterone. Progesterone is known to block cholesterol transport.
- Cells were fixed with 3% paraformaldehyde for 30 minutes at room temperature.
- Neutral lipid droplets in the cells were identified with Nile Red.
- Nile Red staining was examined and recorded with a Zeiss LSM 410 confocal microscope.

Results

- Normal cells: Endocytic uptake of LDL derived cholesterol ester (CE) results in hydrolysis of that CE in lysosomes. The resultant free cholesterol traffics to the endoplasmic reticulum (ER) where it is re-esterified to CE by acyl cholesterol acyl transferase (ACAT). Accumulation of cholesterol esters (neutral lipids) forms intracellular lipid droplets that can be visualized with Nile Red staining.

Figure 4 – A diagram showing the pathway for the formation of Nile Red positive lipid droplets. Functional NPC2 is proposed to play a role in the transport of cholesterol from the lysosomes to the ER.



Discussion

- After LDL uptake, control fibroblasts (normal) have an increase in neutral lipid droplets due to esterification of endocytosed cholesterol by ACAT in the ER. Normal fibroblasts also have the expected decrease of lipid droplets after progesterone treatment. Progesterone blocks egress of LDL derived cholesterol out of lysosomes.
- After LDL uptake, NPC2 null mutant fibroblasts have little neutral lipid droplets compared to control or NPC1 fibroblasts. This result suggests that functional NPC2, which resides in lysosomes, may be necessary for transport of LDL derived cholesterol to the ER.

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General References

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