Lay Summary #1

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

Nicholas Cianciola, Sponsor: Cathleen Carlin

At the cellular level, NPC disease is characterized by the buildup of cholesterol in aberrant late endosomes (LEs) and lysosomes termed lysosomal storage organelles. Mutations in NPC1 or NPC2, the proteins that coordinate the export of cholesterol out of these compartments, blocks the transport of cholesterol out of endosomes to other membranes in the cell, including the endoplasmic reticulum (ER). The ER houses the machinery that regulates cellular cholesterol levels, which senses changes in cholesterol load and modifies the expression of cholesterol regulatory genes accordingly. An increase in ER cholesterol triggers a cascade of reactions aimed at decreasing cellular cholesterol to maintain homeostasis. A second level of control is the activation of acyl coenzyme A: cholesterol acyltransferase (ACAT), which converts free cholesterol into cholesterol esters that can be stored in lipid droplets. NPC mutations interrupt the management of cholesterol levels further enhancing the buildup of cholesterol in the cell. While researchers have shown that NPC1 and NPC2 function in the transport of cholesterol out of endosomes, the mechanism of cholesterol transport to the ER has yet to be determined.

We have previously reported that the adenovirus protein RIDα can rescue the cholesterol storage phenotype when expressed in NPC1 fibroblasts. We hypothesized that RIDα restores cholesterol homeostasis at the level of the ER in addition to alleviating cholesterol storage abnormalities in NPC cells. We are currently studying RIDα in a cell model of NPC1 disease, and have quantified the correction of the NPC phenotype by assaying the cholesterol load of these cells. We found that RIDα causes a modest decrease in both total and free cholesterol. However we were surprised to discover that RIDα induces a significant 33% increase in the amount of stored cholesterol. This result supports our hypothesis that RIDα is directing cholesterol to the ER, but specifically into a pool of cholesterol that is under the control of ACAT. This increase in cholesterol esters is potentially beneficial to NPC disease correction since it has been postulated that stored cholesterol is less toxic to the cell compared to free cholesterol. Future experiments will be aimed at identifying the contribution of ACAT in the correction of the NPC phenotype by RIDα.

We have also made progress on identifying a potential mechanism of cholesterol transport from endosomes to the ER. A recent paper discovered that oxysterol-binding protein related protein 1 (ORP1L) found on late endosomes interacts with the ER protein VAMP-associated protein (VAP) to create LE-ER contacts. We previously discovered that RIDα binds ORP1L, and we now show that RIDα colocalizes with VAP. Furthermore, we found that RIDα expression in NPC1 mutant cells display an increase in the amount of contact sites between VAP and the late endosome markers LAMP1 and LAMP2 under conditions of high cholesterol. These data support our hypothesis that RIDα induces LE-ER contacts through ORP1L/VAP interactions. We are currently testing whether RIDα is directly involved in mediating these LE-ER contacts using a number of different microscopic techniques. Taken together, our data indicates that RIDα is able to overcome the inhibition of ER cholesterol transport caused by NPC1 mutation, perhaps by increasing LE-ER contacts as a mechanism of cholesterol delivery to the ER.