AUTOPHAGY
2017, VOL. 13, NO. 9, 1615–1616
https://doi.org/10.1080/15548627.2017.1339845

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Regulation of SQSTM1/p62 via UBA domain ubiquitination and its role in disease

YouJin Lee and Conrad C. Weihl

Department of Neurology, Washington University School of Medicine, St. Louis, MO, USA

ABSTRACT

Macroautophagy/autophagy can be a selective degradative process via the utilization of various autophagic receptor proteins. Autophagic receptors selectively recognize ubiquitinated cargoes and deliver them to phagophores, the precursors to autophagosomes, for their degradation. For example, SQSTM1/p62 directly binds to ubiquitinated protein aggregates via its UBA domain and sequesters them into inclusion bodies via its PB1 domain. SQSTM1also interacts with phagophores via its LC3-interacting (LIR) motif. However, a regulatory mechanism for autophagic receptors is not yet understood.

ARTICLE HISTORY
Received 30 May 2017
Revised 2 June 2017
Accepted 2 June 2017

KEYWORDS
autophagy; Keap1-Cullin3-Nrf2 pathway; neurodegenerative diseases; p62/SQSTM1; protein aggregates; ubiquitination

Accumulating evidence supports the idea that ubiquitination regulates the function of autophagic receptor proteins. For example, ubiquitination of the autophagic receptor OPTN (optineurin) by HACE1 increases its association with SQSTM1 and enhances autophagic flux. Also, several studies suggest that ubiquitination of SQSTM1 modulates its functions. RNF26 ubiquitinates SQSTM1s UBA domain at the ER membrane leading to an increase in SQSTM1s capture of ubiquitinated endosomes. However, it is not clear which lysine within SQSTM1s UBA domain is ubiquitinated by RNF26.

During xenophagy RNF166 catalyzes K33-lnked ubiquitination of SQSTM1 and recruits SQSTM1 and another autophagic receptor, CALCOCO2/NDP52 to ubiquitinated bacteria. RNF166 may target multiple lysine sites within different domains of SQSTM1 including K91 in the PB1 domain. PARK2/parkin and TRIM21 also ubiquitinate SQSTM1s PB1 domain. PARK2 catalyzes ubiquitination of K13, promoting SQSTM1s degradation. TRIM21 ubiquitinates SQSTM1 at K7 within the PB1 domain, abrogating SQSTM1s oligomerization and sequestering activity. In our recent study, we show that ubiquitination of SQSTM1 at residue K420 within its UBA domain by KEAP1-CUL3 (cullin 3) enhances its function in autophagy. Substitution of K420 with arginine, deletion of the UBA domain or mutation of the KEAP1-interacting (KIR) motif decrease SQSTM1s sequestering activity and consequently the formation of ubiquitinated inclusion bodies. These same mutations significantly decrease SQSTM1s interaction with LC3 and enhance proteotoxicity. In contrast, the overexpression of KEAP1-CUL3 increases SQSTM1 body size and rescues cells from proteotoxicity. Additionally, KEAP1-CUL3 overexpression increases SQSTM1s interaction with LC3 and autophagic degradation. Thus, these data suggest that UBA domain ubiquitination by KEAP1-CUL3 enhances SQSTM1s sequestering activity, phagophore association, and cargo degradation. This model of SQSTM1 suggests that depending upon the domain, PB1 or UBA, ubiquitination can positively or negatively regulate SQSTM1-dependent autophagic degradation.

Notably, ubiquitin conjugation at K420 is likely to be targeted by another molecular complex involving the UBE2D2 and UBE2D3 E2 ligases. This E2 ligase complex directly interacts with SQSTM1 via residues adjacent to the LIR motif. Interestingly, the consequence of E2 ligase-mediated K420 ubiquitination is similar to that of KEAP1-CUL3-mediated ubiquitination.

The UBA domain mediates several functions including sequestering activity, ubiquitin binding, and self-dimerization. UBA domain dimerization inactivates ubiquitin binding because the interfaces for these 2 events are shared. Phosphorylation of the UBA domain disrupts this self-dimerization and increases its binding affinity to ubiquitin. Similarly, a purified SQSTM1 carrying the K420R mutation along with an E409K mutation, which destabilizes the UBA dimerization, increases poly-ubiquitin association in vitro, as compared with purified WT SQSTM1. A purified ubiquitinated WT SQSTM1 (SQSTM1-Ub) also binds more poly-ubiquitin than unmodified WT SQSTM1 in vitro.

The formation and degradation of ubiquitinated protein aggregates require SQSTM1s oligomerization. This sequestering activity is mediated by SQSTM1s UBA domain and PB1 domain that regulates SQSTM1s self-association. This self-association of SQSTM1 is necessary for the formation of a filamentous SQSTM1 scaffold that then allows for the interaction with LC3 and development of the phagophore membrane. The deletion of either the PB1 or UBA domains disrupts the self-association of SQSTM1 and reduces cell viability against proteotoxicity. Our work also shows that UBA domain ubiquitination requires dimerization via the PB1 domain. A PB1 domain

CONTACT YouJin Lee youjin.lee@wustl.edu Department of Neurology, Washington University School of Medicine, 660 South Euclid Avenue, Campus Box 8111, St. Louis, MO 63110, USA.

Punctum to: Lee YL, Chou T-F, Pittman SK, Keith AM, Razani B, Weihl CC. Keap1/Cullin3 modulates p62/SQSTM1 activity via UBA domain ubiquitination. Cell Report 2017;19:188-202.

deletion or a SQSTM1^{D69A} mutation, which blocks oligomerization, prevents ubiquitinattion at K420. This finding suggests that only SQSTM1 dimer can be ubiquitinated by KEAP1-CUL3. However, it is not clear how different post-translational modifications crosstalk to fine tune SQSTM1s dimerization and functions. Also, it remains unclear how SQSTM1 regulates its homo-dimerization and hetero-oligomerization with other autophagy receptor proteins such as NBR1, OPTN and CALCOCO2.

Dominantly inherited deletion or missense mutations of SQSTM1 are predominantly within its UBA domain in several degenerative diseases, including Paget disease of the bone, amyotrophic lateral sclerosis, fronto-temporal dementia, and inclusion body myopathy. The common pathological feature among these different tissues is the accumulation of ubiquitin- and SQSTM1-positive protein aggregates. However, how SQSTM1 mutations contribute to pathogenesis is not clear, but disrupted ubiquitin binding in vitro may explain it. To address that, we examined the ubiquitination status of SQSTM1-disease mutants within the UBA domain. Surprisingly, all disease mutants have diminished ubiquitination, decreasing their sequestering function and cell viability against proteotoxicity. SQSTM1 mutations are recently identified in the KIR and LIR motifs from PDB and ALS patients. These KIR disease mutants decrease the interaction with KEAP1, whereas LIR disease mutants diminish LC3 binding. We speculate that these KIR and LIR mutants may also affect their ubiquitination and decrease their sequestering function.

Modulation of KEAP1-CUL3 may be a therapeutic target. The overexpression of KEAP1-CUL3 in SQSTM1-disease mutant transfected cells rescues cells against polyglutamine toxicity. However, whether KEAP1-CUL3-mediated ubiquitination increases KEAP1 sequestration, resulting in NFE2L2/Nrf2 activation remains unclear. Notably, NFE2L2 activation can be cytoprotective in several diseases including Huntington disease, inflammatory diseases, and cancer. Our current data suggest that SQSTM1 UBA domain ubiquitination modulates selective autophagy and further connects to SQSTM1 to KEAP1-NFE2L2 signaling.