

Lay Summary #5

Activation of an alternative cholesterol homeostatic mechanism in Niemann-Pick disease Type C
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Niemann-Pick disease type C (NPC) is caused by mutations to NPC1 or NPC2 and is characterized by high levels of free cholesterol in late endosomes (LE) and lysosomes (Ly) termed lysosomal storage organelles (LSOs). These mutations disrupt the regulation of cholesterol levels in the cell since there is a blockage in the transport of cholesterol to the endoplasmic reticulum (ER) where the cholesterol regulatory machinery is located. One approach to treat NPC is to restore trafficking of cholesterol to the ER and reengage this regulatory machinery to reestablish equilibrium in the cell. While much is known about the trafficking routes of cholesterol in the cell, there is a lack of understanding of how cholesterol is transported from LE/Ly to the ER. A better understanding of this fundamental transport step could uncover novel targets for NPC treatments.

We have previously shown that expression of the Adenovirus protein RID α rescues the cholesterol storage phenotype in NPC1-mutant cells. Interestingly, expression of RID α in NPC1-mutant cells stimulates the formation of lipid droplets (LD), organelles that store a form of cholesterol called esterified cholesterol. Storage of esterified cholesterol is thought to be less toxic to cells than high levels of free cholesterol, so this outcome can be viewed as a positive step towards rescuing the phenotype. LDs are formed when cholesterol is transported to the ER so that it can be acted upon by the enzyme ACAT, which esterifies cholesterol. Therefore, RID α is able to overcome the loss of NPC1 function and restore the transport of cholesterol to the ER. We have recently shown that RID α function is dependent on two proteins that play a role in communication between LEs and the ER. ORP1L is located on LEs and senses and binds cholesterol and oxysterols, and also interacts with RID α . VAP-A also interacts with ORP1L, and is located on the ER. The interaction of ORP1L with VAP-A causes LEs to come into close contact with the ER, and potentially provides a mechanism for the transport of cholesterol from LEs to the ER. We have shown using fluorescence microscopy that RID α is in close proximity to both LEs (marked by the LE-specific protein LAMP1) and the ER (marked by VAP-A). We have also discovered that experimental reduction of ORP1L or VAP-A protein levels blocks the ability of RID α to induce formation of LDs in NPC1-mutant cells. We have graciously been provided with ORP1L mutants that disrupt the interaction with VAP-A, or the ability of ORP1L to bind cholesterol, and have tested these mutants for their effect on RID α function. Both of these ORP1L mutants completely block the ability of RID α to rescue the NPC1-mutant cholesterol storage phenotype, indicating that RID α function is dependent on the ORP1L-VAP-A interaction and the capacity of ORP1L to bind cholesterol. Collectively, these results suggest for the first time a role for ORP1L in the transport of cholesterol to the ER independent of NPC1, and that the Adenovirus protein RID α may reprogram ORP1L to facilitate cholesterol trafficking.

We are currently working on an experimental method that will allow us to directly test the transport of LDL-cholesterol out of LEs to the ER for re-esterification and storage in LDs. We will then be able to apply this method to specifically examine the mechanism by which RID α restores cholesterol transport to the ER through ORP1L/VAP-A-mediated LE-ER contacts independent of NPC1. Dissecting this pathway will add to our understanding of the mechanism of cholesterol transport to the ER and may provide a unique insight into novel potential targets for NPC therapy.