Enhanced lipid droplet degradation by split-intein-mediated lipid droplet targeting to lysosomes in mammalian cells

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Abstract

Lipid droplets (LDs) are endoplasmic-reticulum-derived neutral lipid storage organelles. An overabundance of LDs in mammalian cells is characteristic of the progression of the metabolic syndrome. Thus, the development of technologies to increase controlled LD breakdown could provide therapy to treat metabolic-associated disorders. Chaperone-mediated autophagy (CMA) plays a key role in LD breakdown. In CMA, LDs are guided to lysosomes where perilipins (PLINs) are degraded so that adipose tissue triglyceride lipase (ATGL) can convert the neutral lipids into free fatty acids for energy or materials for phospholipids. Here, we used a naturally spit intein to target LDs to lysosomes to enhance CMA. DNA constructs were introduced into NIH/3T3 fibroblasts where the C-terminal segment (NpuC) of the split was linked to PLIN2 and the N-terminal segment of the split intein (NpuN) was linked to the lysosomal surface protein LAMP2A. We showed that NpuC-mCherry-PLIN2/LDs colocalized with LAMP2A-NpuN-GFP/lysosomes in NIH/3T3 fibroblasts. These fibroblasts had a ˜4-fold decrease in the number of LDs versus non-transfected control cells and cells expressing a non-cleaving version of the intein system: NpuC(N136A)-mCherry-PLIN2. These results point to the possibility of using technologies to guide entire organelles or protein complexes to lysosomes to control cellular autophagy and upkeep.

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