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Beclin orthologs: integrative hubs of cell signaling, membrane trafficking, and physiology

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The Beclin family, including yeast Atg6 (autophagy-related gene 6), its orthologs in higher eukaryotic species, and the more recently characterized mammalian-specific Beclin 2, are essential molecules in autophagy and other membrane-trafficking events. Extensive studies of Beclin orthologs have provided considerable insights into the regulation of autophagy, the diverse roles of autophagy in physiology and disease, and potential new strategies to modulate autophagy in a variety of clinical diseases. In this review we discuss the functions of Beclin orthologs, the regulation of such functions by diverse cellular signaling pathways, and the effects of such regulation on downstream cellular processes including tumor suppression and metabolism. These findings suggest that Beclin orthologs serve as crucial molecules that integrate diverse environmental signals with membrane trafficking events to ensure optimal responses of the cell to stressful stimuli.

Functional diversity of Beclin orthologs

The lysosomal degradation pathway of autophagy is an evolutionarily conserved pathway that functions in protein and organelle quality control, cellular and tissue homeostasis, differentiation and development, and in diverse aspects of physiology and pathophysiology [1,2]. One of the most-extensively studied autophagy gene products, mammalian Beclin 1 [the ortholog of yeast Atg6/Vps30 (vacuolar protein sorting 30)], is part of a lipid kinase complex that mediates the initial stages of autophagosome formation [3]. Historically, loss-of-function genetic studies with Atg6/Beclin 1 orthologs were crucial in suggesting a role for the autophagy pathway in tumor suppression, dauer development, fruiting body formation, longevity, innate immunity, and the pathophysiology of neurodegenerative and cardiac diseases [1,4,5] (Table 1). Beyond autophagy, there is expanding evidence that Beclin 1 functions in other membrane-trafficking pathways such as endocytic and phagosomes maturation [6–8]. Moreover, a mammalian-specific close cousin of Beclin 1 has been discovered, Beclin 2, that functions in endolysosomal trafficking of G protein-coupled receptors [9]. There is also increasing evidence that diverse signaling pathways, including oncogenic/tumor-suppressive signals, immune signals, and stress-responsive signals, converge on Beclin 1 to regulate autophagy, allowing Beclin 1 to serve as a central hub that coordinates environmental stimuli with downstream physiological outputs. We discuss recent studies related to the functional diversity of Beclin orthologs, their mechanisms of regulation, and the implications of these studies for understanding the role of Beclin orthologs in physiological processes.

Discovery of yeast Atg6/Vps30 and human Beclin 1

Yeast ATG6/VPS30 was discovered in two independent genetic screens, including one to identify genes required for survival during starvation, which led to the discovery of ATG6 [10], and one to identify genes required for endosome to Golgi retrieval of the vacuolar protein sorting receptor, Vps10p, which led to the discovery of VPS30 [11]. In yeast, Atg6/Vps30 is part of two distinct phosphatidylinositol 3-kinase (PI3K) complexes [12]. During autophagy, in complex I, Atg14 interacts with Atg6/Vps30 and targets the PI3K Vps34 and its membrane anchor, Vps15, to the pre-autophagosomal structure (PAS) [13,14]. The newly identified subunit Atg38 bridges the interaction between the Atg14–Atg6 subcomplex and the Vps34–Vps15 subcomplex through dual binding of Atg14 and Vps34 [15]. During vacuolar protein sorting, in complex II, Vps38 is present instead of Atg14 and targets Vps34–Vps15–Atg6 to the endosomal membrane [12].

Beclin 1, the mammalian ortholog of yeast Atg6/Vps30, was discovered in a yeast two-hybrid screen using the anti-apoptotic protein Bcl-2 (B cell lymphoma 2) as bait [16]. Shortly thereafter, Beclin 1 was shown to rescue autophagy, but not vacuolar protein sorting, in yeasts lacking ATG6/VPS30, and to rescue starvation-induced autophagy in human breast carcinoma cells with low endogenous Beclin 1 expression [17]. Subsequently, genetic
loss-of-function studies revealed an evolutionarily conserved role for Atg6/Beclin 1 orthologs in autophagy in multiple eukaryotic species including plants, slime molds, nematodes, fruit flies, and mice [4]. The lack of vacuolar protein sorting function of human Beclin 1 in ATG6 null yeast may simply reflect the sequence divergence between yeast Atg6 and human Beclin 1 (24.4% identity and 39.1% similarity) as Atg6/Beclin 1 orthologs have been shown to
function in endocytosis in multicellular organisms including *A. thaliana* [18], *Drosophila* [19], *C. elegans* [20], and mice [21].

Similar to its role in yeast, mammalian Beclin 1 is part of distinct Vps34 (also known as PI3KC3)-containing complexes (PI3KC3-C1 and PI3KC3-C2) that function differentially in autophagy and endocytic trafficking, respectively (Figure 1) [22–25]. The precise subunits of each complex vary somewhat in different reports but, in general, there is consensus that mammalian Atg14 (also known as Atg14L or Barkor (Beclin 1 interacting autophagy-related key regulator)) functions similarly to yeast Atg14 as an autophagy-specific component that targets Beclin 1–Vps34 to the sites of initiation of phagophore biogenesis, whereas mammalian UVRAG functions similarly to yeast Vps38 as a subunit involved in endocytic maturation [22–25]. In addition to its function in early autophagosomal biogenesis, Atg14 appears on mature autophagosomes where it interacts with autophagic SNAREs [soluble NSF attachment protein (SNAP) receptors], syntaxin-17 and SNAP29 to promote autophagosome-lysosome tethering and fusion [26]. NRBF2 (nuclear receptor binding factor 2), the mammalian counterpart of Atg8, also bridges the interaction between Vps34–Vps15 and Beclin 1–Atg14 [27,28]. UVRAG (UV radiation resistance associated gene) positively regulates Vps34 activity on endosomes and lysosomes [22], and another protein, Rubicon, that lacks an obvious yeast counterpart, counteracts this function of UVRAG [24,25,29,30]. The UVRAG-containing Beclin 1–Vps34 complex is currently believed to function in both endocytic trafficking and autophagosomal maturation, although it is still a matter of debate whether UVRAG functions in autophagosome maturation directly or indirectly through its endocytic functions. In addition to autophagy and endocytic trafficking, mammalian Beclin 1 and its binding partners in the class III PI3K complex also participate in a process termed ‘LC3 (named from microtubule-associated protein 1 light chain 3)–associated phagocytosis’ [7], which involves the maturation of phagosomes containing intracellular pathogens, apoptotic cells, or entotic cells (Figure 2).

**The function of Atg6/Beclin 1 in autophagy**

The central mechanism by which Atg6/Beclin 1 functions in autophagy and probably in endocytic maturation seems to be via the allosteric regulation of organelle-specific lipid kinase activity of the catalytic subunit of Vps34. Atg6/Beclin 1 is a peripheral membrane protein containing an N-terminal intrinsically disordered region [31], a BH3 (Bcl-2 homology 3) domain [32], a coiled-coil domain [33], and a C-terminal BARA (β-α repeated, autophagy-specific) domain [34,35]. It is generally believed that the Vps34–Vps15–Atg6/Beclin 1 complex is recruited to autophagic or endocytic membranes through interaction between the coiled-coil domain of Beclin 1 with either Atg14 or Vps38/UVRAG, thereby specifying the organelar site of Vps34 lipid kinase activity. Atg14 directly interacts with membrane via the hydrophobic surface of an ALPS (amphipathic lipid-packing sensor) motif within its BATS (Barkor/Atg14 autophagosome targeting sequence) domain [36], and UVRAG likely interacts with membrane through its phospholipid-binding C2 domain, or indirectly through its interaction with Bif-1/endophilin B1 that contains an
N-terminal N-BAR (Bin–amphiphysin–Rvs) domain and a C-terminal SH3 (Src-homology 3) domain, which display membrane binding and bending activities [37,38]. Beclin 1 may also bridge the membrane interaction of the PI3KC3 complex through aromatic amino acids at the tip of a surface loop in its BARA domain [34].

Recently, the structure of the PI3KC3-C1 complex was determined by single-particle electron microscopy of ordered regions of the Beclin 1, Atg14, Vps15, and Vps34 subunits, and its dynamics were analyzed by hydrogen exchange [39]. While higher-resolution structures that include the full-length proteins of each of these subunits as well as of NRBF2 will be helpful for a more complete understanding, these studies reveal an architectural and dynamic model that is consistent with a growing body of evidence that modifications in the N terminus of Beclin 1 are crucial for regulating the lipid kinase activity of PI3KC3-C1. According to the model proposed by Baskaran et al., the complex has a V-shaped architecture; Vps15 forms a bridge between Vps34 and an Atg14–Beclin 1 subcomplex; dynamic transitions occur during which the lipid kinase domain of Vps34 is ejected from the complex and Vps15 pivots at the base of the V; and the N-terminal disordered region of Beclin 1 is predicted to reside near the pivot point, thereby allosterically regulating the lipid kinase activity of PI3KC3-C1.

As discussed below and shown in Figure 3, the N-terminal region of Beclin 1 contains many residues that are targets of autophagy regulatory kinases [e.g., ULK1, MAP kinase activated protein kinases 2/3 (MAPKAPK2/ MAPKAPK3 – MK2/MK3), AMPK, DAPK, ROCK1, and MST1], as well as the BH3 domain which mediates its interactions with a crucial family of negative regulators of autophagy, Bcl-2, and its related cellular and viral anti-apoptotic proteins. Most autophagy stimulatory phosphorylation events enhance Beclin 1-associated Vps34 lipid kinase activity, whereas most autophagy inhibitory phosphorylation events as well as Bcl-2 binding inhibit Beclin 1-associated Vps34 lipid kinase activity. Furthermore, Bcl-2 binding, which stabilizes Beclin 1 homodimers, blocks N-terminal autophagy stimulatory phosphorylation (e.g., MK2/MK3-dependent Beclin 1 S90 phosphorylation [40]) whereas Atg14–Beclin 1 heterodimerization enhances N-terminal autophagy stimulatory phosphorylation (e.g., ULK1-dependent Beclin 1 S14 phosphorylation [41], AMPK-dependent Beclin 1 S93 and S96 phosphorylation [42]). Thus, both structural and biochemical data point to a crucial role of regulatory signaling input into Beclin 1 as a key mechanism for regulating Vps34 lipid kinase activity at membranes involved in autophagosomal biogenesis and endocytic maturation. The stimulation of Vps34 lipid kinase activity and resulting phosphatidylinositol 3-phosphate (PI3P) production then allows downstream effectors to complete the processes of membrane extension, cargo recruitment, and autophagosome maturation [43]. Of note (and an important experimental limitation for autophagy detection by Atg8–phosphatidyethanolamine (PE)/LC3-II western blots), Atg6 and Beclin 1 are essential for the recruitment of lipidated Atg8 and LC3, respectively, to the autophagosome, but Atg6 is not essential in yeast [44,45] and Beclin 1 is not essential in mouse embryonic stem cells [46] for the Atg8/LC3 lipidation process itself.

Divergent modes of regulation of Beclin 1 function
Given the complexity of mammals, it is not surprising that mammalian Beclin 1 undergoes multiple layers of regulation in addition to its interactions with other core subunits of the PI3KC3 complexes. Diverse cellular stimuli communicate with the PI3KC3 complexes to modulate autophagy and endocytic trafficking via post-translational modifications of Beclin 1 (e.g., phosphorylation, ubiquitination), sequestration of Beclin 1 in other subcellular locations (e.g., cytoskeleton, Golgi apparatus), or via protein–protein interactions that alter its interactions with other core components of PI3KC3 complexes or directly affect Beclin 1-associated Vps34 lipid kinase activity (Figure 3). The wide and continuously growing spectrum of bona fide Beclin 1-interacting partners (Table 2) that contribute to such regulation underscores the importance of crosstalk with Beclin 1 and autophagy in growth signaling, metabolism, mitophagy, innate
immune signaling, and microbial pathogenesis. At present, it is unclear, given the multitude of post-translational modifications and protein–protein interactions that regulate the function of Beclin 1, what the precise temporal, spatial, and hierarchical relationships are between different modes of Beclin 1 regulation, and how such relationships may be altered in different cell types and physiological/pathophysiological contexts. In the sections below we highlight a few key mechanisms underlying the regulation of Beclin 1 activity that relate to metabolism and cancer, including binding
Table 2. Beclin 1-binding Partners

| Protein                  | Impact on Autophagy                  | Refs       |
|--------------------------|--------------------------------------|------------|
| **PI3KC3 components**    |                                      |            |
| Vps34                    | Required for autophagy               | [3]        |
| Vps15                    | Required for autophagy               | [3]        |
| Atg14/Barkor             | Required for autophagy               | [23–25,53]|
| UVRAG                    | Involved in autophagy induction and/or autophagosome maturation | [22,24,54]|
| AMBRA1                   | Stimulates autophagy                 | [123]      |
| Bif-1/pendophilin B1     | Stimulates autophagy                 | [37]       |
| Rubicon                  | Inhibits autophagy, endosome maturation and autophagosome maturation | [24,25,29,30]|
| NRBF2                    | Stimulates and/or inhibits autophagy | [27,28,124]|
| **Bcl-2 family members** |                                      |            |
| Bcl-2                    | Inhibits autophagy                   | [56,64]    |
| Bcl-XL                   | Inhibits autophagy                   | [56,64]    |
| Bim                      | Inhibits autophagy                   | [52]       |
| **Viral Bcl-2 homologs** |                                      |            |
| Adenovirus E1B19K        | Stimulates autophagy                 | [125]      |
| ASV A179L                | Inhibits autophagy                   | [126]      |
| γHV68 M11                | Inhibits autophagy                   | [127,128]  |
| KSHV vBcl-2              | Inhibits autophagy                   | [56]       |
| **Kinases**              |                                      |            |
| AKT                      | Inhibits autophagy                   | [68]       |
| AMPK<sup>b</sup>         | Stimulates autophagy                 | [42]       |
| DAPK                     | Stimulates autophagy                 | [61]       |
| EGFR                     | Inhibits autophagy                   | [55]       |
| MK2/MK3<sup>b</sup>      | Stimulates autophagy                 | [40]       |
| HER2                     | Inhibits autophagy                   | [129]      |
| MST1                     | Inhibits autophagy                   | [85]       |
| PINK1                    | Stimulates mitophagy                 | [130]      |
| ROCK1                    | Stimulates autophagy                 | [66]       |
| ULK1                     | Stimulates autophagy                 | [41]       |
| **Ubiquitinating/deubiquitinating enzymes** | | |
| A20                      | Inhibits autophagy                   | [131]      |
| PARK2                    | Stimulates mitophagy                 | [132]      |
| TRAF6                    | Stimulates autophagy                 | [131]      |
| USP9X                    | Unknown                               | [133]      |
| WASH                     | Inhibits autophagy                   | [134]      |
| **Immunity-related molecules** | | |
| cGAS                     | Stimulates autophagy                 | [135]      |
| MyD88                    | Stimulates autophagy                 | [62]       |
| NLRP4                    | Inhibits autophagy                   | [136]      |
| PYCARD/ASC               | Unknown                               | [137]      |
| SLAMF1                   | Stimulates LC3-associated phagocytosis | [138]   |
| Trif                     | Stimulates autophagy                 | [62]       |
| TRIM5<sup>a</sup>        | Stimulates autophagy                 | [63]       |
| **Other cellular proteins** |                                      |            |
| 14-3-3                   | Inhibits autophagy                   | [68]       |
| β-Arrestin-1             | Stimulates autophagy                 | [139]      |
| Dapper1 (Dpr1)           | Stimulates autophagy                 | [140]      |
| GLIPR2 (GAPR-1)          | Inhibits autophagy                   | [90]       |
| HMG1B                    | Stimulates autophagy                 | [141]      |
| Ins(1,4,5)P3R            | Inhibits autophagy                   | [142]      |
| LRP1P                    | Inhibits autophagy                   | [143]      |
| nPIST                    | Stimulates autophagy                 | [144]      |
| p53                      | Inhibits autophagy                   | [145]      |
| Rhes                     | Stimulates autophagy                 | [146]      |

**Table 2 (Continued)**

| Protein                  | Impact on Autophagy                  | Refs       |
|--------------------------|--------------------------------------|------------|
| TAB2/TAB3                | Inhibits autophagy                   | [147]      |
| VMP1                     | Stimulates autophagy                 | [148]      |
| **Other microbial proteins** |                                      |            |
| Coronavirus PLP2-TM      | Stimulates autophagy                 | [149]      |
| FMDV 2C                  | Unknown                               | [150]      |
| HCMV TRS1                | Inhibits autophagy                   | [151]      |
| HIV-1 Nef                | Inhibits autophagosome maturation    | [152]      |
| HSV-1 ICP34,5            | Inhibits autophagy and autophagosome maturation | [153,154]|
| Influenza A M2           | Inhibits autophagosome maturation    | [155]      |

<sup>a</sup>Includes only cellular and viral proteins shown to interact with endogenous Beclin 1, unless otherwise noted.

<sup>b</sup>These kinases have been shown to directly phosphorylate Beclin 1 in vitro, but their interaction with Beclin 1 has not been demonstrated.

Regulation of Beclin 1 by Bcl-2

Beclin 1 was originally identified by virtue of its interaction with Bcl-2 [16], and several Bcl-2 family members, including Bcl-2, Bcl-X<sub>L</sub>, and virus-encoded Bcl-2 proteins, inhibit autophagy through a direct interaction with the BH3 domain of Beclin 1 [49]. Unlike other BH3-only proteins, Beclin 1 has no direct effects on the anti-apoptotic function of Bcl-2 or on apoptosis [50], whereas, in contrast, the apoptotic machinery may negatively regulate autophagy through caspase cleavage of Beclin 1 [51] or Bim (Bcl-2 interacting mediator of cell death)-mediated sequestration of Beclin 1 to microtubules [52]. The favored hypothesis is that Bcl-2 family members interact with the dimerized form of Beclin 1 in unstressed cells, and upon exposure to stress stimuli the Bcl-2/Beclin 1 complex, as well as Beclin 1 homodimerization, is disrupted, leading to heterodimerization with Atg14 or UVRAG and enhanced Vps34 lipid kinase activity on distinct membrane structures to activate autophagy or endocytosis [23,24,30,53–55]. Recent evidence also indicates that the binding of Bcl-2 to Beclin 1 blocks a newly defined signaling event both in vitro and in mouse tissues that is essential for initiation of amino acid starvation-induced autophagy, namely, MK2/MK3-mediated Beclin 1 S90 serine phosphorylation [40]. Endoplasmic reticulum (ER)-localized Bcl-2, but not mitochondrion-localized Bcl-2, inhibits autophagy [56], and this is consistent with a crucial role of ER-associated PI3KC3 activity in autophagosome initiation [57,58].

The dissociation of Bcl-2/Beclin 1 represents a central switch that turns autophagy on in response to diverse stress signals, including amino acid starvation [59,60], exercise [60], death signaling molecules (e.g., DAPK) [61], toll-like receptor adaptor signaling molecules (e.g., MyD88 and Trif) [62] and the host restriction molecule, TRIM5<sup>a</sup> (tripartite motif-containing protein 5<sup>a</sup>) [63].
Several different mechanisms regulate the interaction between Bcl-2 and the BH3 domain of Beclin 1, including competitive disruption by BH3-only proteins [64], phosphorylation events within the BH3 domain of Beclin 1 that either promote (e.g., MST1) or inhibit (e.g., DAPK, ROCK1) its binding to Bcl-2 [61,65,66], or by multisite phosphorylation of the non-structured loop of Bcl-2, which prevents its binding to Beclin 1 [59]. During starvation conditions, this is mediated by the stress-signaling molecule, JNK1 (c-Jun N-terminal kinase 1) [59], whereas the kinase that leads to Bcl-2 phosphorylation and disruption of the Bcl-2/ Beclin 1 complex during exercise is not yet known [60].

The physiological relevance of Bcl-2 multisite phosphorylation in regulating stimulus-induced autophagy has been demonstrated in vivo using knock-in mice containing non-phosphorylatable mutations in Bcl-2 (T69A, S70A and S84A; Bcl-2AA) [60]. These mice have normal basal levels of autophagy but are severely deficient in starvation- and exercise-induced autophagy. This deficiency in exercise-induced autophagy in mice with normal basal levels of autophagy and normal tissue development led to the discovery of a novel role of Beclin 1 and autophagy in metabolism. Unlike wild type mice, acute exercise in Bcl-2AA mice fails to result in increased insulin sensitivity in skeletal muscle, as measured by glucose uptake, plasma membrane localization of the glucose transporter Glut4, and activation of AMP-activated protein kinase (AMPK).

Similar results were also observed in mice with allelic loss of the Beclin 1 gene Beclin1 or a hypomorphic allele of another autophagy gene, Atg16L1, suggesting that these phenotypes are indeed likely due to deficient autophagy rather than an unknown effect of the Bcl-2AA mutation. Moreover, chronic exercise has beneficial effects in wild type mice but not in Bcl-2AA mice against high-fat diet-induced diabetes and other metabolic abnormalities. These findings raise the possibility that autophagy may act not only downstream of AMPK signaling but also upstream of AMPK signaling. Such a feed-forward mechanism of autophagy on AMPK activation may help to explain the biochemical mechanisms underlying the more broad observations of beneficial effects of autophagy not only on metabolism but also on tumor-suppression and lifespan extension.

Regulation of Beclin 1 by oncogenic kinases

The discovery of Akt (protein kinase B)-mediated and epidermal growth factor receptor (EGFR)-mediated phosphorylation and inhibition of Beclin 1 provides insights into the biochemical mechanisms connecting cell growth control with autophagy regulation, and also allows dissection of the role of oncogenic signaling mediated inhibition of autophagy in different stages of tumorigenesis. Many oncogenic receptor tyrosine kinases and growth signaling molecules in the class I PI3K signaling pathway have been long known to suppress autophagy [67]; however, because these signals activate mTOR (mechanistic target of rapamycin), which not only inhibits autophagy but also stimulates translation and other processes involved in anabolic growth, the relevance of oncogenic signaling suppression of autophagy per se in tumorigenesis has been unclear.

The oncprotein Akt is activated in the majority of human epithelial tumors, and such activation results in Beclin 1 phosphorylation on serines S234 and S295, the binding of Beclin 1 to 14-3-3 and intermediate filament proteins, its sequestration in the cytoskeleton, and mTOR-independent autophagy inhibition [68]. Importantly, the ability of Akt to transform fibroblasts and the ability of Akt-transformed fibroblasts to form fibrosarcomas when implanted into immunodeficient mice is severely impaired in cells expressing mutant forms of Beclin 1 that are resistant to Akt-mediated phosphorylation and autophagy suppression [68]. This suggests that the suppression of autophagy by oncogenic signaling may directly contribute to oncogenic transformation.

The oncogenic receptor tyrosine kinase, EGFR, is amplified in many solid tumors, and constitutive active mutations in its tyrosine kinase domain are driver mutations in non-small cell lung carcinomas (NSCLCs) that occur in non-smokers [69]. Activated EGFR phosphorylates three Beclin 1 tyrosine residues, Y229, Y233, and Y352, which promotes Beclin 1 homodimerization and Rubicon binding, leading to decreased formation of the autophagy-active Beclin 1-containing PI3KC3-C1 complex [55]. These biochemical data are consistent with predictions from the crystal structure of the Beclin 1 coiled-coil domain, which suggest that it forms a metastable antiparallel dimer that can be disrupted by Atg14 or UVRAG to form heterodimers [33]. The two Beclin 1 tyrosine phosphorylation sites, Y229 and Y233, are at the interface of the Beclin 1 coiled-coil domain and stabilize the homodimers [70]. Constitutive activation of Beclin 1 tyrosine phosphorylation functions as a dominant negative mutant by promoting Beclin 1 homodimerization, and the expression of such a mutant in NSCLC xenografts leads to autophagy suppression, enhanced tumor growth, increased cell proliferation, and tumor dedifferentiation from an adenocarcinoma to a more aggressive adenosquamous phenotype [55]. In addition, the inducible expression of a Beclin 1 tyrosine phosphorysmic mutant in established NSCLC xenografts results in partial chemoresistance to EGFR tyrosine kinase inhibitor therapy [55]. Thus, Beclin 1 not only acts as a suppressor of tumor initiation, but, at least in the setting of NSCLC driven by an active EGFR mutation, also can prevent the progression of established tumors and help to mediate chemotherapeutic responses.

In contrast to active EGFR, which inhibits autophagy through Beclin 1 tyrosine phosphorylation, inactive EGFR may function in basal and starvation-induced autophagy [71]. Inactive EGFR forms an endosomal-localized complex with LAPTM4B (lysosomal protein transmembrane 4b) and Sec5 (also known as EXOC2, exocyst complex component 2), which promotes EGFR association with Rubicon, leading to Beclin 1 dissociation from Rubicon and autophagy initiation. This function of inactive EGFR is postulated to promote tumor cell survival upon serum starvation or metabolic stress; however, this concept has not yet been tested in tumorigenesis in vivo. The opposing regulation of Beclin 1 by inactive EGFR versus active EGFR raises the broader question of whether oncogenic signals may function more generally as ‘on/off’ switches that tightly coordinate autophagic responses with cell growth control.
Regulation of Beclin 1 by tumor-suppressor signaling molecules

Whereas there is emerging evidence that oncogenic signaling molecules may converge on Beclin 1 to suppress autophagy, there is also emerging evidence that tumor-suppressor signaling molecules converge on Beclin 1 to stimulate autophagy. The death-associated protein kinase (DAPK) is a serine/threonine kinase that induces different types of cell death (including autophagic cell death), functions as a tumor-suppressor, and is deleted in many cancer types [72]; this kinase induces autophagy by Beclin 1 threonine T119 phosphorylation, which disrupts its binding to Bcl-2 and Bcl-XL [61]. The liver kinase B1 (LKB1)/AMPK signaling pathway is an important tumor-suppressor signaling pathway that has multiple downstream targets including p53 and inhibition of mTOR [73]. AMPK has also been shown to promote autophagy by phosphorylating components of the autophagy machinery, including the upstream kinase, ULK1 (unc-51 like autophagy activating kinase 1) [74,75], and, during glucose starvation, Beclin 1 serines S93 and S96 [42]. The stress-related kinase, MK3, was originally discovered by virtue of its frequent deletions in small cell lung carcinomas, is lost in a variety of other tumors, and is postulated to have tumor suppressor function [76]. This kinase, as well as its close relative, MK2, is required for amino acid starvation-induced autophagy via direct phosphorylation of Beclin 1 S90 [40]. Furthermore, mutation of Beclin 1 S90 blocks the ability of Beclin 1 to rescue starvation-induced autophagy in Beclin 1-deficient cells and blocks the tumor-suppressor activity of Beclin 1 in MC7 human breast carcinoma xenografts [40]. While further studies are warranted to determine the precise role of Beclin 1 activation/phosphorylation by tumor-suppressor signaling molecules in mediating their tumor-suppressor function, it is notable that environmental stress signals – such as glucose and/or amino acid starvation – may dually activate autophagy and inhibit tumorigenesis. The overlapping requirement of specific post-translational modifications of Beclin 1 for its autophagy and tumor-suppressor activity may suggest a mechanistic link between these two functions, although more general effects on regulation of Vps34 activity in other trafficking events cannot be ruled out.

The discovery of Beclin 2

Another layer of complexity in higher eukaryotic organisms, beyond the diversity of signaling inputs to Beclin 1, has emerged from the identification of mammalian specific autophagy proteins such as AMBRA1 (activating molecule in Beclin 1-regulated autophagy) that activate the Beclin 1-containing PI3KC3-C1 complex (reviewed in [77]) and the recent identification of a mammalian-specific Beclin 1 homolog, Beclin 2 [9]. Beclin 2 shares ~57% sequence identity with Beclin 1 and, similarly to Beclin 2, contains a predicted BH3 domain, a central coiled-coil domain, and a C-terminal BARA domain [9]. Beclin 2 interacts with Atg14, Vps34, UVRAG, and AMBRA1, but not Rubicon, and is required for autophagy [9]. The distinct tissue expression pattern of Beclin 2 versus Beclin 1 [9] may reflect distinct roles of different Beclin family members in different tissues; however, it is unclear why these proteins are not functionally redundant in autophagy in cells that express both proteins given their similarity in the coiled-coil and BARA domains.

The most notable sequence divergence between Beclin 1 and Beclin 2 is in their N-terminal regions. The N terminus of Beclin 2, but not Beclin 1, interacts with GASP1 (G-protein-coupled receptor-associated sorting protein 1), and Beclin 2, but not Beclin 1, is required for the endolysosomal trafficking and degradation of GASP1-dependent G protein-coupled receptors (GPCRs), including the δ-opioid receptor, the cannabinoid type 1 receptor (CB1R), and the non-recycling mutant β-adrenergic receptor [9]. This function of Beclin 2 in lysosomal degradation of particular GPCRs is not shared by other members of the autophagic PI3KC3 complex such as Atg14, Beclin 1, and Vps34. Thus, GPCR endolysosomal trafficking represents the first Vps34 kinase-independent trafficking function described for members of the Atg6/Beclin family. This dual use of a mammalian-specific protein in an autophagy PI3KC3 complex and in autophagy-independent, PI3KC3-independent, endolysosomal degradation of an important class of cell surface receptors may reflect a need for mammalian cells to integrate diverse lysosomal degradation pathways to optimally fine-tune cellular responses to complex environmental signals.

Physiological functions of mammalian Beclin 1 and Beclin 2

Atg6/Beclin 1 null mutations in lower eukaryotes result in several important developmental and disease phenotypes (Table 1), likely reflecting its diverse functions in autophagy, vacuolar protein sorting, and endocytic maturation. Many of these phenotypes, such as defective sporulation in yeasts [10], proper entry into G2/M quiescence during nitrogen starvation in yeasts [78], defective fruiting body formation in Dictyostelium [79], uncontrolled cell death during the plant hypersensitive response [80], and defective dauer development and longevity phenotypes in C. elegans [81,82], are identical to those observed with mutation of other core autophagy genes. However, some phenotypes such as defective pollen germination in plants [18], impaired cell polarity in Drosophila [83], and defects in retrograde transportation from endosomes to Golgi in C. elegans [20] are not shared by other autophagy gene mutant organisms.

In mice, biallelic loss of Beclin 1 results in early embryonic lethality [84,85]. Monoallelic deletion of Beclin 1 results in increased incidence of spontaneous malignancies including lung carcinomas, lymphomas, hepatocellular carcinomas, and breast carcinomas that have basal-like features [84–86]. This latter phenotype is consistent with recent data showing that decreased BECN1 mRNA expression in patients is strongly associated with increased risk of the more aggressive basal-like subtypes of sporadic breast cancer and with worse patient survival [87]. In addition, allelic loss of Beclin1 in mice results in increased susceptibility to Alzheimer’s-like disease, increased severity of Desmin-related cardiomypathy, increased renal fibrosis following ureteral obstruction, increased lung pathology during respiratory syncytial virus (RSV) infection, reduced exercise endurance, and impaired exercise-induced insulin sensitivity, among other phenotypes (Table 1) [5].
Trends in Cell Biology September 2015, Vol. 25, No. 9

It is difficult to ascertain whether most of these phenotypes are due to defects in autophagy or other trafficking functions of Beclin 1 because comparable disease models have rarely been studied in mutant animals with partial loss of function of other autophagy genes. However, tissue-specific deletion of other autophagy genes in mice generally confirms an important role for autophagy in tumor suppression and in protection against neurodegenerative, infectious, cardiac, muscular, and metabolic diseases [1,2]. Nonetheless, a recent report describes a role for Beclin 1 in Rab5 (Ras-related in brain 5) GTPase-associated endosome formation and endosome maturation in vivo in mouse neurons [21]. Thus, the spectrum of phenotypes of Beclin 1-deficient mice most likely reflects its diverse roles in autophagy, endocytic trafficking, and LC3-associated phagocytosis. Although Beclin 1-dependent LC3-associated phagocytosis is thought to be essential for apoptotic corpse clearance [7], the requirement for cell autonomous ATP-production that requires both Beclin 1 and the autophagy protein, ATG5, in apoptotic clearance in mammalian embryonic body cavitiation suggests that the autophagy process itself is important in this aspect of early development [88].

Interestingly, mice with allelic loss of Beclin2 have a metabolic phenotype not shared by mice with allelic loss of Beclin1, even though Beclin1+/− mice are more deficient in autophagy [9]. Beclin2 heterozygous-deficient mice have increased brain levels of CB1R [9] and, consistent with the known effects of excessive CB1R signaling [89], have increased food intake, obesity, impaired glucose tolerance, and decreased insulin sensitivity [9]. These findings suggest that Beclin 2-mediated lysosomal degradation of CB1R, and potentially other GPCRs, may be important in vivo in preventing diseases caused by excessive GPCR signaling. Indeed, the genetic locus that contains human Beclin 2, chromosome 1q43, has been linked to obesity and diabetes in multiple ethnic populations [9], warranting further investigation of a possible role for Beclin 2 as a regulator of body weight and glucose homeostasis in humans.

Therapeutic potential of Beclin 1-derived autophagy activation

Given the broad involvement of autophagy in defense against infection, neurodegenerative disorders, diabetes, cancer, and aging, agents that specifically induce autophagy may have wide therapeutic applications [5]. Proof-of-principle for the concept that enhanced Beclin 1 activity may be therapeutically beneficial is provided by rodent Beclin1 gene therapy studies that have shown a protective effect in different neurodegenerative diseases (α-synuclein models of Parkinson’s disease, spinocerebellar ataxia type 3 disease), cystic fibrosis, K-ras-driven lung tumors, and collagen VI muscular dystrophies [5]. Moreover, the central role of Beclin 1 as a rate-limiting protein in autophagy initiation that is tightly regulated by protein–protein interactions and post-translational modifications provides a unique opportunity to translate knowledge gained from mechanistic studies of its regulation into new therapeutic approaches. Several drugs already in clinical use may activate autophagy at the level of Beclin 1 through disrupting Bcl-2/Beclin 1 interactions, inhibiting Akt, inhibiting EGFR and HER2 (human epidermal growth factor 2), or activating AMPK, and with recent advances in understanding the precise molecular of Beclin 1 regulation it should be possible to design even more specific activators in the future.

Another therapeutic strategy for increasing autophagy by increasing Beclin 1 function has emerged from the identification of a cell permeable peptide, Tat-Beclin 1, composed of 18 amino acids from the BARA domain of Beclin 1, that likely induces autophagy by competitively disrupting the binding of endogenous Beclin 1, and a newly identified negative regulator, the Golgi-associated plant pathogenesis-related protein 1 (GAPR-1, also known as GLIPR2) [90]. Tat-Beclin 1 decreases the accumulation of polyglutamine expansion protein aggregates and the replication of several pathogens (including HIV-1) in vitro, and reduces the mortality of mice infected with chikungunya virus or West Nile virus. Thus, this autophagy-inducing peptide (or derivatives thereof) may have potential efficacy in the treatment of human diseases.

Concluding remarks

Significant recent progress has been made in understanding the architecture of Beclin 1/PI3KC3 complexes, the functional diversity of Beclin 1 and Beclin 2 in different membrane trafficking events, and the stress-induced signaling events that alter the conformation and function of Beclin 1/PI3KC3 complexes. An emerging principle is that different upstream trafficking events, including endocytic maturation, phagosomal maturation, autophagosomal biogenesis, and endolysosomal GPCR protein sorting, which converge at the stage of lysosomal degradation, may all involve protein complexes that contain either Beclin 1 (and associated subunits in the PI3KC3 complex) or Beclin 2. The complex integration of cell signaling molecules that regulate these complexes and their functional outputs is likely to be essential for a wide range of physiological processes.

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