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AUTOPHAGIC PUNCTUM

KLHL20 links the ubiquitin-proteasome system to autophagy termination

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ABSTRACT

Autophagy is a dynamic and self-limiting process. The amplitude and duration of this process need to be properly controlled to maintain cell homeostasis, and excessive or insufficient autophagy activity could each lead to disease states. Compared to our understanding of the molecular mechanisms of autophagy induction, little is known about how the autophagy process is turned off after its activation. We recently identified KLHL20 as a key regulator of autophagy termination. By functioning as a substrate-binding subunit of CUL3 ubiquitin ligase, KLHL20 targets the activated ULK1 and phagophore-residing PIK3C3/VPS34 and BECN1 for ubiquitination and proteasomal degradation, which in turn triggers a destabilization of their complex components ATG13 and ATG14. These hierarchical degradation events cause the exhaustion of the autophagic pool of ULK1 and PIK3C3/VPS34 complexes, thereby preventing persistent and excessive autophagy activity. Impairment of KLHL20-dependent feedback regulation of autophagy enhances cell death under prolonged starvation and aggravates muscle atrophy in diabetic mice, which highlights the pathophysiological significance of this autophagy termination mechanism in cell survival and tissue homeostasis. Modulation of this autophagy termination pathway may be effective for treating diseases associated with deregulation of autophagy activity.

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Autophagy mediates lysosome-dependent degradation and recycling of cytoplasmic components and thus represents a quality control mechanism for cells to cope with various stress conditions. However, uncontrolled autophagy leads to excessive self-consumption, which could generate detrimental effects on cells and tissues. Lenardo's group previously identified a mechanism for attenuating autophagy activity during prolonged nutrient deprivation, which is mediated by MTOR reactivation in response to the regeneration of intracellular nutrients from autolysosome-mediated degradation. However, it remains unclear whether feedback regulation of autophagy could occur at an earlier step during the autophagic process to allow a timely fine-tuning of autophagy activity.

Our recent study uncovers an intriguing link of the ubiquitin-proteasome system with autophagy termination. This study was initiated by the identification of ULK1, a serine/threonine kinase critical for autophagy induction, as an interacting partner of KLHL20, which functions as a substrate adaptor of CUL3 ubiquitin ligase. Subsequent analysis revealed that ULK1 is a direct substrate of CUL3-KLHL20 ubiquitin ligase, and ULK1 ubiquitination by this complex causes its proteasomal degradation. We next went on to investigate whether KLHL20-mediated ULK1 ubiquitination is regulated during the autophagic process. Remarkably, interaction between ULK1 and KLHL20 is increased in response to starvation and amino acid deprivation, which is associated with an enhanced ubiquitination and degradation of ULK1. Mechanistically, this elevated interaction with KLHL20 is mediated by autophosphorylation of ULK1 at S1042 and T1046 residues. In light of this mechanism, depletion of KLHL20 or replacement of wild-type ULK1 with a KLHL20-resistant mutant increases the amplitude and duration of autophagy. These findings support a role for the KLHL20-ULK1 axis in autophagy termination.

The function of KLHL20 in regulating autophagy dynamics is not confined to its action on ULK1. In fact, KLHL20 also mediates autophagy-dependent ubiquitination and degradation of PIK3C3/VPS34 and BECN1. In these cases, targeting of PIK3C3/VPS34 and BECN1 to KLHL20 is attributed to their co-recruitment to phagophores. In response to starvation, KLHL20 is distributed to vesicle-like structures, which are situated at the edge of phagophores, whereas the PIK3C3/VPS34 complex is recruited to phagophores in a ULK1- and ATG14-dependent manner. Failure of the phagophore localization of the PIK3C3/VPS34 complex blocks the recruitment of PIK3C3/VPS34 and BECN1 to KLHL20 for ubiquitination and degradation in autophagic cells. Consistent with previous reports indicating the requirement of ULK1 and PIK3C3/VPS34-BECN1 for the stability of ATG13 and ATG14, respectively, KLHL20 also governs the autophagy-dependent destabilization of ATG13 and ATG14. Our study thus identifies a central role of KLHL20 in autophagy termination by coordinating a hierarchy of degradation events, which lead to the depletion of multiple components in the ULK1 and PIK3C3/VPS34 complexes. Considering that ULK1 autophosphorylation and PIK3C3/VPS34 complex recruitment to phagophores are general phenomena in autophagy, KLHL20-mediated turnover of these

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2 complexes is likely a general mechanism for controlling autophagy dynamics.

Notably, KLHL20 specifically targets the autophagic pool of ULK1 and PIK3C3/VPS34 complex components for degradation. This selectivity would not only prevent nonspecific blockage of other cellular functions elicited by these proteins but also establish a need to activate new pools of ULK1 and PIK3C3/VPS34 for each run of autophagy, a mechanism to facilitate a tight connection of autophagy activity with the nutrient status of cells. It is likely that KLHL20 is a limiting factor for the degradation of ULK1 and PIK3C3/VPS34 complex components. In line with this notion, KLHL20 only appears in ~68% of BECN1-containing phagophores and only a fraction of KLHL20 is recruited to phagophores. Such limited availability of KLHL20 on phagophores may prevent an instant and premature degradation of these autophagic proteins, thus enabling autophagy initiation and autophagosome nucleation to proceed properly.

The pathophysiological significance of this autophagy termination mechanism was also determined in our study. At a cellular level, impairment of KLHL20-dependent autophagy termination potentiates cell death induced by prolonged starvation. At an organism level, *Klhl20* knockout in the skeletal muscle of mice exacerbates muscle atrophy in an experimental diabetes model, and this phenotype is dependent on excessive autophagy activity. These findings underscore the importance of KLHL20-dependent autophagy termination in maintaining cell survival and tissue homeostasis by preventing unrestrained cellular degradation. Deregulation of autophagy has been implicated in a wide range of diseases, such as cancers, neurodegenerative diseases, infectious diseases, and myopathy. Unraveling the autophagy termination mechanism provides a new window for designing therapeutic strategies to intervene in autophagy deregulation-associated diseases.

Disclosure of potential conflicts of interests

No potential conflicts of interest were disclosed.

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