doi:10.1093/jmcb/mjq028
Published online September 15, 2010

Research Highlight

From a Global View to Focused Examination: Understanding Cellular Function of Lipid Kinase VPS34-Beclin 1 Complex in Autophagy

Zhenyu Yue [1,*] and Yun Zhong [2]

1 Department of Neurology & Neuroscience, Mount Sinai School of Medicine, New York, NY 10029, USA
2 Laboratory of Molecular Biology, The Rockefeller University, New York, NY 10021, USA
* Correspondence to: Zhenyu Yue, Tel: + 1-212-241-3155; E-mail: zhenyu.yue@mssm.edu

Phosphoinositide 3 kinase Class III (PIK3C3) or VPS34-Beclin 1 complex plays a key role in the autophagy–lysosome pathway. Previous identification of numerous binding partners for VPS34-Beclin 1 suggested a complex scheme of the autophagy control mechanism. Recent large-scale screening of autophagy network and signaling pathways in mammalian cells not only confirms the previous binding partners, but also reveals additional interactors and intricate connections of VPS34-Beclin 1 complex to other functional groups of autophagy, yielding a wealth of information that will direct future detailed study of the central control mechanism of autophagy mediated by VPS34-Beclin 1 and other regulators.

Autophagy is a cell 'self-digestion' process via lysosomal degradation. The best-known type of autophagy is macroautophagy (hereafter referred to as autophagy), which involves the formation, delivery and degradation of autophagosomes. The physiological function of autophagy is the control of cellular nutrient and organelle homeostasis and can be regulated by various extracellular and intracellular cues (Klionsky and Emr, 2000; Levine and Klionsky, 2004). The precise process of autophagy is not well-understood; current knowledge suggests that the autophagy machinery includes several functional groups that regulate the life cycle of autophagosomes. They include Ulk1/2 protein kinase complex, VPS34-Beclin 1 lipid kinase complex, two ubiquitin-like protein conjugation systems and Atg protein retrieval and recycling system (Ohsumi, 2001; Xie and Klionsky, 2007; Mizushima, 2010; Yang and Klionsky, 2010).

The VPS34-Beclin 1 kinase complex mediates localization of other autophagy proteins to the preautophagosomal structure and participates in the nucleation of autophagosome formation (Kihara et al., 2001; Suzuki et al., 2001). The core component Beclin 1 was initially identified as a Bcl-2 interaction protein, and it was found that the Beclin 1-Bcl-2 interaction negatively regulated autophagy (Liang et al., 1998; Pattingre et al., 2005). The major advance was made towards the understanding of VPS34-Beclin 1 function when several groups simultaneously reported the identification of UVRAG, Atg14L and Rubicon as new binding partners for VPS34-Beclin 1 (Itakura et al., 2008; Sun et al., 2008; Matsunaga et al., 2009; Zhong et al., 2009). These studies shed light on the core composition and the multiple functional complexes of Beclin 1-VPS34 at a constitutive level. Available evidence suggests that Beclin 1-VPS34, by binding to different partners, forms distinctive functional protein complexes and regulates autophagy at different steps.

The role of UVRAG in autophagy, although awaiting to be further clarified, may be related to autophagosome formation and maturation via regulating lipid kinase activity of VPS34 (Liang et al., 2006; Itakura et al., 2008). Moreover, UVRAG interacts with members of class C VPS complex, which mediate the tethering events between endosomes. It was shown that UVRAG also participates in the endocytic pathway (Liang et al., 2008). In contrast, Atg14L clearly promotes autophagosome biogenesis and positively regulates the VPS34 kinase activity (Matsunaga et al., 2009; Zhong et al., 2009). Unlike UVRAG or Atg14L, Rubicon inhibits the kinase activity of VPS34 and blocks the maturation of autophagosomes, thereby negatively regulating autophagic activity (Matsunaga et al., 2009; Zhong et al., 2009). Over-expression of Rubicon induces aberrant endosomes and blocks the degradation of internalized epidermal growth factor receptor following epidermal growth factor treatment, suggesting that it is also involved in the endocytic pathway.

The recent large-scale screening of autophagy proteins, reported by Berhand et al. (2010) in *Nature* and Lipinski et al. (2010) in *Developmental Cell*, significantly expands our view of autophagy machinery on a global level including the VPS34-Beclin 1 with respect to their composition, connectivity and functionality. The large-amount dataset provided in these studies is re-shaping the landscape of autophagy and is expected to have strong impact in future mechanistic analysis of autophagy process and regulation. Behrends et al. (2010) performed proteomic analysis of autophagy interaction network, which unveils a blueprint of 751 interactions

among 409 candidate interacting proteins with extensive connectivity among subnetworks. Many new components in this network are associated with vesicle trafficking, protein or lipid phosphorylation and protein ubiquitination, and they alter autophagic activity when validated with RNA interference analysis. The success of this study is underscored by the findings of a great number of previously identified interacting proteins as well as the novel components which may fulfill the role as the missing links connecting several functional modules in this network. For example, VPS15, UVRAG, Atg14L, Rubicon and Ambra 1, the known binding partners for the VPS34-Beclin 1 complex, were repeatedly found in the subnetwork of VPS34-Beclin 1 using the reciprocal proteomic protocol. At least a dozen new interacting proteins for VPS34 or Beclin 1 were also identified, including NRBF2, DDB1–DDA1 and USP11 that are likely in the VPS34-Beclin 1 complex, and many others as binding proteins seemingly binding to Beclin 1 only. In addition, the VPS34-Beclin 1 network was extended to the interactors of the binding partners of VPS34-Beclin 1 complex after performing secondary screening. Notably, UVRAG interacts with several small rab GTPases; Beclin 1 binds C13orf18 (related to GEF for Rho GTPase) and TBC1D7 (putative GAP for rab GTPase); Ambra 1 associates with DDB1–DDA1–CUL4 which is ubiquitin ligase. Interestingly, Ambra 1 not only interacts with Atg3 and Atg4B, the components in the two ubiquitin-like conjugation systems, but also is linked to Ulk2 through MAP1A/B, which binds LC3 with high affinity (Atg8) (Wang et al., 2006). On one hand, despite lacking a dynamic view of the interactions, the study yields valuable information that promotes a series of hypotheses regarding the functional connectivity of VPS34-Beclin 1 complex to ubiquitin–proteasome system, endocytosis and microtubule-associated transport. On the other hand, it may raise the possibility that VPS34-Beclin 1 complex functions in cellular pathways beyond autophagy (Funderburk et al., 2010). The potential functions of autophagy protein complexes in non-canonical autophagy pathways are not restricted to VPS34-Beclin 1. For example, Ulk1 or Ulk2 has been long known to carry out cellular functions that are not necessarily associated with autophagy (Tomoda et al., 1999, 2004; Chan and Tooze, 2009). However, defining the role of autophagy protein complexes in canonical autophagy pathways vs. non-canonical autophagy pathways will be particularly challenging. Nonetheless, understanding the distinct functions of these complexes will be important for the development of highly specific and potent therapeutic intervention targeted at autophagy pathways (Levine and Kroemer, 2008).

The study by Behrends et al. (2010) demonstrates a powerful analysis using the large-scale proteomics, but certain limitations of this study are noticed. Apart from limited validation of the many interactions, the approach may exclude the identification of transient or unstable interactions. For example, none of the Bcl-2 family members were found in the list of Beclin 1-interaction proteins. In addition, the screening was performed in HEK293T cells, a tumor-like cell line, under a single condition (nutrient rich). Therefore, it cannot provide critical information about induced autophagy, an 'acute' response that may involve the recruitment of distinct sets of proteins directing a rapid enhancement of the autophagic capacity (e.g. increase of autophagosome synthesis and degradation), as well as cell-type or physiological condition-dependent autophagy control.

In another large-scale study, Lipinski et al. (2010) performed genome-wide siRNA screening for autophagy regulators in mammalian cells. They show that a group of growth factors and cytokines regulate autophagy under a nutrient-rich condition via multiple pathways, including MAPK-ERK1/2, Stat3, Akt/Foxo3 and CXCR4/GPCR. Importantly, these cell growth/proliferation-promoting pathways converge on VPS34-Beclin 1 and inhibit its lipid kinase activity, resulting in block of autophagy. Surprisingly, the mTORC1, which plays a key role in nutrient-related cellular response as well as autophagy regulation, is not required for cytokine-mediated autophagy alteration. This result highlights the prevalence of mTORC1-independent autophagy regulation in mammalian system. The overall impact of the study by Lipinski et al. (2010) is the finding that VPS34-Beclin 1 is the central control of autophagic activity by integrating diverse signals, particularly of cell growth and proliferation.

In summary, the two large screenings produced unprecedentedly the wealth of information for autophagy study. The information will guide the future detailed study of the control mechanism mediated by VPS34-Beclin 1 and other regulators in mammalian autophagy. It will also assist in predicting and validating drug targets targeted at autophagy pathways.

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