Lay Summary #2

Activation of an alternative cholesterol homeostatic mechanism in Niemann-Pick disease Type C

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Export of free cholesterol out of late endosomes (LE)/lysosomes (Ly) to the endoplasmic reticulum (ER) is blocked in NPC1 and NPC2 mutant cells. The ER is the site where cells regulate cholesterol levels by a number of different mechanisms including (1) conversion of free cholesterol into esterified cholesterol by acyl coenzyme A: cholesterol acyltransferase (ACAT) and storage in lipid droplets (LDs); (2) changes in the expression of genes involved in the maintenance of cholesterol levels; and (3) degradation of HMG-CoA reductase, the rate limiting enzyme in cholesterol synthesis. High levels of free cholesterol are interpreted by the ER and cause changes that effectively reduce the level of free cholesterol to a base level. The mutations that cause NPC block the ability of the ER to sense high cholesterol levels, leading to increased accumulation of cholesterol and impairment of cellular activities. We have established that RIDα rescues the NPC1 cholesterol storage phenotype, and we are currently working on understanding the mechanism of this action. We hypothesize that RIDα activates a mechanism redundant to NPC1 for the transport of cholesterol out of LE/Ly to the ER.

Previously, we demonstrated that expression of RIDα caused a significant increase in the amount of esterified cholesterol in a CHO cell model of NPC disease (CT43). We now show that RIDα induces an increase in the size and number of LDs in CT43 cells compared to CT43 cells that do not express RIDα. We also observed a dramatic increase in LD formation in NPC1 mutant fibroblasts transiently expressing RIDα compared to fibroblasts in the same field that did not express RIDα. Interestingly, expression of RIDα in NPC2 mutant fibroblasts had no effect on the formation of LDs compared to mock-transfected NPC2 fibroblasts, consistent with our hypothesis that the ability of RIDα to rescue the NPC1 cholesterol storage phenotype is dependent on NPC2. Furthermore, RIDα had no effect on LD formation in normal fibroblasts, indicating that, while RIDα may provide a redundant cholesterol egress mechanism to NPC1, it is unneeded in the presence of functional NPC1. Since esterified cholesterol is produced in the ER by ACAT from newly delivered free cholesterol, the observed increases in esterified cholesterol and LDs in RIDα-expressing cells is evidence that RIDα mediates transport of cholesterol to the ER in the absence of functional NPC1. We are currently performing experiments to specifically test the activity of ACAT, which will further support our hypothesis that RIDα mediates transport of cholesterol to the ER in NPC1 mutant cells.

As stated above, a second outcome of transport of free cholesterol to the ER is the regulation of genes involved in cholesterol homeostasis. Gene transcription is activated by SREBP when free cholesterol levels are low, and blocked when free cholesterol levels are high. We tested the effect of sterol load on the expression of *HMGCR*, the gene for HMG-CoA reductase. In parental CHO cells with wild-type NPC1 and NPC2, sterol-starved cells display a 3.5-fold increase in *HMGCR* expression, while sterol-loaded cells have *HMGCR* expression near basal levels. In CT43 cells that express non-functional NPC1, sterol-starved cells do not show an increase in *HMGCR* expression, while sterol-loaded cells exhibit a 2.5-fold increase. These results demonstrate that CT43 cells are unable to properly sense and regulate cholesterol levels caused by the inhibition of ER cholesterol transport due to NPC1 mutation. Interestingly, expression of RIDα in CT43 cells does not correct *HMGCR* expression levels in sterol-starved and loaded cells. Therefore, while RIDα promotes cholesterol transport to the ER for ACAT-mediated storage, it does not promote cholesterol transport to the ER for SREBP-mediated gene

regulation. These data are supported by research indicating that separate pools of cholesterol regulate ACAT and SREBP responses. We are currently performing additional experiments to support this concept, including studying the degradation of HMG-CoA reductase, a cellular response that is also mediated through SREBP ER cholesterol pools.

The NPC1 mutant fibroblasts used in our studies are a compound heterozygote harboring the P237S mutation and the I1061T mutation in separate alleles. The I1061T mutation causes NPC1 to buildup in the ER since proper trafficking to LE/Ly is inhibited. Recently it has been demonstrated that treatment of these cells with histone deacetylase inhibitors (HDACi) rescues the cholesterol storage phenotype by increasing the expression of NPC1 and forcing escape of NPC1 from the ER to LE/Ly where it can function in cholesterol egress. The relocalization of NPC1 from ER to LE/Ly is one potential mechanism for RIDα-mediated NPC1 rescue. Therefore, we used confocal microscopy to study the localization pattern of NPC1 in normal fibroblasts as well as mock-transfected and RIDα-transfected NPC1 mutant fibroblasts. In normal fibroblasts, we found that NPC1 colocalized with the LE marker LAMP1, but not with the ER markers KDEL and calreticulin. In mock-transfected NPC1 mutant fibroblasts, NPC1 colocalized with both ER markers, but not with the LE marker LAMP1. Similarly, RIDα expression in these cells did not alter the localization of NPC1, as it remained colocalized with the ER markers, and not with the LE marker. These results show that RIDα does not rescue the NPC1 cholesterol storage phenotype by a mechanism similar to HDACi, supporting our hypothesis that RIDα activates a mechanism redundant to NPC1 for the transport of cholesterol out of LE/Ly to the ER. Identification of this redundant mechanism could provide new targets for NPC therapies.