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New functions of a known autophagy regulator: VCP and autophagy initiation

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ABSTRACT

VCP, a conserved ATPase, is involved in several cellular processes, and mutations in this protein are associated with various diseases. VCP also plays a role in autophagosome maturation. However, because a deficiency in autophagosome maturation presents a readily observable phenotype, other roles of VCP in autophagy regulation, in particular in the initial steps of autophagosome formation, may have been overlooked. In a recently published paper, using small-molecule inhibitors, Hill *et al.* showed that VCP regulates autophagy initiation through both stabilization of BECN1 and enhancement of phosphatidylinositol 3-kinase (PtdIns3K) complex assembly.

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VCP/p97 (valosin containing protein), a ubiquitously expressed protein of the ATPases associated with diverse cellular activities protein family, is involved in multiple cellular processes, such as the ubiquitin-proteasome system, mitochondria quality control and endoplasmic reticulum-associated degradation [1]. Given its crucial roles to maintain cellular proteostasis, it is not surprising to see that VCP is associated with several muscular and neural degenerative diseases, such as inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia/IBMPFD and amyotrophic lateral sclerosis [2,3].

Besides the well-characterized functions mentioned above, VCP is also involved in both selective and nonselective autophagy regulation [4–7]. It is worth noting that previous studies on the role of VCP in nonselective autophagy mainly focused on its function in autophagosome maturation; cells with a *VCP* knockdown or those expressing disease-related mutations in VCP display accumulation of immature autophagic vesicles [6,7]. However, because the deficiencies in autophagosome maturation display a striking morphological phenotype, researchers may have missed VCP-associated defects that affect the early steps of autophagy. One way to avoid this problem is through acute inhibition of VCP function. In the paper highlighted here, Hill *et al.* demonstrate new functions of VCP in regulating autophagy initiation using VCP inhibitors combined with a block in autophagosome-lysosome fusion [8].

BECN1 (beclin 1) is one of the most important components of the PtdIns3K complex, which produces phosphatidylinositiol-3-phosphate (PtdIns3P); this phosphoinositide is critical in phagophore biogenesis. The detection of an endogenous interaction between VCP and BECN1 suggests a potential role of VCP in autophagy initiation. Along these lines, inhibiting VCP activity using the specific inhibitor DBeQ [9] in the absence of any other treatment results in a higher level of LC3-II, but a lower level if cells are pretreated with bafilomycin A_1 in both nutrient-rich and starvation conditions. That is, inhibition of VCP alone predominantly results in a block in autophagosome-lysosome fusion (perhaps indirectly through its effect on autophagosome-endosome fusion), resulting in the accumulation of LC3-II; however, if autophagosome-lysosome fusion is first inhibited with bafilomycin A_1, it is now possible to detect a decrease in LC3-II following DBeQ treatment. Significantly, the DBeQ-dependent increase in LC3-II is much larger than the decrease resulting from the combined treatment, which likely explains why the second phenotype had previously been overlooked – a chronic defect in VCP function would result in a substantial accumulation of LC3-II. These findings indicate a role for VCP in regulating both autophagosome formation and maturation. Consistently, after stimulating autophagosome formation with nutrient depletion, there are smaller numbers of LC3 and PtdIns3P puncta in the cells treated with DBeQ and other VCP inhibitors. The starvation-induced accumulation of WIPI2 and ATG16L1, two early autophagy markers that are recruited to the site of phagophore formation through binding to PtdIns3P, is also impaired by DBeQ and other VCP inhibitors, further indicating the deficiency in autophagy initiation when VCP is inhibited.

Inhibiting VCP activity using DBeQ leads to an accelerated degradation of BECN1 and a decreased level of ATXN3 (ataxin 3), a VCP-interacting protein that deubiquitinates and stabilizes BECN1 [10,11], suggesting that VCP may function in autophagy initiation through an ATXN3-dependent stabilization of BECN1. Indeed, overexpressing mutant ATXN3 with a compromised ability to bind VCP does not stabilize BECN1, and this mutation also shows a less efficient rescue of LC3 puncta formation in *ATXN3* knockdown cells

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compared with wild-type ATXN3. Adding VCP and ATXN3 into an *in vitro* system with ATP reduces the level of ubiquitinated BECN1 more significantly than adding VCP or ATXN3 alone. All of these results demonstrate that binding between VCP and ATXN3 is critical for the deubiquitination and stabilization of BECN1, thus promoting autophagy initiation.

In addition to BECN1, VCP also interacts with other core components of PtdIns3K complexes, including ATG14, UVRAG and RUBCN [12]. Focusing more on the ATG14-containing complex, which functions in autophagosome formation, Hill *et al*. found that VCP interacts with ATG14 even in *ATXN3* and *BECN1* knockdown cells, which indicates a distinct role of VCP other than stabilizing BECN1. Increased PtdIns3P production after adding VCP to pre-assembled PtdIns3K complexes suggests that VCP may have a function in enhancing the assembly of the lipid kinase complex. In accordance with this, inhibiting VCP activity impairs the interaction between ATG14 and other PtdIns3K complex components, including PIK3R4/VPS15, PIK3C3/VPS34 and BECN1. This conclusion is further supported by an induction of PtdIns3K complex assembly, shown by the increased co-purification with ATG14, when adding VCP to an *in vitro* system with individually purified PtdIns3K components. The authors also observed a higher molecular mass VCP band, which exists in total cell lysates, but is enriched following ATG14 affinity isolation. This second band suggests that there may be some post-translational modifications on VCP, which are enriched in, and may be important for, the population of the protein that interacts with the PtdIns3K complex.

In summary, by acutely inhibiting VCP activity, the authors discovered a novel function of VCP in regulating autophagy initiation through stabilizing BECN1 and facilitating the assembly of the PtdIns3K complex. Actually, DBeQ has been used to test the role of VCP in autophagy regulation in one of the previous studies [9]. However, if the cells are treated with DBeQ and a lysosome inhibitor such as bafilomycin A_1 simultaneously, the effect on the early step of autophagy was still masked, possibly because the VCP inhibitor function affecting autophagosome maturation is too rapid. By first inhibiting lysosome function through bafilomycin A_1 pre-treatment followed by an acute inactivation of VCP activity, this study provides a new insight into the role of this disease-related protein in regulating autophagy and may also give us a new direction to explore how disease-related mutations in VCP affects autophagy regulation.

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