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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 disease, infection and cancer. Cells respond with extensive ubiquitination of damaged lysosomes, which triggers selective macroautophagy/autophagy of the whole organelle, termed lysophagy. We screened an 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 reduces association of the autophagy receptor SQSTM1/p62 and the LC3-decorated phagophore, and prevents recruitment of the ubiquitin-targeted AAA-ATPase VCP/p97 that facilitates lysophagy. Even in unchallenged cells, UBE2QL1 depletion leads to MTOR dissociation and TFEB activation, and mutation of the homolog UBC-25 destabilizes lysosomes in *C. elegans*, indicating that UBE2QL1 is critical for maintaining lysosome integrity in addition to lysophagy.

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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, OPTN, CALCOCO2/NDP52), which link it to LC3-positive phagophores. Subsequent engulfment of the damaged lysosome followed by 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 [1]. Using immunofluorescence and microscopy-based readouts, we identified UBE2QL1 as the major regulator of lysosomal ubiquitination upon organelle 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 results 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 correlates 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 reveals that both K48 ubiquitin chains and UBE2QL1 are located in the lysosomal lumen, suggesting that ubiquitination initiates on the lumenal side of lysosomal membrane proteins. Ubiquitination of damaged lysosomes is 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 SCARB2/LIMP2, NPC1, LAMP1, and LAMP2 that can be potential UBE2QL1 ubiquitination targets. We also identified cytosolic galectins LGALS1, LGALS3 and LGALS8. The fact that galectins bind to lumenal glycans concurs with the EM data detecting UBE2QL1 and K48 ubiquitin chains in the lumen of damaged lysosomes. Moreover, we identified 2 autophagy receptors (SQSTM1 and TAX1BP1). Because the involvement of TAX1BP1 had not been previously described in lysophagy, we confirmed its translocation to damaged lysosomes by immunofluorescence microscopy.

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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 abrogates 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 reduces SQSTM1 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 is compromised as detected with the LGALS3 assay. LLOMe-induced lysosomal damage causes strong recruitment of LGALS3 in both control and UBE2QL1-depleted cells. However, whereas control cells are able to clear damaged lysosomes in a period of 10 h, a significant number of LGALS3-positive organelles persist 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 causes partial dissociation of MTOR from lysosomes and dephosphorylation of TFEB, which translocates to the nucleus to induce expression 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 homolog UBC-25 accumulate permeable lysosomes as detected by decoration with overexpressed GFP-LGALS3. This defect is aggravated in double-mutant worms lacking both UBC-25 and the lysosome stabilizing protein SCAV-3 (a homolog of SCARB2/LIMP2).

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. Because 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 has 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.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Reference

[1] Koerver L, Papadopoulos C, Liu B, et al. The ubiquitin-conjugating enzyme UBE2QL1 coordinates lysophagy in response to endolysosomal damage. EMBO Rep. 2019 Aug;21:e48014. PMID: 31432621.