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Inhibition of HDACs 1, 2, And 3 Is Necessary for Clearance of Cholesterol Accumulation in NPC1 in Fibroblasts.

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Niemann-Pick disease type C (NPC) is a rare genetic cholesterol storage disorder caused by mutations in the NPC1 protein. Mutations in this trans-membrane protein lead to a block in normal cholesterol efflux from the late endosomes and lysosomes. Pan-histone deacetylase inhibitors (HDACi) such as Vorinostat (SAHA) can correct the cholesterol accumulation phenotype in NPC1 patient fibroblast cells. We screened 125 HDAC inhibitors that targeted either individual HDACs or classes of HDACs to identify which were necessary for cholesterol clearance in NPC1 patient-derived fibroblast cells. We used a high-throughput microscopy screening assay to identify compounds that resulted in cholesterol clearance from these cells. We have determined that the important targets for HDAC inhibition for cholesterol clearance in NPC1 fibroblasts are HDACs 1, 2, and 3.

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A novel approach to analyze lysosomal dysfunctions through subcellular proteomics and lipidomics: the case of NPC1 deficiency.

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Superparamagnetic iron oxide nanoparticles (SPIONs) have mainly been used as cellular carriers for genes and therapeutic products, while their use in subcellular organelle isolation remains underexploited. We engineered SPIONs targeting distinct subcellular compartments. Dimercaptosuccinic acid-coated SPIONs are internalized and accumulate in late endosomes/lysosomes, while aminolipid-SPIONs reside at the plasma membrane. These features allowed us to establish standardized magnetic isolation procedures for these membrane compartments with a yield and purity permitting proteomic and lipidomic profiling. We validated our approach by comparing the biomolecular compositions of lysosomes and plasma membranes isolated from wild-type and Niemann-Pick disease type C1 (NPC1) deficient cells. Our lipidomics analysis revealed the buildup of several species of glycerophospholipids and other storage lipids in late endosomes/lysosomes of NPC1 KO cells. While the plasma membrane proteome remained largely invariable, we observed pronounced alterations in several proteins linked to autophagy and lysosomal catabolism reflecting vesicular transport obstruction and defective lysosomal turnover resulting from NPC1 deficiency. Thus the use of SPIONs provides a major advancement in fingerprinting subcellular compartments, with an increased potential to identify disease-related alterations in their biomolecular compositions.