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SQSTM1/p62 (sequestosome 1) senses cellular ubiquitin stress through E2-mediated ubiquitination

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ABSTRACT

The alterations in cellular ubiquitin (Ub) homeostasis, known as Ub stress, feature and affect cellular responses in multiple conditions, yet the underlying mechanisms are incompletely understood. We recently reported that the macroautophagy/autophagy receptor SQSTM1/p62, functions as a novel Ub sensor to activate autophagy upon Ub^+ stress (upregulation of the Ub level). First, SQSTM1 was found to undergo extensive ubiquitination and activate autophagy under Ub^+ stress induced by prolonged Bortezomib (BTZ) treatment, Ub overexpression or by heat shock. Mechanistically, Ubiquitination of SQSTM1 disrupts its dimerization of the UBA domain, switching it from an auto-inhibitory conformation to recognize poly-ubiquitinated cargoes, promoting autophagic flux. Interestingly, Ub^+ stress-responsive SQSTM1 ubiquitination is mediated by Ub conjugating enzymes, UBE2D2/3, in a unique E2-dependent manner. Our work has thus revealed a novel mechanism for how SQSTM1 senses cellular Ub stress conditions and regulates selective autophagy in response to diverse intrinsic or extrinsic challenges.

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Ubiquitin (Ub) is a 76-amino acid polypeptide ubiquitously distributed in all tissues of eukaryotic organisms. Ubiquitination, the reaction of attaching Ub to a substrate protein or Ub itself to form Ub conjugates, regulates the stability, function, localization and protein-protein interactions of the substrate. Intracellular Ub (both free and in conjugates) is estimated to be at a level of approximately 500 pmol/mg cell lysate, thus being one of the most abundant proteins in a typical cell. Maintaining the cellular pools of Ub conjugates and free Ub constitutes an essential part of cellular Ub homeostasis, which is subjected to highly dynamic but strict regulation. Alteration in Ub homeostasis, termed Ub stress, is critically implicated in many important biological, pathological or therapeutic conditions such as heat shock, aging, microbial infection, neurodegenerative diseases, or chemotherapy.

Indeed, changes in cellular Ub homeostasis could actually unify otherwise dramatically diverse conditions. For example, Ub^+ stress has been observed typically during prolonged proteasomal inhibition and heat shock, where the pools of free Ub or Ub conjugates are often substantially upregulated. So far there has been accumulating evidence that genetic manipulations of Ub homeostasis at cell or organism levels could have a significant impact on multiple fundamental aspects of cell activities, including cell proliferation, aging, drug responses, etc., although the mechanisms are largely unknown. It is interesting to note that researchers who overexpress Ub in cells to facilitate detection of ubiquitination, often almost totally ignore whether and how overexpression of Ub might potentially bring certain unnoticed complications in their specific studies.

In our most recent work led by Peng & Yang et al, we reported that prolonged proteasomal inhibition, heat shock and Ub overexpression (exceeding ~500 μM), which were termed as Ub^+ stress to highlight the common feature that Ub homeostasis was dramatically upregulated, could efficiently induce autophagy dependent on the autophagy receptor SQSTM1/p62. Interestingly, SQSTM1 was found to be increasingly ubiquitinated during all 3 autophagy-inducing conditions. Subsequently, SQSTM1 was found to specifically interact with 2 E2 Ub conjugating enzymes, UBE2D2 or UBE2D3, using a common E2-interacting region (EIR). Evidence was also presented to demonstrate that these E2s could support SQSTM1 ubiquitination both in vitro and in the cell. Multiple Lys (K) residues in SQSTM1 were mapped as the sites for this E2-supported ubiquitination, which include K420 in the UBA domain of SQSTM1. Mutation of these sites into Arg (K-to-R substitution), deletion of the EIR in SQSTM1, or genetic ablation of both E2s in the cells was found to efficiently abolish both SQSTM1 ubiquitination and autophagy activated during the above Ub^+ stress conditions. Previously, multiple groups have reported that UBA domains in SQSTM1 form stable dimers prominently involving the intermolecular hydrogen bonds formed between E409 in one SQSTM1 molecule and K420 in

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another, which would prevent SQSTM1 from binding to the polyUb chain in autophagy cargos. Indeed, our in vitro polyUb-binding assays indicate that unmodified SQSTM1 can bind to polyUb chains much more poorly than SQSTM1 polyubiquitinated in the presence of the E2s. Consistently, electron microscopy and dynamic light scattering analyses indicate that polyubiquitinated SQSTM1 might predominantly form a large complex with polyUb chains (~2-fold larger in diameter) than unmodified SQSTM1, suggesting that SQSTM1 might have indeed adopted an open conformation upon E2-supported polyubiquitination. By discovering E2-supported ubiquitination of SQSTM1 as a novel mechanism for activating its autophagy receptor function under Ub^+ stress conditions, our work has thus revealed a unique "sensor" function of SQSTM1 in modulating autophagy as part of the cellular responses to prolonged proteasomal inhibition, heat shock or Ub overexpression.

Last but not least, because overexpression of Ub could activate SQSTM1-dependent autophagy, researchers whose experiments routinely involve Ub overexpression should take caution when interpreting data, particularly because activation of autophagy, however inadvertently, would profoundly affect many aspects of cellular activities, thus causing unanticipated complications.

Now that SQSTM1 appears to be a novel but bona fide sensor for cellular Ub^+ stress, it is intriguing to consider whether and how other autophagy receptors such as NBR1 or TOLLIP might also have similar functions in orchestrating cellular responses to diverse intrinsic and extrinsic challenges. Ub sensors such as SQSTM1 might serve as potential targets for pharmacological interventions in autophagy-related disease.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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