Cholesterol Transport through Lysosome-Peroxisome Membrane Contacts: Concerns about the Unexpected Connection

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To the Editor:

Gaining a better insight into the mechanisms governing intracellular lipid transport is of fundamental importance to medical science. Recently, Song and coworkers reported in *Cell* the existence of dynamic membrane contact sites between lysosomes and peroxisomes that mediate cholesterol transport from the former to the latter organelle (Chu et al., 2015). In addition, the authors argue that these contact sites are formed by interaction of lysosomal synaptotagmin VII (SYT7) with phosphatidylinositol 4,5 bisphosphate ($PI(4,5)P_2$) on the peroxisomal membrane and that cholesterol is accumulating in cultured cells from patients and mice with diverse peroxisomal disorders. The authors employed an elegant genome-wide RNAi screen in HeLa cells to detect genes that affect cholesterol trafficking from lysosomes to the plasma membrane. To validate the unexpected enrichment of various genes involved in peroxisome biogenesis and metabolism, they designed an organelle co-precipitation method to identify components of, and functionally characterize, the lysosome-peroxisome membrane contacts observed by advanced microscopy at the biochemical level. However, to our surprise, this assay was based on the affinity capturing of peroxisomes in HeLa cells expressing EGFP-His_6-SKL on Ni-Sepharose. Indeed, given that SKL-tagged variants of EGFP are routinely used in the peroxisome field as *bona fide* marker proteins for the peroxisome lumen, EGFP-His_6-SKL is physically shielded from the Ni-affinity matrix by the peroxisomal membrane and cannot be used to study direct interactions between the peroxisomal and lysosomal compartments. As such, all conclusions deriving from this particular experimental setup need to be interpreted with care, including the cholesterol transfer from lysosomes to peroxisomes via (lysosomal) Syt7-(peroxisomal)$PI(4,5)P_2$ membrane contact sites. Also, we are very puzzled by the authors' observations that a knockdown of *PEX1*, *PEX3*, *PEX6*, *PEX10*, or *PEX26* results in fewer peroxisome-lysosome contact sites and the accumulation of cholesterol, but not in a cytosolic mislocalization of EGFP-His_6-SKL. Of course, the latter finding may be explained by the fact that residual amounts of these peroxins are sufficient to maintain a functional peroxisomal protein import machinery. However, if this is indeed the case, it may well be that the *in cellulo* decrease in lysosome-peroxisome contact sites can be attributed to a reduction in peroxisome number, a well-known consequence of *PEX* gene disruption (Steinberg et al., 2006), rather than to altered peroxisome properties.

The authors translate their findings by showing massive cholesterol accumulation (as determined by filipin staining) in skin fibroblasts from patients with either peroxisome biogenesis defects or ABCD1 deficiency and in tissues of *Abcd1* knockout mice. Here it is important to point out that other investigators have already reported that cholesterol is enriched in the late endosomal/lysosomal compartment of skin fibroblasts from peroxisome biogenesis disorder patients (Thai et al., 2001). Importantly, these authors link this

abnormality to a deficiency in plasmalogens, ether phospholipids that depend on peroxisomes for their synthesis. Others have shown that plasmalogen deficiency, one of the biochemical hallmarks of malfunctioning peroxisomes, interferes with the transport of cholesterol from the plasma membrane or endocytic compartments to the endoplasmic reticulum in Chinese hamster ovary cells (Munn et al., 2003). Hence, it is essential to take into consideration that silencing of peroxins can affect the plasma membrane cholesterol content by routes other than cholesterol transport from lysosomes to peroxisomes.

Concerning the findings related to ABCD1, a peroxisomal transporter necessary for import of a subset of substrates of the peroxisomal β-oxidation pathway, it is remarkable that no other essential peroxisomal β-oxidation enzymes were discovered in the screen nor tested in the validation assays, and that no logical links with a shortage of $PI(4,5)P_2$ in the peroxisomal membrane were proposed. The authors' assumption that perturbed cholesterol trafficking was overlooked as a key to the pathogenesis in *ABCD1* and *PEX* gene-related disorders is worth further investigation. However, cholesterol metabolism was investigated intensively in ABCD1 deficiency in the 1970's, subsequent to the discovery of the presence of very-long-chain fatty acid cholesterol esters in adrenals of X-ALD patients. To our knowledge, except for one, never confirmed publication (Burton & Nadler, 1974), no abnormalities in cholesterol metabolism were found in fibroblasts (Yavin et al., 1976). In addition, cholesterol levels were normal in liver and brain tissue of PEX-deficient mice (Vanhorebeek et al., 2001). Moreover, readers should be aware that there are vast differences between the phenotypes of ABCD1-, PEX- or NPC1-deficient patients and those of their respective mouse models. Finally, in contrast to what is stated by the authors, *Abcd1* knockout mice do not develop CNS demyelination (Kemp et al., 2012).

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