VCP/p97 modulates PtdIns3P production and autophagy initiation

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Abstract

VCP/p97 is an essential multifunctional protein implicated in a plethora of intracellular quality control systems, and abnormal function of VCP is the underlying cause of several neurodegenerative disorders. We reported that VCP regulates the levels of the macroautophagy/autophagy-inducing lipid phosphatidylinositol-3-phosphate (PtdIns3P) by modulating the activity of the BECN1 (beclin 1)-containing phosphatidylinositol 3-kinase (PtdIns3K) complex. VCP stimulates the deubiquitinase activity of ATXN3 (ataxin 3) to stabilize BECN1 protein levels and also interacts with and promotes the assembly and kinase activity of the PtdIns3K complex. Acute inhibition of VCP activity impairs autophagy induction, demonstrated by a diminished PtdIns3P production and decreased recruitment of early autophagy markers WIPI2 and ATG16L1. Thus, VCP promotes autophagosome biogenesis, in addition to its previously described role in autophagosome maturation.

Keywords: ATXN3, autophagy initiation, beclin 1, PI(3)P, PI3K, VCP/p97

The human AAA+ ATPase VCP/p97 (valosin containing protein) is highly conserved across species. VCP has an essential role in many pathways related to the ubiquitin-

proteasome system (UPS), including endoplasmic reticulum-associated protein degradation (ERAD), but is also central to non-proteolytic functions of ubiquitin signaling, including cell cycle regulation and Golgi biogenesis. Mutations in the *VCP* gene cause a dominantly inherited, multisystemic inclusion body myopathy with early-onset Paget disease and frontotemporal dementia (IBMPFD), amyotrophic lateral sclerosis 14, with frontotemporal dementia (ALS14) and Charcot-Marie-Tooth disease 2Y (CMT2Y). Recently, a loss-of-function mutation in VCP was also implicated in dementia associated with MAPT/tau accumulation.

At the cellular level, loss of VCP leads to extensive accumulation of ubiquitinated proteins, suggesting that clearance of these proteins is impaired. Indeed, loss of VCP activity impairs autophagic flux as well as compromise the UPS. The presence of IBMPFD mutant VCP variants in cells leads to the accumulation of LC3, SQSTM1/p62 and LAMP1- and LAMP2-positive vesicles, indicating VCP involvement in autophagosome maturation. The observed phenotype has been partially explained by VCP involvement in multiple steps of endocytosis, which could in turn affect autophagic flux.

We recently identified a novel role for VCP in autophagy initiation using small molecule inhibitors and genetic approaches [1] (**Figure 1**). In both basal and autophagy-stimulating conditions (starvation, torin1 treatment), VCP inhibition blocks the increase in LC3 puncta and recruitment of early autophagic markers including WIPI2 (WD repeat domain, phosphoinositide interacting 2) and ATG16L1. Importantly, loss of VCP activity reduces the formation of PtdIns3P, an early signal acting upstream of WIPI2 and LC3 recruitment to phagophores. This could be explained by our observations that VCP activity is essential for the proper function of the PtdIns3P-producing PtdIns3K complex I, composed of BECN1, ATG14, NRBF2, PIK3C3/VPS34 and PIK3R4/VPS15.

VCP interacts with the deubiquitinase ATXN3 and we previously revealed that ATXN3, interacts with and stabilizes BECN1, thereby regulating autophagy. Indeed, using BECN1 affinity purification and *in vitro* binding studies, we identified VCP as a novel interactor of BECN1. We further showed that ATXN3 binding to VCP stimulates the protective deubiquitinase activity of ATXN3 in BECN1 stabilization. This protects BECN1 from proteasomal degradation and increases its levels, thereby stabilizing PtdIns3P-producing PtdIns3K complexes.

In addition to the ATXN3-dependent role, we showed that VCP interacts directly with additional members of the PtdIns3K complex I and that the presence of active VCP stimulates the assembly of this complex. We found that VCP independently interacts with UVRAG and RUBCN/rubicon, suggesting that VCP could also form a complex with PtdIns3K

complex II and III. In this study we focused on the ATG14-containing complex I, as we aimed to understand the role of VCP in autophagosome formation. Decreasing VCP activity with different inhibitors causes less VCP to be co-immunoprecipitated with components of the PtdIns3K complex I and also decreases the levels of assembled PtdIns3K complexes found in the cell. These findings were verified also *in vitro*, where we showed that addition of VCP to individually purified PtdIns3K proteins stimulates type I PtdIns3K complex assembly. We found that the ATPase activity of VCP stimulates the assembly and activity of the PtdIns3K complex to increase production of PtdIns3P and thereby regulate the extent of autophagy initiation.

These functions of VCP in BECN1-dependent autophagy initiation were identified through inhibition of lysosomal activity followed by acute inhibition of VCP activity. By combining acute inhibition of VCP with the lysosomal inhibitor bafilomycin A_1, we were able to demonstrate a decrease in the production of LC3-positive autophagosomes. This was correlated with a decrease in early autophagic markers adding a new role to the list of VCP-dependent processes. As prolonged depletion of VCP also causes a blockage in autophagy maturation, the accumulation of immature LC3-positive autophagic vesicles would mask any effect on autophagy initiation and had thus been unrecognized in previous studies using genetic approaches. Our findings may also provide additional explanation for the diverse and severe phenotypes seen in diseases with compromised VCP function via impaired autophagy initiation.

References:

[1] Hill, S.M., Wrobel, L., Ashkenazi, A. *et al.* VCP/p97 regulates Beclin-1-dependent autophagy initiation. *Nat Chem Biol* (2021). https://doi.org/10.1038/s41589-020-00726-x

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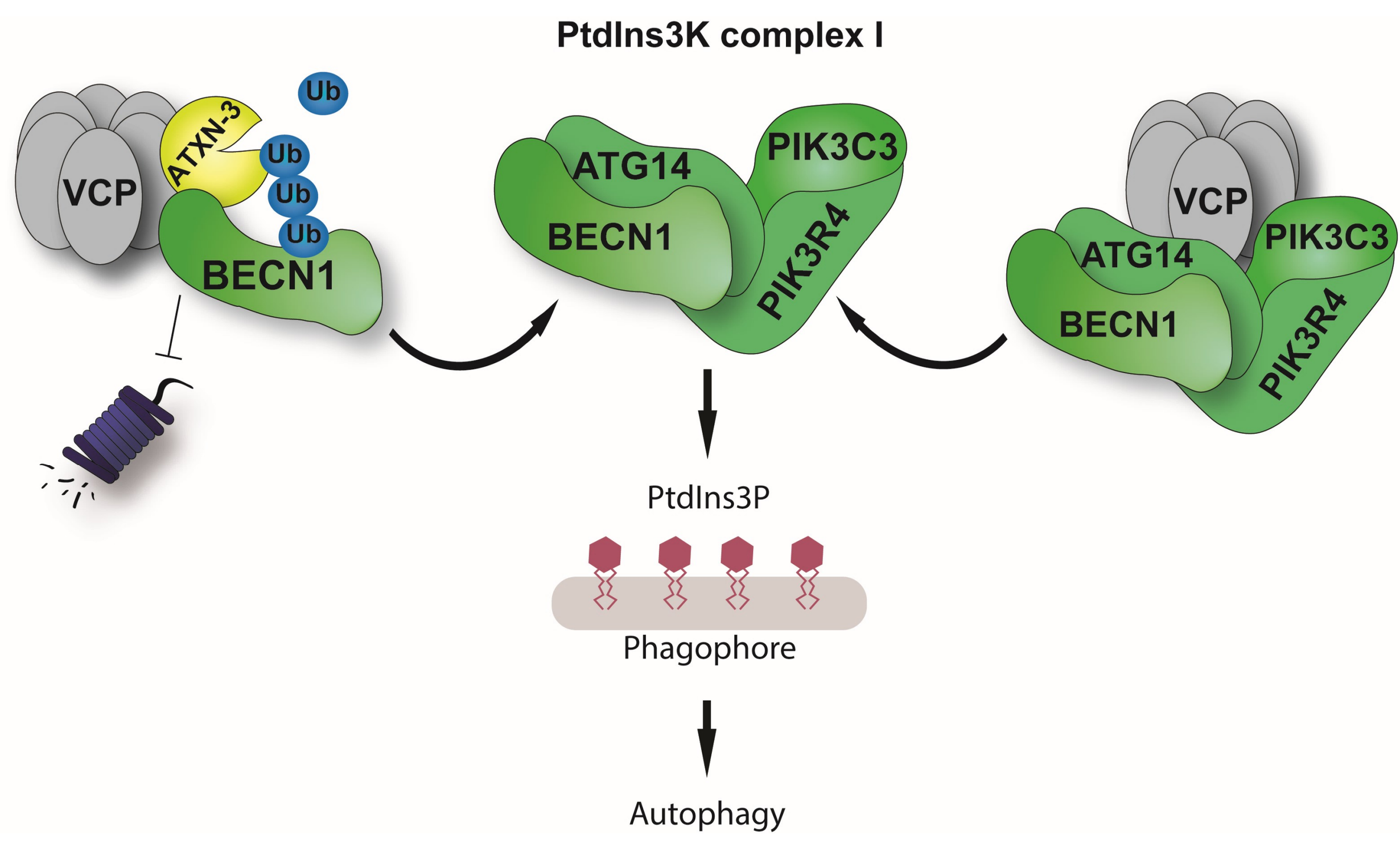
Declaration of interests

DCR is a consultant for Aladdin Healthcare Technologies Ltd and Nido Biosciences. None of the other authors have competing interests.

Figure legend

Figure 1. VCP has a dual role in autophagy initiation. VCP binds ATXN3 and BECN1 to stimulate deubiquitination of BECN1, thereby protecting this protein from degradation and stabilizing its levels as a part of the PtdIns3K complex. VCP also binds to other components of the type I PtdIns3K complex, promoting assembly of the entire complex and enhancing PtdIns3K complex activity. This results in the increase in PtdIns3P production and autophagy stimulation.


PtdIns3K complex I
Ub
ATXN-3
Ub
Ub
Ub
VCP
BECN1
ATG14
BECN1
PIK3C3
PIK3R4
VCP
ATG14
BECN1
PIK3C3
PIK3R4
PtdIns3P
Phagophore
Autophagy