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Commentary Open Access

Talk of the Titans: Overview of Crosstalk between Autophagy and the Ubiquitin-proteasome System

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Commentary

E3 ligases attach the ubiquitin (Ub)-tag to the cellular proteins and mark them for degradation in the proteasome [1]. Since everything that is created must be destroyed, the question arises: how are E3 ligases that are apt in degrading their targets themselves degraded? Two modes of E3 ligases degradation have been proposed so far: self-catalyzed ubiquitination and/or ubiquitination by an exogenous E3 ligase [2]. Recently, we proposed a novel mode of E3 ligase regulation that is centered on the ability of autophagy machinery to degrade Really-Interesting New Gene (RING)-domain E3 ligases [3]. This commentary briefly summarizes the current understanding of E3 ligase degradation and highlights our recent study in which we demonstrated the role of autophagy-associated transmembrane protein EI24 as a bridging molecule between the UPS and autophagy by regulating the degradation of several E3 ligases with RING-domains [4].

Several layers of regulations are imposed on E3 ligases owing to their important role in maintaining the proteome homeostasis in the cells. Same kind of posttranslational modification in two different E3 ligases could have completely different outcomes regarding the fate of the substrate. CBL RING E3 ligases are phosphorylated by their activated substrates, tyrosine kinases, that inturn increases the affinity of E3 ligases towards the kinases through phosphotyrosine-binding domain present in E3 ligases, accelerating the degradation of the substrate [5]. On the other hand, neural precursor cell-expressed developmentally downregulated 4-2 (NEDD4-2) phosphorylation decreases its affinity towards its substrate epithelial sodium channel (ENaC) [6]. Interestingly, activation of fibroblast growth factor (FGF) receptor 1 (FGFR1) results in the activation of NEDD4 and this phosphorylation activates the E3 ligase activity of NEDD4 towards FGFR1 [7]. Thus, same kind of posttranslational modification on the same E3 ligase could have different outcomes regarding the fate of the substrate depending on the signaling involved. Ubiquitination is another posttranslational modification employed by E3 ligases to degrade their substrates including themselves and other E3 ligases. One of the most characterized regulations of E3 ligase degradation is through self-catalyzed ubiquitination independent of the substrate or in other instances requires the binding to the substrate. The classical example of substrate-independent self-ubiquitination of E3 ligase is RING-domain dependent self-ubiquitination and degradation of MDM2 proto-oncogene (MDM2) [8]. However, E3 ligases that catalyze their self-ubiquitination can also be subjected to degradation by an external E3 ligase. For an example, MDM2 is targeted for proteasome-dependent degradation by an external ligase p300-CBP-associated factor (PCAF) [9]. Thus, based on the cellular context, the degradation of a particular E3 ligase can be executed through completely different routes. In case of MDM2, the general consensus is that under physiological levels, PCAF is the E3 ligase of choice for MDM2. Once the MDM2 protein levels reaches a threshold, self-destructive mode of MDM2 is activated leading to self-ubiquitination and proteasome-dependent degradation [2].

Substrates are also known to exert their effect on E3 ligases by either promoting or suppressing functional activity of E3 ligases. In one example of substrates contributing to their own suicide, activated Tyrosine kinases phosphorylate CBL E3 ligases and activate their E3 ligase activity towards these kinases. In this case, the fate of both substrate and E3 ligase is proteasomal degradation [10]. There are also other cases where the binding of the substrates has inhibitory effect on the self-ubiquitination and has protective effect on E3 ligases. The degradation of cell division cycle 4 (Cdc4) by F-box proteins follows this modality by creating the competitive environment between the E3 ligase and substrate for ubiquitin binding and inhibiting the self-ubiquitination of E3 ligase [11].

There also exists a group of E3 ligase that is unable to destruct on its own, thus, need to be exclusively ubiquitinated and degraded by an external E3 ligase. For an example, RING1B is ubiquitinated by E6-AP to be degraded in the proteasome. For RING1B, self-ubiquitination does not promote its own destruction, rather serves as a signal to monoubiqutinate histone H2A [12].

Regulation of RING-E3 by Autophagy: a Novel Route of E3 Ligase Degradation in Autophagy-dependent Manner

The classical view that UPS and autophagy are independent protein degradation machineries are challenged by several recent studies that show that these pathways crosstalk with each other [13]. In this light, we asked, if there was an alternative pathway for E3 ligase degradation beyond self-ubiquitination or ubiquitination by other E3 ligases. Evidences collected over a span of several years pointed to the possibility of systematic targeting of RING-domain E3 ligases through the autophagy pathway [3]. First, while studying the function of autophagy-associated transmembrane protein EI24, we observed that EI24 promoted autophagy-dependent degradation of RING E3 ligases, including TNF receptor associated factor 2 (TRAF2) and TRAF5 leading to the suppression of epithelial-to-mesenchymal transition (EMT) and tumor progression by dampening RELA proto-oncogene/NF-kB subunit (RELA/NFKB p65) activity [14]. Using genetically engineered mouse model, we also observed that EI24-induced RING-domain dependent degradation of tripartite motif containing 41 (TRIM41/RINCK1) results in protein kinase c α (PKCα)

stabilization leading to the development of 7,12-dimethylbenz [a]-anthracene (DMBA)-12-O-tetradecanoylphorbol-13-acetate (TPA)-induced skin carcinogenesis in mice [15]. Since EI24 is an essential autophagy gene in C. elegans and mice [16], and we observed RING-domain dependent degradation of several E3 ligases by EI24, we hypothesized that EI24 could be the molecule that connects E3 ligases to the autophagy machinery.

To evaluate the mechanism of RINCK1 degradation by EI24, we first examined whether EI24-mediated degradation of RINCK1 occurs via the UPS or autophagy pathway. RINCK1 degradation by EI24 could be rescued by the inhibition of autophagy or by deleting the RING-domain but not by inhibiting the proteasome. These results indicated that EI24 recognizes the RING domain that is present in the majority of E3 ligases and degrades them using the autophagy pathway, unraveling another dimension of RING-domain E3 ligase regulation. We then tested the susceptibility of more RING-domain to be degraded by EI24. We found that out of 20 RING-domain E3 ligases tested, 14 (70%) were degraded by EI24 (TRAF2, TRAF5, RINCK1, RINCK2, TRIM1, TRIM3, TRIM4, TRIM6, TRIM21, TRIM2, TRIM28, TRAF6, CIAP1, and MDM2), whereas 6 (30%) were not (TRIM5, TRIM8, TRIM20, Parkin, XIAP, and CIAP2). To generalize this observation in a broader context, we separated the E3 ligases into two groups: those that are susceptible (Group 1) and resistant (Group 2) to EI24-mediated degradation and searched for gene expression differences between Group 1 and 2 that could potentially contribute to EI24-mediated autophagic degradation susceptibility. Using a multi-block partial least square-discriminant analysis (MPLS-DA) [17,18] with which two different EI24 gene expression datasets could be effectively integrated [14,19], we are able to successfully separate Group 1 from Group 2 and 161 E3 ligases (predicted Group [pGroup] 1) were predicted to be EI24 targets and 64 E3 ligases (pGroup 2) were predicted to be non-targets. More importantly, the bioinformatic separation of E3 ligases into pGroup 1 and 2 was validated experimentally showing that pGroup1 E3 ligases were degraded and pGroup2 E3 ligases were not degraded by EI24, indicating the high degree of sensitivity and specificity of our model. Thus, we concluded that the RING domain, which is present in the majority of E3 ligases, acts as an 'eat-me' signal for EI24-mediated autophagic degradation. Our study strongly supports the idea of integration of the autophagy machinery with the UPS, indicating that these protein degradation pathways are not as independent as previously suggested.

One might ask, what could be the biological implication of autophagy-dependent degradation of RING-domain E3 ligases by autophagy? Since protein degradation is very important to the health of the cells, there should always be a line of communication that exists between autophagy and UPS. In case of failure to clear the E3 ligases by the loss of UPS activity, that could be detrimental to the cells, ability of autophagy to degrade E3 ligases could act as a backup mechanism. Only if these titans of protein degradation communicate with each other and function in a coordinated fashion, the cellular machinery can execute its activities properly.

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