Lay Summary #3

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

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Many processes occur to maintain proper balance of cholesterol levels inside the cell. Cholesterol enters the cell in the form of LDL and is trafficked through endosomes, where NPC1 and NPC2 coordinate the transport of cholesterol out of the late endosome (LE)/lysosome (Ly). From there, cholesterol can be delivered to the endoplasmic reticulum (ER), which houses much of the cellular machinery that regulates cholesterol homeostasis. Cholesterol is also transported to mitochondria, where it can be converted to oxysterols that also regulate cholesterol pathways in the ER. These ER regulatory pathways mediated by cholesterol and oxysterols include 1) regulation of gene expression by cholesterol through SREBP/SCAP, 2) esterification of cholesterol by acyl coenzyme A: cholesterol acyltransferase (ACAT) and storage in lipid droplets (LDs), 3) regulation of gene expression by oxysterols through LXR, and 4) targeted degradation of HMG-CoA reductase (HMGR), the rate-limiting enzyme in cholesterol synthesis, by oxysterols through INSIG. Oxysterols can also regulate SREBP target genes through an interaction with INSIG. Mutations to NPC1 or NPC2 impair the transport of cholesterol to the ER, and perturb the mechanisms that regulate cholesterol homeostasis.

We have previously shown that expression of the adenovirus protein RIDα rescues the cholesterol storage phenotype of NPC1 patient fibroblasts by increasing storage of cholesterol in LDs. Restoration of cholesterol storage is blocked when RIDα-expressing cells are given an inhibitor of ACAT, revealing the importance of ACAT in RIDα-mediated NPC1 rescue. We have also shown that RIDα has no effect on SREBP target gene expression in NPC1 mutant cells. Since SREBP regulation can occur through cholesterol or oxysterol sensing, we sought to understand the role of RIDα on LXR target gene regulation. We have found that RIDα has no effect on LXR target gene expression, indicating RIDα has no effect on cholesterol transport to mitochondria for oxysterol production. We are also testing the effect of RIDα on HMGR degradation, and have been troubleshooting our experimental conditions. In summary, our data indicates that RIDα mediates delivery of cholesterol to the ER in NPC1 mutant cells to a pool that is sensitive to ACAT only. These results have implications for NPC treatment since stored cholesterol is less toxic to the cell compared to free cholesterol.

While RIDα rescues the cholesterol storage phenotype of NPC1 mutant cells and increases LD formation, it has no effect in NPC2 mutant cells. We had previously shown that RIDα colocalizes with lysobisphosphatidic acid (LBPA), an unconventional phospholipid found in LEs that is closely coupled to cholesterol levels, in NPC1 patient fibroblasts. To help understand why RIDα does not rescue the NPC2 cholesterol storage phenotype, we tested RIDα-expressing NPC2 cells for LBPA colocalization, and found that RIDα does not colocalize with LBPA in these cells. These data support our previous hypothesis that RIDα function is dependent on NPC2, but also uncovers a role for LBPA in RIDα-mediated NPC1 rescue as well.

Finally, we recently published a review on the similarities between the cholesterol storage phenotype of NPC and that of cystic fibrosis with one of our colleagues at Case Western Reserve University, Tom Kelley. The review focuses on what we can learn from cystic fibrosis that we can apply to NPC and the possibility of finding common therapeutic targets. We have been invited to list this publication in the next edition of Global Medical Discovery Series, a "service [that] alerts the scientific community to breaking journal articles considered to be of importance to the drug discovery process."