

REVIEW

The autophagy effector Beclin 1: a novel BH3-only protein

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BH3 domains were originally discovered in the context of apoptosis regulators and they mediate binding of proapoptotic Bcl-2 family members to antiapoptotic Bcl-2 family members. Yet, recent studies indicate that BH3 domains do not function uniquely in apoptosis regulation; they also function in the regulation of another critical pathway involved in cellular and tissue homeostasis called autophagy. Antiapoptotic Bcl-2 homologs downregulate autophagy through interactions with the essential autophagy effector and haploinsufficient tumor suppressor, Beclin 1. Beclin 1 contains a BH3 domain, similar to that of Bcl-2 proteins, which is necessary and sufficient for binding to antiapoptotic Bcl-2 homologs and required for Bcl-2-mediated inhibition of autophagy. This review will summarize the evidence that the BH3 domain of Beclin 1 serves as a key structural motif that enables Bcl-2 to function not only as an antiapoptotic protein, but also as an antiautophagy protein.

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Introduction

Autophagy is the primary cellular pathway by which long-lived proteins, cytoplasmic organelles and intracellular pathogens undergo degradation. The pathway involves sequestration of these cellular constituents in double- or multimembrane cytoplasmic vesicles called autophagosomes, with subsequent delivery to the lysosome, where they are degraded and recycled (Klionsky and Emr, 2000; Levine and Klionsky, 2004). Autophagy promotes cellular survival by enabling cells to maintain macromolecular synthesis and energy homeostasis during nutrient deprivation and other forms of cellular stress; it also functions in differentiation and development, antiaging, innate and adaptive immunity, and

tumor suppression (Levine and Klionsky, 2004; Shintani and Klionsky, 2004; Levine and Kroemer, 2008; Mizushima *et al.*, 2008). The disruption of autophagy has been implicated in a wide variety of diseases including cancer, neurodegenerative disorders, skeletal and cardiac myopathies, cancer, inflammatory bowel disease and infectious diseases (Levine and Kroemer, 2008).

The autophagy pathway is conserved among all eukaryotes, and in the last decade many autophagy effectors (called Atg proteins) as well as major protein regulators have been identified (Levine and Klionsky, 2004; Xie and Klionsky, 2007). Regulators that induce autophagy include tumor suppressors, such as PTEN, TSC1 and TSC2 complexes, and DAPk; stress-activated signaling molecules, such as c-Jun N-terminal kinase 1 (JNK1), and those that respond to low energy (for example, AMP kinase) or endoplasmic reticulum (ER) stress (for example, PERK, eIF2 α -kinase and IRE1), and molecules involved in innate immune signaling, such as toll-like receptors and immunity-related GTPases (Escalante *et al.*, 2009). Proteins that inhibit autophagy include oncogenes, such as class I phosphatidylinositol 3-OH kinase (PI3K), Akt, Ras, TOR and Bcl-2 (Pattingre and Levine, 2006; Maiuri *et al.*, 2008). The tumor suppressor gene, *p53*, has been reported to play a dual role in autophagy (Levine and Abrams, 2008; Tasdemir *et al.*, 2008b), with some studies suggesting transcription-dependent and transcription-independent positive regulation of autophagy (Feng *et al.*, 2005; Crighton *et al.*, 2006) and other studies suggesting the negative regulation of autophagy by wild type and mutant forms of *p53* in the cytoplasm (Morselli *et al.*, 2008; Tasdemir *et al.*, 2008a, 2008c).

The molecular structures and mechanism(s) of action of the autophagy effectors have not been completely elucidated, but most function by participating in multi-protein complexes responsible for vesicle induction, nucleation, elongation, docking and fusion with the lysosome and degradation (Levine and Klionsky, 2004; Levine and Kroemer, 2008; Mizushima *et al.*, 2008). Some autophagy regulators induce autophagy through the phosphorylation of key components of the induction complex, such as Atg1 and Atg13. The process by which targets are selected for autophagy is not understood, and may be different for different targets. Autophagy induction triggers the conversion of phosphatidylinositol to phosphatidylinositol 3-phosphate (PI3P) by the

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vesicle nucleation or class III PI3K complex, and induces the recruitment of Atg9 and associated mitochondrial lipids to the growing vesicle (He *et al.*, 2008). PI3P serves as a signal to recruit proteins required for vesicle elongation (Obara and Ohsumi, 2008), which involves two ubiquitin-like conjugation pathways. One pathway results in the conjugation of Atg12 to Atg5 and the formation of the Atg12–Atg5–Atg16 complex, which then assists in the second pathway, resulting in the conjugation of phosphatidylethanolamine to Atg8/LC3, and the incorporation of this lipidated form of Atg8/LC3 into the growing phagophore. The docking and fusion of the completed autophagosome with the lysosome is directed by proteins such as LAMP2 and Rab7, which are also involved in other vesicle trafficking pathways. Ultimately, the autophagosome, along with its contents, is degraded by lysosomal hydrolases. The degraded contents are recycled by the cell to maintain macromolecular synthesis and energy homeostasis, which is important for survival during nutrient deprivation and other forms of cellular stress.

The essential autophagy effector, Beclin 1

Beclin 1, initially isolated as a Bcl-2-interacting protein (Liang *et al.*, 1998), shares 30% sequence identity with yeast, Atg6/Vps30, which participates in a protein complex essential for autophagy in yeast (Kametaka *et al.*, 1998). Beclin 1 was among the first mammalian autophagy effectors to be identified and along with the yeast Vps34 homolog, class III PI3K (Kihara *et al.*, 2001), and the yeast Vps15 homolog, p150 (Panaretou *et al.*, 1997), forms a complex responsible for autophagic vesicle nucleation in mammals (Aita *et al.*, 1999; Liang *et al.*, 1999; Kihara *et al.*, 2001). The precise mechanism by which the Beclin 1/class III PI3K complex mediates vesicle nucleation is unclear.

The Beclin 1 yeast ortholog, Atg6/Vps30, was also independently discovered in a genetic screen for proteins involved in vacuolar protein sorting (Seaman *et al.*, 1997), suggesting that Beclin 1 may also function in other class III PI3K-dependent membrane trafficking events. In a manner somewhat parallel to yeast, two distinct complexes, each of which contains class III PI3K, p150 and Beclin 1, have recently been identified in mammalian cells (Itakura *et al.*, 2008). One complex, considered the equivalent of the yeast complex I that is essential for autophagy, includes a human ortholog of yeast Atg14 that is required for autophagy. The other complex, considered the equivalent of the yeast complex II that functions in the vacuolar protein sorting pathway, includes ultraviolet irradiation resistance-associated gene (UVRAG), which is proposed to be the human ortholog of yeast, Vps38. Although Mizushima and colleagues (Itakura *et al.*, 2008) found that the UVRAG-containing Beclin 1/class III PI3K complex is not required for autophagosome formation, these findings contradict an earlier report that UVRAG is required for autophagy (Liang *et al.*, 2007). Further,

Bif-1, which binds to UVRAG and regulates membrane curvature (Takahashi *et al.*, 2007), and Ambra1, which binds to Beclin 1 in a complex containing UVRAG (Fimia *et al.*, 2007), have each been shown to upregulate the autophagy function of the Beclin 1/class III PI3K complex. Thus, additional studies are needed to clarify the role of distinct human Atg14- and UVRAG-containing complexes in autophagy and other membrane trafficking processes.

Definitive evidence for autophagy-independent functions of Beclin 1 in mammalian cells is still lacking, and two studies showed that Vps34/class III PI3K-dependent trafficking events, such as the proteolytic processing of procathepsin D in route from the trans-Golgi network to lysosomes or the postendocytic sorting of the epidermal growth factor receptor were intact in cells without functional Beclin 1 (Furuya *et al.*, 2005b; Zeng *et al.*, 2006). However, in plants, Ohsumi and colleagues (Fujiki *et al.*, 2007) postulated an autophagy-independent function of *ATG6/VPS30*, as *ATG6/VPS30* mutants, unlike other *Arabidopsis thaliana* *ATG* gene mutants were defective in pollen germination. Furthermore, the embryonic phenotype of *beclin 1* null mice is more severe than that of other autophagy gene-deficient mice (for example, *Atg5*^{-/-}, *Atg7*^{-/-} mice; Levine and Kroemer, 2008), suggesting that *beclin 1*-regulated processes other than autophagy may be essential in early mammalian development. Net, given the presence of distinct Beclin 1/class III PI3K complexes in mammalian cells, the pleiotropic effects of Beclin 1 orthologs in lower eukaryotes, and the differences between phenotypes of *ATG6* or *beclin 1* versus other mutant *ATG* genes in plants and mice, it seems likely that mammalian Beclin 1 also functions in other membrane-trafficking processes besides autophagy.

Despite these possible autophagy-independent functions of Beclin 1, the best-characterized function of Beclin 1 is its role in autophagy. The autophagy function of Atg6/Beclin 1 is highly conserved throughout eukaryotic evolution and is presumed to be important in mediating many of its biological effects. Genetic knockdown or knockout studies of *beclin 1*, or its orthologs in lower eukaryotes have revealed several important phenotypes, many of which are likely related to autophagy, as the loss-of-function mutations of *ATG6/beclin 1* are phenocopied by null mutations in other *ATG* genes. Like all yeast *ATG* genes, *ATG6* is essential for survival during starvation and yeast sporulation (Levine and Klionsky, 2004). Like other plant *ATG* genes, *A. thaliana* or *Nicotiana benthamiana* *beclin 1* is essential for the prevention of premature chlorosis and the restriction of programmed cell death during the innate immune response (Liu *et al.*, 2005; Patel and Dinesh Kumar, 2008). Like other *Caenorhabditis elegans* *ATG* genes, *bec-1* is essential for dauer development (Melendez *et al.*, 2003); survival during starvation (Hars *et al.*, 2007); lifespan extension because of caloric restriction, insulin- signaling pathway mutations or p53 mutations (Melendez *et al.*, 2003; Hars *et al.*, 2007; Jia and Levine, 2007; Hansen *et al.*, 2008; Tavernarakis *et al.*, 2008; Tóth *et al.*, 2008); and embryonic development (Takacs-Vellai *et al.*, 2005).

Studies in targeted mutant mice have revealed several important functions of *beclin 1*. Mice with biallelic loss of *beclin 1* are early embryonically lethal (Qu *et al.*, 2003; Yue *et al.*, 2003), and mice with monoallelic loss of *beclin 1* have an increased incidence of spontaneous tumorigenesis (Qu *et al.*, 2003; Yue *et al.*, 2003), display abnormal proliferation of mammary epithelial cells and germinal center B lymphocytes (Qu *et al.*, 2003), and have increased susceptibility to neurodegeneration (Pickford *et al.*, 2008) and desmin-related cardiomyopathy (Tannous *et al.*, 2008). In humans, monoallelic deletions of *beclin 1* are frequently observed in sporadic breast, ovarian and prostate carcinoma (Aita *et al.*, 1999), and decreased Beclin 1 expression in the central nervous system is linked to susceptibility to Alzheimer's disease (Pickford *et al.*, 2008). Thus, taken together, evidence is emerging that Beclin 1 plays an essential role in tumor suppression, development, aging prevention, innate immunity, neuroprotection and cardioprotection.

An antiautophagic function for antiapoptotic Bcl-2 proteins

As noted, Beclin 1 was originally isolated as a Bcl-2-interacting protein (Liang *et al.*, 1998), and several antiapoptotic members of the Bcl-2 family, such as Bcl-2 and Bcl-X_L, as well as viral Bcl-2 homologs encoded by oncogenic γ -herpesviruses, such as Kaposi's sarcoma-associated herpesvirus (KSHV) ν Bcl-2 and γ HV68 M11, bind to Beclin 1 and are negative regulators of its autophagy function (Liang *et al.*, 1998; Pattingre *et al.*, 2005; Maiuri *et al.*, 2007; Sinha *et al.*, 2008; Ku *et al.*, 2008a). For example, human MCF7 breast carcinoma cells that typically express low levels of Beclin 1 do not show starvation-induced upregulation of autophagy unless Beclin 1 is ectopically expressed. This Beclin 1-mediated rescue of starvation-induced autophagy in MCF7 cells is inhibited by the exogenous expression of human Bcl-2 and Bcl-X_L (Liang *et al.*, 1998; Pattingre *et al.*, 2005), as well as viral Bcl-2 homologs, KSHV Bcl-2 (Liang *et al.*, 1998; Pattingre *et al.*, 2005) and γ HV68 M11 (Sinha *et al.*, 2008; Ku *et al.*, 2008a). These results have now been extended to numerous cell lines in numerous studies, and thus, it appears that the overexpression of cellular and viral Bcl-2 homologs is a potent inhibitor of autophagy in diverse settings (Sinha *et al.*, 2008; Ku *et al.*, 2008a). Furthermore, endogenous Bcl-2 also likely regulates autophagy, as autophagy-competent Hela cells undergo a twofold increase in levels of starvation-induced autophagy upon Bcl-2 siRNA knockdown (Liang *et al.*, 1998; Pattingre *et al.*, 2005). Finally, enforced Bcl-2 expression can also inhibit autophagy *in vivo*, because transgenic mice that express Bcl-2 under the control of a cardiac-specific promoter exhibit decreased levels of starvation-induced autophagy in cardiomyocytes (Liang *et al.*, 1998; Pattingre *et al.*, 2005).

The antiautophagy function of Bcl-2 appears to be exerted at the ER. Although an initial report suggested that endogenous Beclin 1 localized entirely to the trans-Golgi network (Kihara *et al.*, 2001), subsequent studies

showed that endogenous Beclin 1, similar to ectopically expressed Beclin 1, also localizes to mitochondria and ER (Liang *et al.*, 1998; Pattingre *et al.*, 2005). Only ER-targeted Bcl-2, and not mitochondrial-targeted Bcl-2, can inhibit starvation-induced autophagy, suggesting that only the interaction of Bcl-2 and Beclin 1 at the ER negatively regulates autophagosome formation. At least in certain cell types, such as HT-29 human colon carcinoma cells, enforced Bcl-2 expression decreases the amount of class III PI3K/Vps34 that coimmunoprecipitates with Bcl-2 and decreases Beclin 1-associated class III PI3K activity (Liang *et al.*, 1998; Pattingre *et al.*, 2005). It is not yet known how the ER-localized Bcl-2-Beclin 1 complex regulates the formation and/or activity of the Beclin 1/class III PI3K complex.

The binding of endogenous Bcl-2 antiapoptotic homologs to Beclin 1 may regulate basal autophagy levels and cell survival. In multiple different cell lines, including MCF7 cells that lack a functional caspase 3, the enforced expression of wild-type Beclin 1 does not increase basal levels of autophagy or induce cell death (Liang *et al.*, 1998; Pattingre *et al.*, 2005). Yet, the enforced expression of Bcl-2-binding defective mutants of Beclin 1 induces increased levels of autophagy, as well as cell death that is blocked by siRNA against a downstream autophagy gene, *atg5*, but not by caspase inhibitors. Thus, Bcl-2 homologs appear to function as a rheostat, which maintain homeostatic levels of autophagy that promote cell survival while preventing excessive levels of autophagy that promote cell death. It is not yet clear whether this antiautophagy function of Bcl-2 homologs contributes to their antideath role in physiological settings. Although the role of autophagy as a cell death pathway is controversial (Kroemer and Levine, 2008), it is nonetheless tempting to speculate that Bcl-2 homologs may serve as inhibitors of multiple death pathways, including not only apoptosis, but also autophagy.

Beclin 1 contains a BH3 domain that is sufficient and essential for binding to Bcl-2 homologs

Beclin 1 is predicted to consist of at least three domains within the evolutionarily conserved C-terminal two-thirds of the protein. The first of these is a coiled-coil domain, which shares ~25% of its sequence identity with coiled-coil domains from proteins such as myosin, and includes a poorly conserved leucine zipper. This domain is involved in heterodimerization with UVRAG, another coiled-coil domain-containing protein involved in vesicle trafficking (Liang *et al.*, 2006). The first 10 residues of the leucine zipper also constitute a nuclear export signal essential for the cytoplasmic localization of Beclin 1, as well as its function in autophagy and tumor suppression (Liang *et al.*, 2001). The structure and molecular function of the remaining domains in this evolutionarily conserved region have not been identified, but these domains are required for interactions with Vps34/class III PI3K, Beclin 1-dependent PI3K activity,

autophagy (Furuya *et al.*, 2005b), and peripheral membrane association (Liang *et al.*, 1998). Although this evolutionarily conserved region of Beclin 1 is essential for the process of autophagy, the molecular mechanisms by which it mediates autophagy are not understood.

The N-terminal one-third of Beclin 1 is the most variable in sequence, with equivalent regions from evolutionarily distant orthologs sharing less than 15% sequence identity. This variable N-terminal region of Beclin 1 may serve to regulate protein interactions and/or the autophagy function of the evolutionarily conserved C-terminal region. Truncation experiments showed that residues 88–140 of human Beclin 1 are sufficient for binding to antiapoptotic Bcl-2 homologs (Liang *et al.*, 1998; Pattingre *et al.*, 2005). Within this region, recent structural, mutagenic and biochemical analyses show that residues 108–127 constitute a BH3

domain, similar to that required for the binding of proapoptotic proteins to antiapoptotic Bcl-2 homologs (Figure 1; Feng *et al.*, 2007; Maiuri *et al.*, 2007; Oberstein *et al.*, 2007). Consistent with the variable sequence of the N-terminal regions of Beclin 1, the molecular mechanisms of the regulation of Beclin 1 may also vary among different eukaryotes; for example, yeast lack Bcl-2 homologs suggesting that Bcl-2 binding to the N-terminal region of Atg6/Beclin 1 is not conserved.

Typically, a BH3 domain is defined as a four-turn amphipathic α -helix, bearing the sequence motif: Hy-X-X-Hy-K/R-X-X-Sm-D/E-X-Hy, in which Hy are hydrophobic residues and Sm represents small residues, typically glycine (Figure 1a). However, this domain is often difficult to identify from sequence alignments alone, as there are no invariant residues and even the pattern of residues is poorly conserved. Thus, combined analyses of sequence, structure, and a common molecular mechanism of binding to Bcl-2 homologs, may be required to identify these domains. Residues of human Beclin 1 that correspond to the residues conserved among BH3 domains are L112, L116, K117, G120, D121 and F123 (Figure 1a). Structural evidence obtained from crystal structures of peptides corresponding to the human Beclin 1 residues 108–127 bound to Bcl-X_L (Oberstein *et al.*, 2007), or from standard heteronuclear multidimensional NMR spectroscopy of variously labeled Bcl-X_L-Beclin 1 (104-131) fusion protein (Feng *et al.*, 2007) showed that like BH3 domains from proapoptotic proteins, these Beclin 1 residues fold into a four-turn α -helix when bound to Bcl-2 homologs (Figure 1b).

Proapoptotic Bcl-2 proteins are grouped into two categories: (1) the multidomain proapoptotic proteins that contain three BH domains, BH4, BH3 and BH1; and (2) the BH3-only proapoptotic proteins that contain only the BH3 domain. In both these groups, the BH3 domain is required for interaction with antiapoptotic Bcl-2 proteins. Antiapoptotic viral and cellular Bcl-2 homologs have very divergent sequences, but share similar three-dimensional structures consisting of a central hydrophobic α -helix surrounded by six amphipathic helices, delineated into four BH domains: BH4, BH3, BH1 and BH2 (Muchmore *et al.*, 1996; Loh *et al.*, 2005). A hydrophobic groove on the molecular surface of the antiapoptotic cellular Bcl-2 homologs (Sattler *et al.*, 1997; Petros *et al.*, 2000; Liu *et al.*, 2003), as well as viral Bcl-2 homologs, such as KSHV Bcl-2 (Huang *et al.*, 2002) and γ HV68 M11 (Loh *et al.*, 2005), is responsible for binding BH3 domains of proapoptotic proteins (Figure 2a) (Sattler *et al.*, 1997; Petros *et al.*, 2000; Huang *et al.*, 2002, 2003; Liu *et al.*, 2003; Loh *et al.*, 2005). However, the structurally analogous surface groove of EBV BHRF1 is occluded and cannot bind BH3 domains (Huang *et al.*, 2003). Recent structural data have shown that the BH3 domain of Beclin 1 also binds in the BH3-binding groove of Bcl-X_L (Figure 2b; Feng *et al.*, 2007; Oberstein *et al.*, 2007) and γ HV68 M11 (Figure 2c) (Sinha *et al.*, 2008; Ku *et al.*, 2008a).

Residues conserved among BH3 domains participate in the binding interface with the antiapoptotic Bcl-2

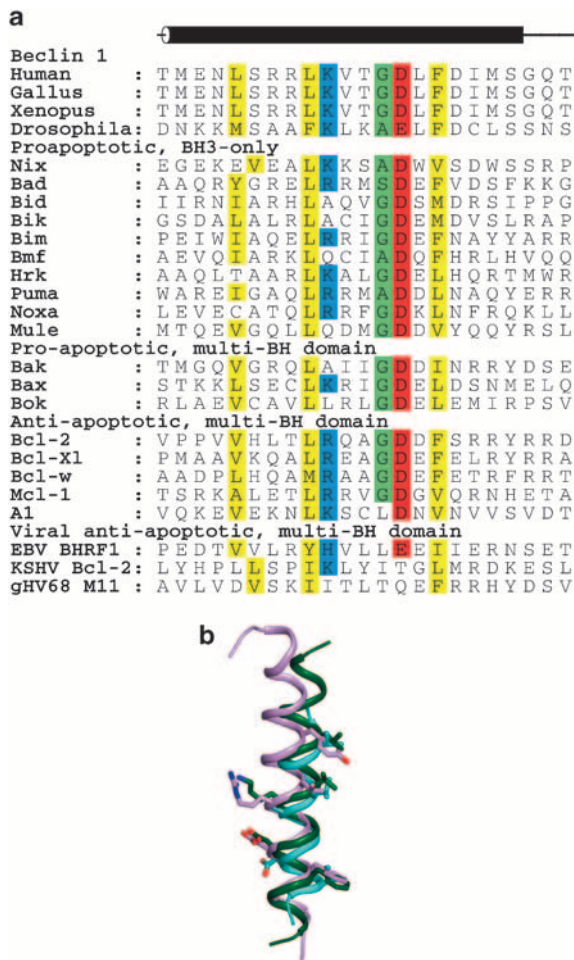


Figure 1 Comparison of BH3 domains from Beclin 1 and different proapoptotic proteins. (a) Sequence alignment. Backgrounds of conserved residues are colored by residue type: yellow hydrophobic, green small, blue basic and red acidic. (b) Structural superposition. BH3 domains are represented by different colored ribbons; Beclin 1 (dark green.); Bad (violet) and Bak (teal). Residues conserved among BH3 domains that are involved in binding to the antiapoptotic Bcl-2 homologs are shown in atomic detail. All molecular figures were prepared with the program PYMOL (<http://www.pymol.org>).

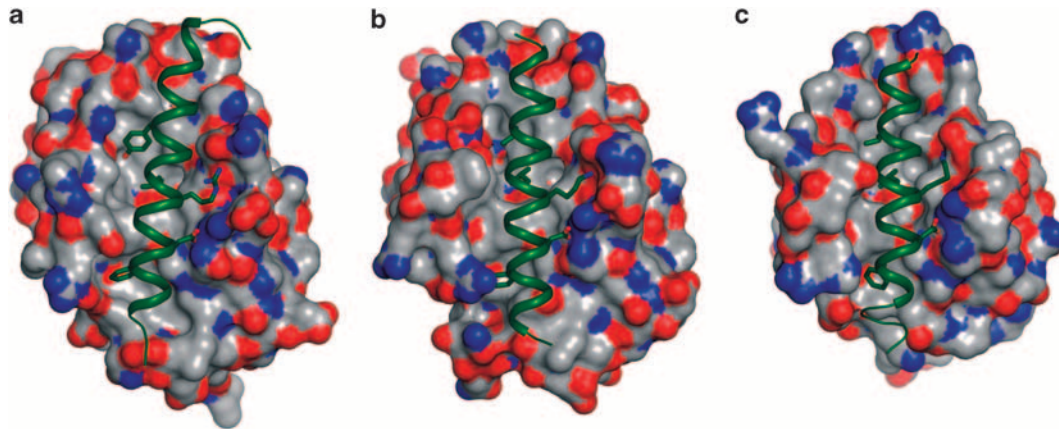


Figure 2 Binding of BH3 domains to Bcl-2 homologs. Molecular surfaces of M11 and Bcl- X_L are colored by atom type—oxygen red, nitrogen blue, sulfur green and carbon gray. BH3 domains are displayed in dark green ribbon with conserved residues involved in binding to antiapoptotic Bcl-2 homologs shown in atomic detail (a) Bad bound to Bcl- X_L . (b) Beclin 1 bound to Bcl- X_L . (c) Beclin 1 bound to M11.

homologs (Figures 1b and 2). The three hydrophobic residues conserved among the BH3 domains are packed against hydrophobic residues that line the BH3-binding groove of the antiapoptotic Bcl-2 homologs. The main chain atoms of the remaining two conserved residues, usually a glycine–aspartate pair, pack in an antiparallel manner against the main chain atoms of a glycine–arginine pair that is highly conserved among the BH1 domains of antiapoptotic Bcl-2 homologs. Further, the conserved BH3 domain aspartate ion pairs with the BH1 domain arginine conserved among the antiapoptotic Bcl-2 homologs. These interactions are preserved in the structures of the Beclin 1 BH3 domain bound to either Bcl- X_L (Figure 2b; Feng *et al.*, 2007; Oberstein *et al.*, 2007) or γ HV68 M11 (Figure 2c; Sinha *et al.*, 2008; Ku *et al.*, 2008a).

Several studies have identified residues within the BH3 domain of Beclin 1 required for binding to Bcl-2 homologs as well as residues within the BH3-binding groove of the Bcl-2 homologs required for binding to Beclin 1. Mutational analyses of Beclin 1 residues conserved among the BH3 domains show that the Beclin 1 BH3 domain is necessary for binding to Bcl-2. Selected substitution of key Beclin 1 residues, such as L112A, L116A, L116E, L116Q, G120E, D121A and F123A, either reduce or abrogate the interaction between Beclin 1 and either Bcl-2 or Bcl- X_L , as tested by qualitative binding assays, such as analytical size exclusion chromatography, affinity pull-downs and coimmunoprecipitation assays (Feng *et al.*, 2007; Maiuri *et al.*, 2007; Oberstein *et al.*, 2007). Similarly, alanine substitution of Beclin 1 residues L112, L116 and F123 reduce or prevent coimmunoprecipitation with γ HV68 M11 (Sinha *et al.*, 2008). Quantitative binding assays, such as isothermal calorimetry or fluorescence anisotropy measurements further confirm that the Beclin 1 BH3 domain residue mutants such as L116A and F123A, have unmeasurable affinity for Bcl- X_L or Bcl-2 (Feng *et al.*, 2007; Oberstein *et al.*, 2007).

As predicted, mutations of residues within the BH3-binding groove of the Bcl-2 homologs also block

interactions with Beclin 1. For example, mutations of conserved glycines in the BH1 domain of the Bcl-2 homologs, G145A for Bcl-2 (Pattingre *et al.*, 2005) and G138A for Bcl- X_L (Feng *et al.*, 2007; Oberstein *et al.*, 2007), abolish binding to wild-type Beclin 1 in coimmunoprecipitation assays. Similarly, double mutations of conserved γ HV68 M11 residues G86A + R87A (Sinha *et al.*, 2008; Ku *et al.*, 2008a) in the BH1 domain, or of residues Y60A + L74A (Sinha *et al.*, 2008) lining the hydrophobic groove, prevent γ HV68 M11 binding to wild-type Beclin 1. Thus, the combined structural, biochemical and mutagenic analyses show that Beclin 1 contains a BH3 domain that is sufficient and essential for binding to Bcl-2 homologs. Further, the BH3 domains from Beclin 1 and various proapoptotic proteins bind to the Bcl-2 homologs by very similar mechanisms.

Although interactions of the BH3 domain residues appear to be conserved in each interacting BH3 domain–Bcl-2 homolog pair, the contribution of each of these residues to the total affinity of binding varies significantly between each pair of interactions. For instance, the conserved Beclin 1 BH3 domain residues, G120 and D121, are not essential for binding to γ HV68 M11 (Sinha *et al.*, 2008). As suggested by NMR studies examining residues of γ HV68 M11 affected by binding to the BH3 domain of Beclin 1 versus BH3 domains from the proapoptotic proteins, Bad or Bax (Sinha *et al.*, 2008), the variation in amino acids constituting the BH3 domains, as well as in the residues lining the BH3-domain-binding groove of the interacting Bcl-2 homologs, probably result in a wide range of binding affinities for each pair of interactions, enforcing a degree of specificity in these interactions.

Mechanisms of Bcl-2-mediated inhibition of Beclin 1-dependent autophagy

The BH3 domain of Beclin 1, and interactions between the BH3 domain of Beclin 1 and Bcl-2 homologs, are essential for the antiautophagy activity of Bcl-2

proteins. Two major lines of evidence suggest that Bcl-2 proteins inhibit autophagy by binding to the BH3 domain of Beclin 1. First, mutation of residues in either Beclin 1 or Bcl-2 homologs that block Beclin 1–Bcl-2 homolog interactions, without exception, also block the ability of Bcl-2 to inhibit autophagy. Bcl-2, Bcl-X_L and viral Bcl-2s inhibit autophagy when wild-type Beclin 1 is expressed, but not when Bcl-2 binding-defective Beclin 1 mutants are expressed. (Liang *et al.*, 1998; Pattingre *et al.*, 2005). Conversely, Bcl-2 homolog mutants that cannot bind to Beclin 1, such as Bcl-2 G145A (Pattingre *et al.*, 2005), Bcl-X_L G138A (Maiuri *et al.*, 2007) and γ HV68 M11 G86A + R87A or Y60A + L74A (Sinha *et al.*, 2008; Ku *et al.*, 2008a), are defective in autophagy inhibition. Second, studies using the peptidomimetic inhibitor, ABT-737, a small molecule designed to bind to the BH3-binding groove of Bcl-2 or Bcl-X_L (Oltersdorf *et al.*, 2005), show that ABT-737 competitively inhibits the binding of peptides corresponding to the Beclin 1 BH3 domain to Bcl-X_L, and abrogates the inhibition of autophagy by Bcl-X_L (Maiuri *et al.*, 2007), providing further evidence that Bcl-2-mediated downregulation of autophagy depends on binding through the Bcl-X_L BH3-binding groove.

Although the research reviewed above conclusively demonstrates that the binding of the Beclin 1 BH3 domain to Bcl-2 homologs is essential for the Bcl-2-mediated downregulation of autophagy, as yet, we do not understand how this interaction results in autophagy inhibition. In mammals, Beclin 1, class III PI3K and p150 (Kihara *et al.*, 2001; Furuya *et al.*, 2005b), and either Atg14 (Itakura *et al.*, 2008) or UVRAG (Liang

et al., 2007), Bif-1/Endophilin 1 (Takahashi *et al.*, 2007) and Ambra1 (Fimia *et al.*, 2007) have been shown to be essential components of the multiprotein, membrane-associated, autophagosome nucleation complex. The binding of Beclin 1 to Bcl-2 may inhibit the interaction of Beclin 1 with class III PI3K, although class III PI3K does not appear to interact directly with the Beclin 1 BH3 domain (Pattingre *et al.*, 2005; Ku *et al.*, 2008b). Furthermore, both Beclin 1 and Bif-1 can form homooligomers (Takahashi *et al.*, 2007; Ku *et al.*, 2008b), and the binding of Bcl-2 homologs may also prevent higher-order oligomerization of Beclin 1 (Liang *et al.*, 2008). Therefore, it appears likely that the binding of Bcl-2 homologs to Beclin 1 sterically hinders its interactions with other essential components of the autophagy nucleation complex, inhibiting the autophagy-inducing function of Beclin 1 (Figure 3). However, the molecular mechanism(s) by which Beclin 1 functions in autophagy and consequently, the exact mechanism(s) by which the Beclin 1–Bcl-2 interaction disrupts the autophagy function of Beclin 1 remain to be understood.

Regulation of Bcl-2-mediated inhibition of Beclin 1-dependent autophagy

Two major mechanisms have been identified that regulate Bcl-2/Bcl-X_L binding to Beclin 1 and Bcl-2/Bcl-X_L inhibition of Beclin 1-dependent autophagy (Figure 3), including JNK1-mediated Bcl-2 phosphorylation and competitive disruption of Bcl-2/Bcl-X_L binding to Beclin 1 by other BH3 domains. Earlier, we showed

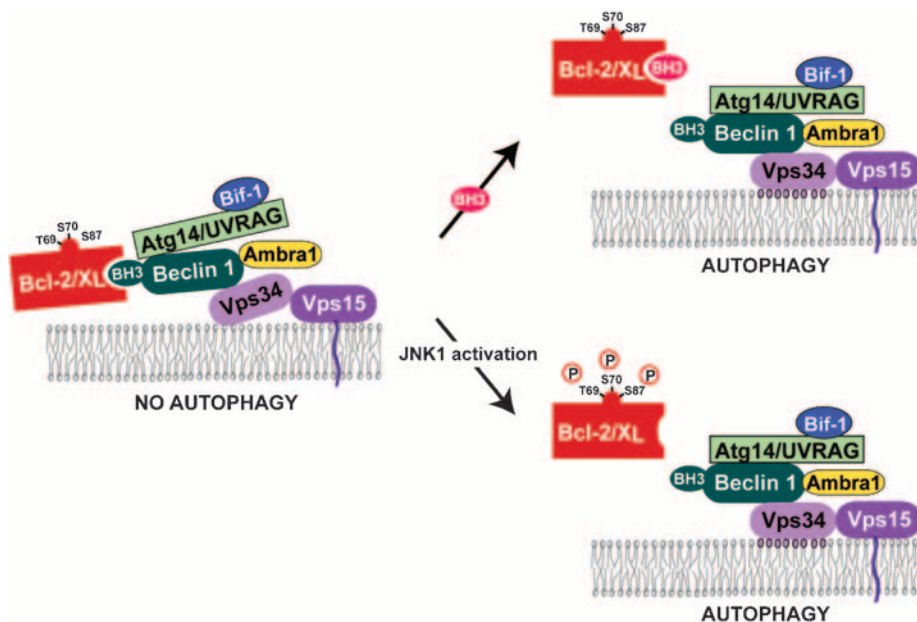


Figure 3 Schematic representation of the role of the Beclin 1 BH3 domain in the regulation of autophagy. The vesicle nucleation complex consists of Vps34 (also known as class III PI3K) and activators, such as Beclin 1, either Atg 14 or UVRAG, Ambra1 and Bif-1. The binding of Bcl-2 homologs to the BH3 domain of Beclin 1 prevents the assembly of an autophagy-competent complex (left). Disruption of the Beclin 1–Bcl-2 homolog interaction, either by competitive displacement of the Beclin 1 BH3 domain by other BH3 domain-containing proteins (upper right) or due to JNK1-mediated phosphorylation of Bcl-2 (lower right) results in the assembly of an autophagy-competent complex. The lipid kinase activity of this complex then catalyses the conversion of PI to PI3P (shown with violet circles) resulting in autophagy.

that the Bcl-2–Beclin 1 interaction is regulated by nutrient status, with the highest levels of interaction in nutrient-rich conditions (when autophagy is suppressed) and the lowest levels of interaction in starvation conditions (when autophagy is maximally stimulated; Liang *et al.*, 1998; Pattingre *et al.*, 2005). More recently, we showed that the starvation-induced activation of JNK1 causes multisite phosphorylation at residues T69, S70 and S87 in the unstructured loop between the BH4 and BH3 domains of Bcl-2, which leads to the dissociation of Bcl-2 and Beclin 1 (Wei *et al.*, 2008a). Bcl-2 is not phosphorylated in cells lacking JNK1, or in which JNK1 is inhibited. In these cells, starvation-induced dissociation of Bcl-2 and Beclin 1 and starvation-induced autophagy are abrogated. Similarly, in cells that express a T69A + S70A + S87A Bcl-2 mutant that cannot be phosphorylated instead of wild-type Bcl-2, there is no starvation-induced dissociation of Bcl-2 and Beclin 1, and no starvation-induced autophagy. Conversely, the expression of constitutively activated JNK1 leads to Bcl-2 phosphorylation, the lack of Bcl-2 and Beclin 1 binding during normal growth conditions, and increased basal levels of autophagy. Further, a multisite T69E + S70E + S87E Bcl-2 phosphomimetic mutant does not bind to Beclin 1 and does not inhibit autophagy (Wei *et al.*, 2008a). Thus, multisite phosphorylation of the Bcl-2 unstructured loop by JNK1 is responsible for the disruption of Bcl-2–Beclin 1-binding during starvation, and subsequent autophagy activation. Interestingly, viral Bcl-2s lack this unstructured loop with analogous phosphorylation sites and constitutively inhibit the autophagy function of Beclin 1 (Wei *et al.*, 2008a), suggesting that they have evolved mechanisms to escape normal physiological regulation of Bcl-2–Beclin 1 interactions.

The competitive binding of BH3 domains to Bcl-2 homologs represents another mechanism by which Bcl-2/Bcl-X_L-mediated inhibition of Beclin-1-dependent autophagy can be modulated. This was shown experimentally by the competitive displacement of Beclin 1 bound to Bcl-2 or Bcl-X_L by Bad, a BH3-only proapoptotic protein. As discussed above, nutrient deprivation reduces the amount of Beclin 1 that coimmunoprecipitates with Bcl-2/Bcl-X_L, resulting in increased levels of starvation-induced autophagy. This correlates with an increase in the amount of Bad that coimmunoprecipitates with Bcl-2/Bcl-X_L (Maiuri *et al.*, 2007). Increased levels of autophagy in response to starvation are not observed upon siRNA-mediated knockdown of *bad* in human HeLa cells or in *bad* knockout mouse embryonic fibroblasts (MEFs), but starvation-induced autophagy is restored in these cells upon treatment with ABT-737, a BH3 domain peptidomimetic. Finally, enforced expression of Bad, but not a Bcl-2-binding defective Bad mutant, is sufficient to induce autophagy, both in normal conditions and upon caspase inhibition.

Therefore, various BH3 domain-containing proteins that either bind with higher affinity to Bcl-2 homologs than does the BH3 domain of Beclin 1, or that are present in substantially higher concentrations, may competitively displace the Beclin 1 BH3 domain bound to Bcl-2, leading to abrogation of Bcl-2/Bcl-X_L-mediated inhibition of

Beclin 1-dependent autophagy. Indeed, in addition to Bad, other BH3-only proteins, such as Nix/Bnip3 (Daido *et al.*, 2004; Chang *et al.*, 2007; Hamacher-Brady *et al.*, 2007; Schweers *et al.*, 2007; Zhang *et al.*, 2008), Bik (Rashmi *et al.*, 2008), Noxa, Puma and BimEL (Abedin *et al.*, 2007) have been shown to induce autophagy and may, in a similar manner, function as competitive inhibitors of Beclin 1–Bcl-2/Bcl-X_L interactions. Thus, the BH3 domain not only functions directly in autophagy regulation by mediating interactions between Beclin 1 and antiapoptotic Bcl-2 homologs, it may also function, in the context of the BH3 domain-containing proapoptotic Bcl-2 proteins, in the indirect modulation of autophagy by influencing the interaction of Beclin 1 with Bcl-2/Bcl-X_L.

In addition to the two regulatory mechanisms discussed above, it is likely that other mechanisms, such as regulation of Beclin 1 and Bcl-2 expression and changes in the subcellular localization of either protein and/or post-translational modifications of Beclin 1, also modulate the interaction of Beclin 1 with Bcl-2 homologs as well as Bcl-2 homolog-mediated inhibition of Beclin 1-dependent autophagy. These potential regulatory mechanisms are important areas of future research.

The BH3 domain: a structural motif integrating the regulation of autophagy and apoptosis

BH3 domain-containing proteins bind with differential affinity to various Bcl-2 homologs, and may competitively affect the binding of other BH3 domain-containing proteins to Bcl-2. The competitive binding of the BH3 domain-containing proteins may be further modulated by the combined effect of post-translational modifications as well as spatial and temporal variations in the concentrations of each of the BH3 domain-containing proteins. Thus, Beclin 1 and proapoptotic BH3 domain-containing proteins may each indirectly regulate apoptosis and autophagy, respectively. As noted, proapoptotic BH3-only proteins induce autophagy by competitively disrupting the low-affinity Beclin 1–Bcl-2/Bcl-X_L interaction (Maiuri *et al.*, 2007). An important question is whether, through this mechanism, autophagy is invariably activated in cells exposed to apoptotic stimuli. A corollary question is whether the proapoptotic BH3-only proteins can also serve a prosurvival function by activating an adaptive autophagy response, and/or an apoptosis-independent pro-death function by activating toxic levels of autophagy that lead to self-cannibalization.

Bcl-2 phosphorylation and its differential effect on the binding of different BH3 domain-containing proteins, provide a mechanism to further fine-tune the regulation of these cellular events. This concerted regulation has been investigated only in the context of cellular response to nutrient starvation (Wei *et al.*, 2008b), although similar parallel mechanisms may well regulate the response to other stress-inducing stimuli. Nutrient deprivation results in increasing levels of JNK1-mediated Bcl-2 multisite phosphorylation, with levels

of phosphorylated Bcl-2 dependent upon the duration of starvation. This may lead to the differential Bcl-2 phosphorylation level-dependent dissociation of different BH3 domain-containing proteins bound to Bcl-2 (Wei *et al.*, 2008b). For example, Wei *et al.* (2008b) showed that early after starvation, low levels of multisite Bcl-2 phosphorylation result in increased dissociation of the Bcl-2–Beclin 1 complex and increased Beclin 1-dependent autophagy, which may be an attempt to promote cell survival. However, after prolonged starvation, when autophagy can no longer keep cells alive, higher levels of multisite phosphorylated Bcl-2 lead to its dissociation from the BH3 domain containing proapoptotic proteins, such as Bax, concurrent with caspase 3 activation and apoptosis (Wei *et al.*, 2008b). Thus, the differential affinities of Bcl-2 for the BH3 domain of Beclin 1 versus that of proapoptotic Bcl-2 family members may provide a mechanism for post-translational modifications of Bcl-2 to temporally coordinate the dual regulation of autophagy and apoptosis by Bcl-2.

Beclin 1 as a novel BH3-only protein

The first structure of the complex of Bcl-X_L bound to the minimal Beclin 1 region required for binding to Bcl-2 homologs, indicated that the Bcl-2-binding region constituted a BH3 domain, which bound to Bcl-X_L in a manner similar to BH3 domains from proapoptotic proteins (Oberstein *et al.*, 2007). On the basis of this structural evidence, the authors postulated that Beclin 1 may function as a proapoptotic BH3-only protein. Further, it was suggested that the role of Beclin 1 in tumor suppression may be explained by this proposed proapoptotic role, rather than by its proautophagic function. However, to date, there is no physiologically relevant evidence of a proapoptotic function for Beclin 1 or that such a proposed function contributes to its tumor suppressor action.

Mauri *et al.* (2007) found that, while the microinjection of high concentrations of a peptide corresponding to the BH3 domain of Beclin 1 induces Bax-dependent apoptosis, enforced overexpression of full-length Beclin 1 fails to trigger mitochondrial membrane permeability transition or apoptosis. This suggests that some region outside of the BH3 domain of Beclin 1 may exert a preventive, antiapoptotic function that 'neutralizes' the potential apoptogenic effects of the isolated BH3 domain. Furthermore, the reduced expression or knockdown of mammalian Beclin 1 has been shown to increase, rather than decrease, apoptosis in several different settings, such as in HeLa cells subjected to nutrient deprivation (Boya *et al.*, 2005); endothelial cells treated with angiogenesis inhibitors (Nguyen *et al.*, 2007; Ramakrishnan *et al.*, 2007); chondrocytes subjected to hypoxia (Bohensky *et al.*, 2007); conjunctival cell death upon exposure to benzalkonium chloride or ultraviolet radiation (Bohensky *et al.*, 2007); HEPG2 cells after antiFas antibody or doxorubicin treatment (Daniel *et al.*, 2006); MEFs treated with C(2)-ceramide (Fujiwara *et al.*, 2008); human myeloid leukemia cells treated with retinoic acid or vitamin D3 (Wang,

2008); human bronchial epithelial cells exposed to cigarette smoke extract (Kim *et al.*, 2008); cultured hepatoma cells (Harada *et al.*, 2008); various human carcinoma cell lines upon irradiation (Apel *et al.*, 2008); and renal tubular epithelial cells after cisplatin injury (Yang *et al.*, 2008). In addition, increased apoptosis has been reported in nematodes with a null mutation in the *C. elegans* beclin 1 ortholog, *bec-1* (Takacs-Vellai *et al.*, 2005).

Similarly, there is no evidence that Beclin 1 functions as a tumor suppressor by playing a proapoptotic role. In tumor-prone epithelial cells in *beclin 1*^{+/-} mice or in tumor-prone immortalized epithelial cells derived from such mice, there is no evidence of decreased apoptosis with allelic loss of *beclin 1* (Karantza-Wadsworth *et al.*, 2007; Mathew *et al.*, 2007; Qu *et al.*, 2007). Yet, in the setting of *beclin 1* allelic loss, epithelial cells display a defect in cell growth control (Qu *et al.*, 2003) and are more prone to DNA damage and chromosomal instability (Karantza-Wadsworth *et al.*, 2007; Mathew *et al.*, 2007), suggesting that apoptosis-independent functions of Beclin 1 contribute to its tumor suppressor action. Thus, it does not appear that the BH3 domain enables Beclin 1 to function physiologically as a BH3-only proapoptotic protein, or that such a proposed role contributes to its tumor suppressor activity.

Another theoretical argument for a proapoptotic function of Beclin 1, in addition to the observation that it is a BH3-only protein, is based on the observed competitive inhibition of BH3 domains from different BH3-only proteins in binding to Bcl-2 antiapoptotic homologs. As discussed, the competitive inhibition of Beclin 1 binding to Bcl-2 homologs by BH3-only proapoptotic proteins is a mechanism by which these proteins indirectly upregulate autophagy (Feng *et al.*, 2007; Oberstein *et al.*, 2007). Similarly, it is theoretically possible that the Beclin 1 BH3 domain might induce apoptosis by the competitive inhibition of Bcl-2 binding to BH3 domains of proapoptotic Bcl-2 proteins. However, this seems unlikely because all cellular Bcl-2 homologs bind BH3 domains of specific subsets of proapoptotic proteins with substantially higher affinity compared with their affinity for the Beclin 1 BH3 domain (Sinha *et al.*, 2008). Instead, it seems more likely that the BH3 domain of Beclin 1 serves as a structural motif that enables the coordinate regulation of apoptosis and autophagy. Thus, although the formal possibility that Beclin 1 may function as a proapoptotic protein in certain settings remains, we propose that Beclin 1 is a novel type of BH3-only protein, in which the BH3 domain enables the antiapoptotic Bcl-2 homologs to inhibit autophagy (through direct binding) and enables the proapoptotic BH3-only homologs to stimulate autophagy (through competitive inhibition of binding to Bcl-2/Bcl-X_L).

Bcl-2 inhibition of Beclin 1-dependent autophagy: a potential mechanism of oncogenesis?

Bcl-2 was the first cellular protein shown to function as an oncoprotein by blocking apoptotic cell death, rather

than by increasing cellular proliferation (McDonnell *et al.*, 1989; Vaux *et al.*, 1998). In light of emerging evidence that Beclin 1 and the autophagy pathway function as tumor suppressors (Edinger and Thompson, 2003; Ogier-Denis and Codogno, 2003; Gozuacik and Kimchi, 2004; Shintani and Klionsky, 2004; Furuya *et al.*, 2005a; Jin, 2006; Levine, 2006; Pattingre and Levine, 2006), a critical unanswered question is whether the antiautophagy activity of Bcl-2 also contributes to its oncogenic activity. Although in normal cells, autophagy is activated by the disruption of Bcl-2–Beclin 1 interactions; Bcl-2 overexpression, a common occurrence in human cancers, can also suppress the physiological activation of autophagy both in cultured cells and in mice (Liang *et al.*, 1998; Pattingre *et al.*, 2005). Furthermore, KSHV vBcl-2, and likely other viral Bcl-2s encoded by other oncogenic γ -herpesviruses, irreversibly bind to Beclin 1 and function as constitutive inhibitors of autophagy, because they lack the unstructured loop between the BH4 and BH3 domain that undergoes regulatory phosphorylation (Wei *et al.*, 2008a). Therefore, it is plausible that cellular Bcl-2 overexpression and viral Bcl-2s disrupt the tumor suppressor activity of the autophagy pathway by blocking the Beclin 1 function.

It should be noted that not only is Beclin 1 a haploinsufficient tumor suppressor, but other proteins that positively regulate the Beclin 1-class III PI3K complex, including UVRAG, Bif-1 and Ambr1 also function in negative growth control and/or tumor suppression (Levine and Kroemer, 2008). Thus, the Beclin 1–class III PI3K vesicle nucleation complex is probably an important nexus in autophagy control and tumor suppression, including the suppression of lymphomagenesis. B-cell lymphomas are the most common spontaneous tumors that arise in mice with either monoallelic deletion of *beclin 1* (Qu *et al.*, 2003; Yue *et al.*, 2003) or biallelic loss of *bif-1* (Takahashi *et al.*, 2007). Therefore, it is likely that cellular Bcl-2, which contributes to lymphomas in patients and mouse models, and viral Bcl-2s, may exert their oncogenic activity, at least in part, through autophagy inhibition. Specific agents that disrupt Bcl-2/Bcl-X_L–Beclin 1 BH3 domain binding without disrupting Bcl-2/Bcl-X_L–proapoptotic BH3 protein binding, and/or mutations in Bcl-2/Bcl-X_L that selectively disrupt its interaction with Beclin 1, will be required to address this hypothesis.

A related question is whether, beyond tumor suppression, cellular and viral Bcl-2 homologs also regulate other processes involving Beclin 1 and autophagy, such as apoptotic corpse clearance, innate immunity, the prevention of neurodegenerative disease and adaptive and maladaptive responses in heart disease (Levine and Kroemer, 2008). At a minimum, given the central role of Beclin 1 and autophagy in intracellular pathogen degradation (xenophagy), and activation of innate and adaptive immunity (Schmid *et al.*, 2006; Levine and Deretic, 2007; Orvedahl and Levine, 2008), it seems likely that viral Bcl-2 targeting of the Beclin 1 function may contribute to γ -herpesvirus pathogenesis. Indeed, autophagy inhibition by another herpesvirus-encoded

protein, herpes simplex virus type I ICP34.5, which binds to a different region of Beclin 1, has been shown to be essential for herpes simplex virus type I-induced lethal encephalitis (Orvedahl *et al.*, 2007). A high research priority will be to determine whether the cellular and viral Bcl-2 homologs also contribute to human disease through binding to the BH3 domain of Beclin 1 and subsequent autophagy inhibition.

Bcl-2–Beclin 1 interactions: a novel therapeutic target for autophagy induction?

Beclin 1 is a central player in autophagosome formation, and Bcl-2/Bcl-X_L function as important regulators of levels of Beclin 1-mediated autophagy. Thus, the Bcl-2/Bcl-X_L–Beclin 1 complex represents an important therapeutic target for autophagy modulation. Compounds that disrupt the complex will lead to increased autophagy and may be useful for the prevention and/or treatment of certain diseases, such as aging, certain cancers, infectious diseases and neurodegenerative diseases. Compounds that stabilize the complex may be useful in scenarios in which it may be desirable to inhibit autophagy, such as in established tumors in which autophagy is considered a prosurvival mechanism.

The BH3 peptidomimetics were originally developed in the context of targeted cancer therapies designed to increase apoptosis in tumor cells by disrupting interactions between antiapoptotic Bcl-2 homologs and proapoptotic BH3-only proteins. Not surprisingly, given the central role of BH3 domains in Bcl-2 regulation of both apoptosis and autophagy, they also constitute a class of drugs that can induce autophagy by disrupting the interaction between Bcl-2 homologs and the BH3 domain of Beclin 1. The first example of such an inhibitor was ABT-737 (Oltersdorf *et al.*, 2005), which specifically targets antiapoptotic Bcl-2 homologs that bind the Bad BH3 domain with high affinity. Assays measuring the binding of synthetic peptides to purified recombinant Bcl-X_L *in vitro* show that ABT-737 also competitively inhibits the binding of Beclin 1 BH3 peptides, with an IC₅₀ in the micromolar range (Maiuri *et al.*, 2007). Consistent with this finding, in cells resistant to the proapoptotic action of ABT-737, pretreatment with this inhibitor abolishes the immunoprecipitation of Beclin 1 with Bcl-2 or Bcl-X_L and induces high levels of autophagy (Maiuri *et al.*, 2007). ABT-737-induced autophagy cannot be inhibited by Bcl-2 or Bcl-X_L overexpression, yet is abolished upon either transfection with Mcl-1, which does not bind ABT-737, or by the siRNA-mediated knockdown of Beclin 1. Together these results clearly show that competitive disruption of the Beclin 1 interaction with Bcl-2 or Bcl-X_L by small molecule inhibitors suffices to induce autophagy (Maiuri *et al.*, 2007). Further refinements of BH3 peptidomimetics, based on structural analyses of Bcl-2 homolog/Beclin 1 BH3 domain complexes, may enable an increase in the specificity and/or potency of autophagy induction by this class of agents.

At present, it is controversial whether the proautophagy action of BH3 peptidomimetics should be enhanced or reduced in developing agents for cancer therapy. Autophagy induction may promote the survival of tumor cells, thereby counteracting or limiting the efficacy of apoptosis induction by these compounds. However, excessive autophagy may also promote cell death through self-cannibalization and help kill tumor cells. Indeed, recent evidence has shown that an experimental BH3 mimetic, obatoclax, kills glucocorticoid-resistant leukemic cells in a manner that is independent of Bax and Bak, but dependent on Atg5, Atg7 and Beclin 1 (Bornhauser *et al.*, 2008). Therefore, different BH3 mimetics may induce either autophagy-dependent cell survival or autophagy-dependent cell death, depending on the magnitude of autophagy induction. It will be important to determine the optimal levels of autophagy induction by BH3 peptidomimetics not only for the treatment of specific cancers, but also in the potential treatment of other diseases in which autophagy stimulation may be beneficial.

Conclusion

In conclusion, the research we have reviewed here shows that the essential autophagy effector, Beclin 1, contains a BH3 domain with a conserved molecular mechanism of

References

- Abedin M, Wang D, McDonnell M, Lehmann U, Kelekar A. (2007). Autophagy delays apoptotic death in breast cancer cells following DNA damage. *Cell Death Differ* **14**: 500–510.
- Aita VM, Liang XH, Murty VVVS, Pincus DL, Yu W, Cayanis E *et al.* (1999). Cloning and genomic organization of *beclin 1*, a candidate tumor suppressor gene on chromosome 17q21. *Genomics* **59**: 59–65.
- Apel A, Herr I, Schwarz H, Rodemann H, Mayer A. (2008). Blocked autophagy sensitizes resistant carcinoma cells to radiation therapy. *Cancer Res* **68**: 1485–1494.
- Bohensky J, Shapiro I, Leshinsky S, Terkhorn S, Adams C, Srinivas V. (2007). HIF-1 regulation of chondrocyte apoptosis: induction of the autophagic pathway. *Autophagy* **3**: 207–214.
- Bornhauser BC, Bonapace L, Schmitz M, Schrappe M, Walensky LD, Bourquin J-P. (2008). The BH3 mimetic obatoclax overcomes glucocorticoid-resistance by activating autophagic cell death. Seventh International Euroconference on Mechanisms of cell death and disease: Advances in therapeutic intervention and drug development. In: McDonnell TJ, Hickman JA and Debatin K-M (eds). Cascais: Portugal, pp Poster 28.
- Boya P, Gonzalez-Polo R-A, Casares N, Perfettini J-L, Dessen P, Larochette N *et al.* (2005). Inhibition of macroautophagy triggers apoptosis. *Mol Cell Biol* **25**: 1025–1040.
- Chang C, Yang M, Liu H, Lin Y, Lei H. (2007). Concanavalin A induces autophagy in hepatoma cells and has a therapeutic effect in a murine *in situ* hepatoma model. *Hepatology* **45**: 286–296.
- Crighton D, Wilkinson S, O'Prey J, Syed N, Smith P, Harrison P *et al.* (2006). DRAM, a p53-induced modulator of autophagy, is critical for apoptosis. *Cell* **126**: 121–124.
- Daido S, Kanzawa T, Yamamoto A, Takeuchi H, Kondo Y, Kondo S. (2004). Pivotal role of the cell death factor BNIP3 in ceramide-induced autophagic cell death in malignant glioma cells. *Cancer Res* **64**: 4286–4293.
- Daniel F, Legrand A, Pessayre D, Vadrot N, Descatoire V, Bernuau D. (2006). Partial Beclin 1 silencing aggravates doxorubicin- and Fas-induced apoptosis in HepG2 cells. *World J Gastroenterol* **12**: 2895–2900.

binding to antiapoptotic Bcl-2 homologs. However, despite this conserved mechanism and unlike the canonical BH3 domain-containing proteins, Beclin 1 does not appear to play a significant role in apoptosis. Rather, studies with the Beclin 1 BH3 domain point to a new function of BH3 domains in enabling the antiapoptotic Bcl-2 homologs to inhibit autophagy, another fundamental cellular pathway involved in tissue homeostasis. Thus, the Beclin 1 BH3 domain facilitates the coordinate regulation of autophagy, primarily a cell survival pathway, and apoptosis, a cell death pathway, by Bcl-2 homologs. Perhaps, through the common molecular mechanism of binding to 'antiapoptotic' Bcl-2 homologs, the simple BH3 domain has, in fact, complex cellular functions.

Conflict of interest

S Sinha has declared no conflict of interest. B Levine is a consultant/an advisor for Novus Biologicals (Littleton, CO, USA).

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- Edinger AL, Thompson CB. (2003). Defective autophagy leads to cancer. *Cancer Cell* **4**: 422–424.
- Escalantine A, Chaumorcet M, Codogno P. (2009). Macroautophagy signaling and regulation. In: Levine B (ed). *Autophagy in Infection and Immunity* (in press).
- Feng W, Huang S, Wu H, Zhang M. (2007). Molecular basis of Bcl-X_L's target recognition versatility revealed by the structure of Bcl-X_L in complex with the BH3 domain of Beclin-1. *J Mol Biol* **372**: 223–235.
- Feng Z, Zhang H, Levine AJ, Jin S. (2005). The coordinate regulation of the p53 and mTOR pathways in cells. *Proc Natl Acad Sci USA* **102**: 8204–8209.
- Fimia G, Stoykova A, Romagnoli A, Giunta L, Bartolomeo S, Nardacci R *et al.* (2007). Ambral regulates autophagy and development of the nervous system. *Nature* **447**: 1121–1125.
- Fujiki Y, Yoshimoto K, Ohsumi Y. (2007). An Arabidopsis homolog of yeast ATG6/VPS30 is essential for pollen germination. *Plant Physiol* **143**: 1132–1139.
- Fujiwara K, Daido S, Yamamoto A, Kobayashi R, Yokoyama T, Aoki H *et al.* (2008). Pivotal role of the cyclin-dependent kinase inhibitor p21WAF1/CIP1 in apoptosis and autophagy. *J Biol Chem* **283**: 388–397.
- Furuya N, Liang X, Levine B. (2005a). Autophagy and cancer. In: Klionsky DJ (ed) *Autophagy* pp 242–255.
- Furuya N, Yu J, Byfield M, Pattingre S, Levine B. (2005b). The evolutionarily conserved domain of *beclin 1* is required for Vps34 binding, autophagy, and tumor suppressor function. *Autophagy* **1**: 41–47.
- Gozuacik D, Kimchi A. (2004). Autophagy as a cell death and tumor suppressor mechanism. *Oncogene* **23**: 2891–2906.
- Hamacher-Brady A, Brady N, Logue S, Sayen M, Jinno M, Kirshenbaum L *et al.* (2007). Response to myocardial ischemia/reperfusion injury involves Bnip3 and autophagy. *Cell Death Diff* **14**: 146–157.
- Hansen M, Chandra A, Mitic L, Onken B, Driscoll M, Kenyon C. (2008). A role for autophagy in the extension of lifespan by dietary restriction in *C. elegans*. *PLoS Genet* **4**: e24.

- Harada M, Strnad P, Toivola D, Omary M. (2008). Autophagy modulates keratin-containing inclusion formation and apoptosis in cell culture in a context-dependent fashion. *Exp Cell Res* **314**: 1753–1764.
- Hars E, Qi H, Ryazanov A, Jin S, Cai L, Hu C *et al.* (2007). Autophagy regulates ageing in *C. elegans*. *Autophagy* **3**: 93–95.
- He C, Baba M, Cao Y, Klionsky D. (2008). Self-interaction is critical for Atg9 transport and function at the phagophore assembly site during autophagy. *Mol Biol Cell* **19**: 5506–5516.
- Huang Q, Petros AM, Virgin HW, Fesik SW, Olejniczak ET. (2002). Solution structure of a Bcl-2 homolog from Kaposi sarcoma virus. *Proc Nat Acad Sci* **99**: 3428–3433.
- Huang Q, Petros AM, Virgin HW, Fesik SW, Olejniczak ET. (2003). Solution structure of the BHRF1 protein from Epstein-Barr virus, a homolog of human Bcl-2. *J Mol Biol* **332**: 1123–1130.
- Itakura E, Kishi C, Inoue K, Mizushima N. (2008). Beclin 1 forms two distinct phosphatidylinositol 3-kinase complexes with mammalian Atg14 and UVRAG. *Mol Biol Cell* **19**: 5360–5372.
- Jia K, Levine B. (2007). Autophagy is required for dietary restriction-mediated life span extension in *C. elegans*. *Autophagy* **3**: 597–599.
- Jin S. (2006). Autophagy, mitochondrial quality control, and oncogenesis. *Autophagy* **2**: 80–84.
- Kametaka S, Okano T, Ohsumi M, Ohsumi Y. (1998). Apg14p and Apg6/Vps30p form a protein complex essential for autophagy in the yeast, *Saccharomyces cerevisiae*. *J Biol Chem* **273**: 22284–22291.
- Karantza-Wadsworth V, Patel S, Kravchuk O, Chen G, Mathew R, Jin S *et al.* (2007). Autophagy mitigates metabolic stress and genome damage in mammary tumorigenesis. *Genes Develop* **21**: 1621–1635.
- Kihara A, Kabeya Y, Ohsumi Y, Yoshimori T. (2001). Beclin-phosphatidylinositol 3-kinase complex functions at the trans-golgi network. *EMBO Rep* **2**: 330–335.
- Kim HP, Wang X, Lee S-J, Huang M-H, Wang Y, Ryter SW *et al.* (2008). Autophagic proteins regulate cigarette smoke induced apoptosis: Protective role of heme oxygenase-1. *Autophagy* **4**: 887–895.
- Klionsky DJ, Emr SD. (2000). Autophagy as a regulated pathway of cellular degradation. *Science* **290**: 1717–1721.
- Kroemer G, Levine B. (2008). Autophagic cell death: the story of a misnomer. *Nat Rev Mol Cell Biol* **9**: 1004–1010.
- Ku B, Woo J-S, Liang C, Lee K-H, Hong H-S, Xiaofei E *et al.* (2008a). Structural and biochemical bases for the inhibition of autophagy and apoptosis by viral Bcl-2 of murine γ -herpesvirus 68. *PLoS Path* **4**: e25.
- Ku B, Woo J-S, Liang C, Lee K-H, Jung JU, Oh B-H. (2008b). An insight into the mechanistic role of Beclin 1 and its inhibition by prosurvival Bcl-2 family proteins. *Autophagy* **4**: 519–520.
- Levine B. (2006). Unraveling the role of autophagy in cancer. *Autophagy* **2**: 65–66.
- Levine B, Abrams J. (2008). p53: The Janus of autophagy? *Nat Cell Biol* **10**: 637–639.
- Levine B, Deretic V. (2007). Unveiling the roles of autophagy in innate and adaptive immunity. *Nat Rev Immunol* **7**: 767–777.
- Levine B, Klionsky DJ. (2004). Development by self-digestion: Molecular mechanisms and biological functions of autophagy. *Dev Cell* **6**: 463–477.
- Levine B, Kroemer G. (2008). Autophagy in the pathogenesis of disease. *Cell* **132**: 27–42.
- Liang C, Xiaofei E, Jung JU. (2008). Downregulation of autophagy by herpesvirus Bcl-2 homologs. *Autophagy* **4**: 268–272.
- Liang C, Feng P, Ku B, B-H O, Jung JU. (2007). UVRAG: a new player in autophagy and tumor cell growth. *Autophagy* **3**: 69–71.
- Liang C, Feng P, Ku B, Dotan I, Canaani D, Oh BH *et al.* (2006). Autophagic and tumour suppressor activity of a novel Beclin1-binding protein UVRAG. *Nat Cell Biol* **8**: 688–699.
- Liang XH, Jackson S, Seaman M, Brown K, Kempkes B, Hibshoosh H *et al.* (1999). Induction of autophagy and inhibition of tumorigenesis by Beclin 1. *Nature* **402**: 672–676.
- Liang XH, Kleeman LK, Jiang HH, Gordon G, Goldman JE, Berry G *et al.* (1998). Protection against fatal Sindbis virus encephalitis by Beclin 1, a novel Bcl-2-interacting protein. *J Virol* **72**: 8586–8596.
- Liang XH, Yu J, Brown K, Levine B. (2001). Beclin 1 contains a leucine-rich nuclear export signal that is required for its autophagy and tumor suppressor function. *Cancer Res* **61**: 3443–3449.
- Liu X, Dai SC, Zhu Y, Marrack P, Kappler JW. (2003). The structure of a Bcl-xL/Bim fragment complex: implications for Bim function. *Immunity* **19**: 341–352.
- Liu Y, Schiff M, Czymmek K, Tallozy Z, Levine B, Dinesh-Kumar SP. (2005). Autophagy regulates programmed cell death during the plant innate immune response. *Cell* **121**: 567–577.
- Loh J, Huang Q, Petros AM, Nettesheim D, van Dyk LF, Labrada L *et al.* (2005). A surface groove essential for viral Bcl-2 function during chronic infection *in vivo*. *PLoS Path* **1**: 80–91.
- Maiuri M, Le Toumelin G, Criollo A, Rain J, Gautier F, Juin P *et al.* (2007). Functional and physical interaction between Bcl-X_L and a BH3-like domain in Beclin-1. *EMBO J* **26**: 2527–2539.
- Maiuri M, Tasdemir E, Criollo A, Morselli E, Vicencio J, Carnuccio R *et al.* (2008). Control of autophagy by oncogenes and tumor suppressor genes. *Cell Death Differ* **16**: 87–93.
- Mathew R, Kongara S, Beaudoin B, Karp C, Bray K, Degenhardt K *et al.* (2007). Autophagy suppresses tumor progression by limiting chromosomal instability. *Genes Dev* **21**: 1367–1381.
- McDonnell T, Deane N, Platt F, Nunez G, Jaeger U, McKearn JP *et al.* (1989). bcl-2-immunoglobulin transgenic mice demonstrate extended B cell survival and follicular lymphoproliferation. *Cell* **57**: 79–88.
- Melendez A, Tallozy Z, Seaman M, Eskelinen E-L, Hall DH, Levine B. (2003). Autophagy genes are essential for dauer development and life-span extension in *C. elegans*. *Science* **301**: 1387–1391.
- Mizushima N, Levine B, Cuervo A, Klionsky D. (2008). Autophagy fights disease through cellular self-digestion. *Nature* **451**: 1069–1075.
- Morselli E, Tasdemir E, Maiuri M, Galluzzi L, Kepp O, Criollo A *et al.* (2008). Mutant p53 protein localized in the cytoplasm inhibits autophagy. *Cell Cycle* **7**: 3056–3061.
- Muchmore SW, Sattler M, Liang H, Meadows RP, Harlan JE, Yoon HS *et al.* (1996). X-ray and NMR structure of human Bcl-X_L, an inhibitor of programmed cell death. *Nature* **381**: 335–341.
- Nguyen T, Subramanian I, Kelekar A, Ramakrishnan S. (2007). Kringle 5 of human plasminogen, an angiogenesis inhibitor, induces both autophagy and apoptotic death in endothelial cells. *Blood* **109**: 4793–4802.
- Obara K, Ohsumi Y. (2008). Dynamics and function of PtdIns(3)P in autophagy. *Autophagy* **4**: 952–954.
- Oberstein A, Jeffrey PD, Shi Y. (2007). Crystal structure of the Bcl-X_L-Beclin 1 peptide complex: Beclin 1 is a novel BH3 only protein. *J Biol Chem* **282**: 13123–13132.
- Ogier-Denis E, Codogno P. (2003). Autophagy: a barrier or an adaptive response to cancer. *Biochim Biophys Acta* **1603**: 113–128.
- Oltersdorf T, Elmore SW, Shoemaker AR, Armstrong RC, Augeri DJ, Belli BA *et al.* (2005). An inhibitor of Bcl-2 family proteins induces regression of solid tumours. *Nature* **435**: 677–681.
- Orvedahl A, Alexander D, Tallozy Z, Sun Q, Wei Y, Zhang W *et al.* (2007). HSV-1 ICP34.5 confers neurovirulence by targeting the Beclin 1 autophagy protein. *Cell Host Microbe* **1**: 23–35.
- Orvedahl A, Levine B. (2008). Eating the enemy within: Autophagy in infectious diseases. *Cell Death Differ* **16**: 57–69.
- Panaretou C, Domin J, Cockcroft S, Waterfield MD. (1997). Characterization of p150, an adaptor protein for the human phosphatidylinositol (PtdIns) 3-kinase. Substrate presentation by phosphatidylinositol transfer protein to the p150 PtdIns 3-kinase complex. *J Biol Chem* **272**: 2477–2485.
- Patel S, Dinesh-Kumar S. (2008). Arabidopsis ATG6 is required to limit the pathogen-associated cell death response. *Autophagy* **4**: 20–27.
- Pattinger S, Levine B. (2006). Bcl-2 inhibition of autophagy: A new route to cancer? *Cancer Res* **66**: 2885–2888.

- Pattingre S, Tassa A, Qu X, Garuti R, Liang XH, Mizushima N *et al.* (2005). Bcl-2 antiapoptotic proteins inhibit Beclin 1-dependent autophagy. *Cell* **122**: 927–939.
- Petros AM, Nettseheim DG, Wang Y, Olejniczak ET, Meadows RP, Mack J *et al.* (2000). Rationale for Bcl-xL/BAD peptide complex formation from structure, mutagenesis, and biophysical methods. *Protein Sci* **9**: 2528–2534.
- Pickford F, Masliah E, Britschgi M, Lucin K, Narasimhan R, Jaeger P *et al.* (2008). The autophagy-related protein Beclin 1 shows reduced expression in early Alzheimer disease and regulates amyloid beta accumulation in mice. *J Clin Invest* **118**: 2190–2199.
- Qu X, Yu J, Bhagat G, Furuya N, Hibshoosh H, Troxel A *et al.* (2003). Promotion of tumorigenesis by heterozygous disruption of the *beclin 1* autophagy gene. *J Clin Invest* **112**: 1809–1820.
- Qu X, Zou Z, Sun Q, Luby-Phelps K, Cheng P, Hogan R *et al.* (2007). Autophagy gene-dependent clearance of apoptotic cells during embryonic development. *Cell* **128**: 833–836.
- Ramakrishnan S, Nguyen T, Subramanian I, Kelekar A. (2007). Autophagy and angiogenesis inhibition. *Autophagy* **3**: 512–515.
- Rashmi R, Pillai S, Vijayalingam S, Ryerse J, Chinnadurai G. (2008). BH3-only protein BIK induces caspase-independent cell death with autophagic features in Bcl-2 null cells. *Oncogene* **27**: 1366–1375.
- Sattler M, Liang H, Nettseheim D, Meadows RP, Harlan JE, Eberstadt M *et al.* (1997). Structure of the Bcl-X_L-Bak peptide complex: recognition between regulators of apoptosis. *Science* **275**: 983–986.
- Schmid D, Dengjel J, Schoor O, Stevanovic S, Munz C. (2006). Autophagy in innate and adaptive immunity against intracellular pathogens. *J Mol Med* **84**: 194–202.
- Schweers R, Zhang J, Randall M, Loyd M, Li W, Dorsey F *et al.* (2007). NIX is required for programmed mitochondrial clearance during reticulocyte maturation. *Proc Natl Acad Sci USA* **104**: 19500–19505.
- Seaman MN, Marcusson EG, Cereghino JL, Emr SD. (1997). Endosome to Golgi retrieval of the vacuolar protein sorting receptor, Vps10p, requires the function of the VPS29, VPS30, and VPS35 gene products. *J Cell Biol* **137**: 79–92.
- Shintani T, Klionsky DJ. (2004). Autophagy in health and disease: a double-edged sword. *Science* **306**: 990–995.
- Sinha S, Colbert CL, Becker N, Wei Y, Levine B. (2008). Molecular basis of the regulation of Beclin 1-dependent autophagy by the γ -herpesvirus 68 Bcl-2 homolog M11. *Autophagy* **4**: 989–997.
- Takacs-Vellai K, Vellai T, Puoti A, Passannante M, Wicky C, Streit A *et al.* (2005). Inactivation of the autophagy gene *bec-1* triggers apoptotic cell death in *C. elegans*. *Curr Biol* **15**: 1513–1517.
- Takahashi Y, Coppola D, Matsushita N, Cualing H, Sun M, Sato S *et al.* (2007). Bif-1 interacts with Beclin 1 through UVRAG and regulates autophagy and tumorigenesis. *Nat Cell Biol* **9**: 1142–1151.
- Tannous P, Zhu H, Johnstone J, Shelton J, Rajasekaran N, Benjamin I *et al.* (2008). Autophagy is an adaptive response in desmin-related cardiomyopathy. *Proc Natl Acad Sci USA* **105**: 9745–9750.
- Tasdemir E, Maiuri M, Galluzzi L, Vitale I, Djavaheri-Mergny M, D'Amelio M *et al.* (2008a). Regulation of autophagy by cytoplasmic p53. *Nat Cell Biol* **10**: 676–687.
- Tasdemir E, Maiuri M, Morselli E, Criollo A, D'Amelio M, Djavaheri-Mergny M *et al.* (2008b). A dual role of p53 in the control of autophagy. *Autophagy* **4**: 810–814.
- Tasdemir E, Maiuri M, Orhon I, Kepp O, Morselli E, Criollo A *et al.* (2008c). p53 represses autophagy in a cell cycle-dependent fashion. *Cell Cycle* **7**: 3006–3011.
- Tavernarakis N, Pasparaki A, Tasdemir E, Maiuri M, Kroemer G. (2008). The effects of p53 on whole organism longevity are mediated by autophagy. *Autophagy* **4**: 870–873.
- Tóth M, Sigmond T, Borsos E, Barna J, Erdélyi P, Takács-Vellai K *et al.* (2008). Longevity pathways converge on autophagy genes to regulate life span in *Caenorhabditis elegans*. *Autophagy* **4**: 330–338.
- Vaux D, Cory S, Adams J. (1998). Bcl-2 gene promotes haemopoietic cell survival and cooperates with c-myc to immortalize pre-B cells. *Nature* **335**: 440–442.
- Wang J. (2008). Beclin 1 bridges autophagy, apoptosis and differentiation. *Autophagy* **4**: 947–948.
- Wei Y, Pattingre S, Sinha S, Bassik M, Levine B. (2008a). JNK-mediated phosphorylation of Bcl-2 regulates starvation-induced autophagy. *Mol Cell* **30**: 678–688.
- Wei Y, Sinha S, Levine B. (2008b). Dual role of JNK1-mediated phosphorylation of Bcl-2 in autophagy and apoptosis regulation. *Autophagy* **4**: 949–951.
- Xie Z, Klionsky DJ. (2007). Autophagosome formation: core machinery and adaptations. *Nat Cell Biol* **9**: 1102–1109.
- Yang C, Kaushal V, Shah S, Kaushal G. (2008). Autophagy is associated with apoptosis in cisplatin injury to renal tubular epithelial cells. *Am J Physiol Renal Physiol* **294**: F777–F787.
- Yue Z, Jin S, Yang C, Levine AJ, Heintz N. (2003). *Beclin 1*, an autophagy gene essential for early embryonic development, is a haploinsufficient tumor suppressor. *Proc Natl Acad Sci* **100**: 15077–15082.
- Zeng X, Overmeyer J, Maltese W. (2006). Functional specificity of the mammalian Beclin-Vps34 PI 3-kinase complex in macroautophagy versus endocytosis and lysosomal enzyme trafficking. *J Cell Sci* **119**: 259–270.
- Zhang H, Bosch-Marce M, Shimoda L, Tan Y, Baek J, Wesley J *et al.* (2008). Mitochondrial autophagy is an HIF-1-dependent adaptive metabolic response to hypoxia. *J Biol Chem* **283**: 10892–10903.