Regulation of lysosome integrity and lysophagy by the ubiquitin-conjugating enzyme UBE2QL1

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Abstract:

Lysosomal membrane permeabilization or full rupture of lysosomes is a common and severe stress condition that is relevant for degenerative diseases, infection and cancer. Cells respond with extensive ubiquitination of damaged lysosomes, which triggers selective macroautophagy of the whole organelle, termed lysophagy. We screened a siRNA library targeting human E2-conjugating enzymes and identified UBE2QL1 as critical for efficient lysosome ubiquitination after chemically-induced lysosomal damage. UBE2QL1 translocates to lysosomes upon damage and associates with autophagy regulators. Loss of UBE2QL1-mediated ubiquitination reduced association of the autophagy receptor p62 and the LC3-decorated phagophore, and prevented recruitment of the ubiquitin-targeted AAA-ATPase VCP/p97 that facilitates lysophagy. Even in unchallenged cells, UBE2QL1 depletion led to mTOR dissociation and TFEB activation, and mutation of the homolog UBC-25 destabilized lysosomes in *C. elegans*, indicating that UBE2QL1 is critical for maintaining lysosome integrity in addition to lysophagy.

Main text:

Lysosomes are membranous subcellular organelles specialized for the digestion of cellular and endocytosed material. However, many agents and conditions can destabilize the integrity of lysosomal membranes, which can lead to leakage of lysosomal proteases into the cytosol and lysosomal cell death. Cells can repair limited permeabilization of the lysosomal membrane through the ESCRT machinery, but heavily damaged lysosomes are removed through the process of selective macroautophagy termed lysophagy. Lysophagy is triggered by ubiquitination of lysosomal proteins. This, in turn, recruits autophagic receptors (SQSTM1, TAX1BP1, optineurin, NDP52), which link it to LC3-positive autophagosomal structures. Subsequent engulfment of damaged lysosome and subsequent fusion of the resulting autophagosomes with intact lysosomes clears the damaged organelles.

In our recent publication, we screened an siRNA library for E2 ubiquitin-conjugating enzymes required for efficient ubiquitination of lysosomes damaged by the lysosomotropic agent L-Leucyl-L-Leucine methyl ester (LLOMe) in HeLa cells. Using immunofluorescence and microscopy-based readouts, we identified UBE2QL1 as the major regulator of lysosomal ubiquitination upon damage. In unchallenged cells, UBE2QL1 is distributed throughout the cytosol, but translocates

to lysosomes upon damage. This translocation is specific because UBE2QL1 is not recruited to depolarized mitochondria.

LLOMe treatment induces modification of lysosomes with K48- and K63-linked ubiquitin chains as detected with chain-specific antibodies. Depletion of UBE2QL1 resulted in a severe reduction of K48-chains on LAMP1-containing organelles, and to a lesser extent of K63-chains. Of note, K63-linked chains are the first to appear at 30 min following LLOMe treatment and peak at 1 h. In contrast, UBE2QL1 recruitment correlated with the delayed K48 ubiquitination that peaks at 2-3 h after damage indicating that UBE2QL1 primarily mediates K48-linked ubiquitination on lysosomes. Interestingly, immuno-electron microscopy revealed that both K48 ubiquitin chains and UBE2QL1 are located in the lysosomal lumen, suggesting that ubiquitination initiates on the luminal side of lysosomal membrane proteins. Ubiquitination of damaged lysosomes was rescued in UBE2QL1-depleted cells by overexpression of wild-type UBE2QL1, but not of a catalytically-inactive mutant (UBE2QL1-C88S).

For further insight, we fused an engineered ascorbate peroxidase (APEX2) to the C-terminus of UBE2QL1 and screened for the proteins found in its vicinity after damage by proximity biotinylation followed by quantitative mass spectrometry. Significant hits were lysosomal transmembrane proteins LIMP2/SCARB2, NPC1, LAMP1, LAMP2 that could be potential UBE2QL1 ubiquitination targets. We also identified cytosolic galectins (LGALS1, LGALS3 and LGALS8). The fact that galectins bind to luminal glycans concurs with the EM data detecting UBE2QL1 and K48 ubiquitin chains in the lumen of damaged lysosomes. Moreover, we identified two autophagy receptors (p62/SQSTM1 and TAX1BP1). Since the involvement of TAX1BP1 had not been previously described in lysophagy, we confirmed its translocation to damaged lysosomes by immunofluorescence microscopy.

One of the top hits of the APEX2 screen was the AAA-ATPase VCP/p97 and its cofactor PLAA. We had previously shown that VCP/p97 is recruited to damaged lysosomes by K48 ubiquitination and that it facilitates lysophagy in an as yet unknown manner. Of note, depletion of UBE2QL1 and concomitant loss of K48-linked ubiquitination abrogated VCP/p97 translocation suggesting that one key function of UBE2QL1 is to recruit the AAA-ATPase to damaged lysosomes. In addition, we observed that the loss of UBE2QL1-mediated ubiquitination reduced SQSTM1/p62 recruitment to damaged lysosomes, and consequently the association of LC3-positive phagophores. Depletion of UBE2QL1 does not affect LC3 lipidation nor autophagic flux in general. Consistent with the defect in phagophore formation around damaged lysosomes in UBE2QL1-depleted cells, lysophagy was compromised as detected with the LGALS3 assay. LLOMe-induced

lysosomal damage caused strong recruitment of LGALS3 in both control and UBE2QL1-depleted cells. However, whereas control cells were able to clear damaged lysosomes in a period of 10 h, a significant number of LGALS3-positive organelles persisted in UBE2QL1-depleted cells.

In addition to the role in the clearance of acutely damaged lysosomes, we found that UBE2QL1 is important for maintaining lysosomal homeostasis in normal conditions. Loss of UBE2QL1 caused partial dissociation of mTOR from lysosomes and dephosphorylation of TFEB, which translocates to the nucleus to induce biogenesis of lysosomal genes. Indeed, we observed an expanded LAMP1-containing compartment and higher levels of LAMP1 protein in cells depleted of UBE2QL1. The role of UBE2QL1 in maintaining lysosomal homeostasis was confirmed *in vivo* in *C. elegans.* Mutant worms, deficient for the UBE2QL1 homologue UBC-25 accumulated LGALS3-permeable lysosomes. This defect was aggravated in double mutant worms lacking both UBC-25 and the lysosome stabilizing protein SCAV-3 (the ortholog of LIMP2/SCARB2).

The finding that the ubiquitin-conjugating enzyme UBE2QL1 regulates lysophagy in interesting, but raises a number of questions. How UBE2QL1 is recruited to lysosomes is not understood. Since cytosolic galectins translocate into permeabilized lysosomes and are proposed to act as damage sensors, we hypothesized that they mediate UBE2QL1 recruitment. However, depletion of LGALS3 and LGALS8 had no effect on UBE2QL1 translocation to damaged lysosomes, indicating a different recruitment mechanism. Potential UBE2QL1 ubiquitination targets need to be identified and confirmed. Moreover, how UBE2QL1 is involved in the maintenance of lysosomal homeostasis in unchallenged cells also requires further investigation.