Lay Summary #4

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

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Niemann-Pick disease type C is an autosomal recessive neurodegenerative disorder caused by mutations to NPC1 or NPC2. These proteins are located in the late endosome (LE)/lysosome (Ly) at the end of a network of vesicles that transports internalized cargo throughout the cell, and they work together to export cholesterol out of this compartment. Cholesterol is transported to the endoplasmic reticulum (ER) where it regulates a number of pathways that control cholesterol levels inside the cell. When NPC1 or NPC2 function is disrupted, cholesterol levels in LE/Ly increase because transport of cholesterol out of this compartment and to the ER is blocked. Therefore, the machinery located in the ER that regulates cholesterol levels cannot sense this increase in cholesterol, and continues production of cholesterol, thus compounding the issue. Although much is known about the transport of cholesterol inside the cell, we still do not know how cholesterol is trafficked from LE/Ly to the ER. Better understanding of this important step in cholesterol trafficking could lead to new targets for NPC therapies.

We have previously shown that expression of the Adenovirus protein RIDα in NPC1-mutant fibroblasts rescues the cholesterol storage phenotype and also induces an increase in the formation of lipid droplets (LDs), compartments that store a form of cholesterol. Cholesterol storage is viewed as beneficial to the overall health of the cell because high free cholesterol levels are more toxic than stored cholesterol. Our lab has also shown that RIDα interacts with a protein called ORP1L, which binds cholesterol and is found on LEs. ORP1L function is controlled by Rab7, which is a master regulator of transport to and from LEs. ORP1L interacts with a protein located on the ER called VAP-A, and this interaction creates membrane contact sites between the LE and ER. These contact sites could provide a mechanism for cholesterol transport because cholesterol could “flip” from the LE to the ER. Since RIDα interacts with ORP1L, we asked whether ORP1L or VAP-A had a role in the ability of RIDα to rescue the cholesterol storage phenotype in NPC1-deficient cells. We performed siRNA knockdown of ORP1L or VAP-A in NPC1-deficient cells that also expressed RIDα. We found that removal of ORP1L or VAP-A blocked the ability of RIDα to rescue the NPC1 cholesterol storage phenotype, and also blocked the RIDα-induced increase in LD formation in NPC1-deficient cells. Furthermore, we stained cells with antibody specific for RIDα, as well as antibody to LAMP1 (which marks LEs) and VAP-A (which marks the ER), and found that RIDα is located simultaneously in close apposition to both the ER and LE. This is evidence that RIDα mediates formation of membrane contact sites between the ER and LE. Taken together, these date suggest for the first time a role for ORP1L in cholesterol transport, and indicate a role for ORP1L and VAP-A in rescue of the NPC1 cholesterol storage phenotype by RIDα.

Cholesterol transport at membrane contact sites is a hypothesis that is also shared for cholesterol trafficking from the ER to mitochondria. In both cases, this proposed mechanism would not require the function of an unknown cholesterol carrier protein, thus providing a simple explanation for cholesterol transport. We hypothesize that RIDα rescues the cholesterol storage phenotype in NPC1-deficient cells by forming LE-ER contact sites with ORP1L and VAP-A, and that these are sites for cholesterol transport to the ER. Since RIDα functions independent of NPC1, this proposed cholesterol trafficking mechanism may be redundant to that of NPC1 and NPC2. We will continue to examine the mechanism by which RIDα rescues the NPC1 cholesterol storage phenotype, and will strive to solidify the notion that ORP1L plays an important role in mammalian cholesterol trafficking.