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REVIEW ARTICLE

Bcl-2 and Bcl-xL play important roles in the crosstalk between autophagy and apoptosis

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Autophagy and apoptosis play important roles in the development, cellular homeostasis and, especially, oncogenesis of mammals. They may be triggered by common upstream signals, resulting in combined autophagy and apoptosis. In other instances, they may be mutually exclusive. Recent studies have suggested possible molecular mechanisms for crosstalk between autophagy and apoptosis. Bcl-2 and Bcl-xL, the well-characterized apoptosis guards, appear to be important factors in autophagy, inhibiting Beclin 1-mediated autophagy by binding to Beclin 1. In addition, Beclin 1, Bcl-2 and Bcl-xL can cooperate with Atg5 or Ca^{2+} to regulate both autophagy and apoptosis. Thus, Bcl-2 and Bcl-xL represent a molecular link between autophagy and apoptosis. Here, we discuss the possible roles of Bcl-2 and Bcl-xL in apoptosis and autophagy, and the crosstalk between them.

Introduction

To become cancerous, a cell needs to overcome a number of failsafe mechanisms [1]. It must evade apoptotic and autophagic cell death to survive. Antiapoptotic Bcl-2 family proteins such as Bcl-2 and Bcl-xL are frequently overexpressed in cancers [2,3]. They inhibit apoptosis by binding to Bax or Bak. Bcl-2 and Bcl-xL are also well known for their anti-autophagy abilities [4]. Prolonged nutrient deprivation can invoke autophagy, an evolutionarily conserved process for bulk degradation of cytoplasmic components, including large molecules and organelles [5]. Autophagy is initially induced to prolong cell survival, but when taken to extremes, it causes cell death. Bcl-2 and Bcl-xL suppress autophagy by binding to the protein Beclin 1, which is required for the initiation of autophagasome formation in autophagy [6]. Thus, Bcl-2 and Bcl-xL can help cells to evade autophagic cell death. They can prolong the survival of growth factor-dependent cells when deprived of their obligate growth factors.

The mechanisms of apoptosis and autophagy are different, and involve fundamentally distinct sets of regulatory and executioner molecules [7–9]. The crosstalk between apoptosis and autophagy is therefore complex in nature, and sometimes contradictory, but surely critical to the overall fate of the cell [10]. In some cellular settings, autophagy can serve as a cell survival pathway to suppress apoptosis [11]. On the other hand, autophagy can lead to cell death, either in collaboration with apoptosis or as a back-up mechanism when apoptosis is defective [10]. Recent studies have revealed that autophagy may play an important role in the regulation of cancer development and

Abbreviations
AMPK, AMP-activated protein kinase; BH, Bcl-2 homology; CAMKK-β, calcium/calmodulin-dependent kinase kinase-β; ER, endoplasmic reticulum; CpG ODN, CpG oligodeoxynucleotide; Hsp, heat shock protein; JNK, c-Jun N-terminal kinase; MMP, mitochondrial membrane permeabilization; mTOR, mammalian target of rapamycin; PI3K, phosphoinositide 3-kinase.

progression. Whether autophagy represents a mechanism for resisting apoptosis or a mechanism for initiating a nonapoptotic form of programmed cell death remains unclear [12–14].

Recently, researchers have found that Bcl-2 and Bcl-xL cooperate with many other substances, such as Ca^{2+} and Atg5, to regulate both autophagy and apoptosis [15–18]. This review discusses current opinions on how Bcl-2 and Bcl-xL are involved in the molecular events. The crosstalk between the autophagy and apoptosis may redefine the roles of Bcl-2 and Bcl-xL in oncogenesis and tumor progression. It may be useful for future improvement of cancer treatment by modulating the two processes.

Bcl-2 and Bcl-xL in the apoptosis

Bcl-2 and Bcl-xL inhibit apoptosis

The Bcl-2 protein family was discovered by analysis of the t(14–18) chromosomal translocation breakpoint in B-cell follicular lymphoma [19], and it has grown to ~ 20 members. All Bcl-2 family proteins contain at least one of the four conserved α-helical motifs known as Bcl-2 homology (BH) domains (BH1–BH4) [20]. The family members are further classified into three groups. One group inhibits apoptosis and possesses all four BH domains, including Bcl-2, Bcl-xL, Bcl-w, Mcl-1, Bcl-B and A1. The proapoptotic proteins are divided into two distinct groups: the multidomain proteins, containing three BH domains (Bax, Bak and Bok); and the BH3-only proteins (Bad, Bid, Bim, Bmf, Bik, Hrk, Noxa and Puma) [21], which have a conserved BH3 domain that can bind to the antiapoptotic Bcl-2 proteins to promote apoptosis (Table 1; Fig. 1).

The molecular surface of the multidomain antiapoptotic Bcl-2/Bcl-xL proteins possesses a hydrophobic cleft, the BH3-binding groove, formed by apposition of the BH1, BH2 and BH3 domains, which can accommodate BH3 domains from proapoptotic Bcl-2 protein family members, hence activating BH123 proteins and/or neutralizing BH1234 proteins [22]. In response to apoptotic stimuli, Bax/Bak translocates to the mitochondrial membrane, facilitating the release of cytochrome *c* from the mitochondrial intermembrane space into the cytosol [23–25]. Bcl-xL and Mcl-1, but not Bcl-2, have been shown to target Bak, whereas all of the antiapoptotic members interact with Bax to inhibit apoptosis [26–29].

The antiapoptotic function of Bcl-2 in immune cells is significantly dependent on its association with heat shock protein (Hsp)90β. Under CpG oligodeoxynucleotide (CpG ODN) treatment, dissociation of these two proteins inhibits the antiapoptotic activity of Bcl-2 by initiating the release of cytochrome *c* from mitochondria into the cytosol and increasing the activities of caspase 3 and caspase 7, resulting in apoptosis of mouse RAW264.7 macrophages [30]. Other studies found that Hsp90β, but not Hsp90α, was associated with Bcl-2 during apoptosis in rat basophilic leukemia (RBL-2H3) cells and bone marrow-derived mast cells from C57BL/6 mice, induced by CpG-B ODN. Inhibition of Hsp90β suppressed the CpG-B ODN-induced association of Hsp90β with Bcl-2, and impaired the inhibitory effect on the release of cytochrome *c* as well as the activation of caspase 3 [31]. These studies thus reveal that without Hsp90β, but not without Hsp90α, the antiapoptotic ability of Bcl-2 is lost in immune cells.

Table 1. Bcl-2 family members.

Bcl-2 family member	Whole name of the member
Prosurvival family members that contain four BH domains	
Bcl-2	B-cell lymphoma-2
Bcl-xL	BCL-2-like protein
Bcl-W	BCL-2-like-2
Mcl-1	Myeloid cell leukemia sequence-1
Bcl-B	BCL-2-like-10
A1	BCL-2-related protein A1
Proapoptotic family members that contain three BH domains	
Bax	BCL-2-associated X protein
Bak	BCL-2-antagonist/killer-1
Bok	BCL2-related ovarian killer
Proapoptotic BH3-only proteins	
Bad	BCL-2 antagonist of cell death
Bid	BH3-interacting domain death agonist
Bim	BCL-2-like-11
Bmf	BCL-2-modifying factor
Bik	BCL-2-interacting killer
Hrk	Harakiri (also known as death protein-5)
Noxa	Phorbol-12-myristate-13-acetate-induced protein 1
Puma	BCL-2-binding component-3

Bcl-2 and Bcl-xL in apoptosis induction

Bcl-2 is best known for preventing apoptosis; however, it could induce apoptosis [32]. One mechanism for

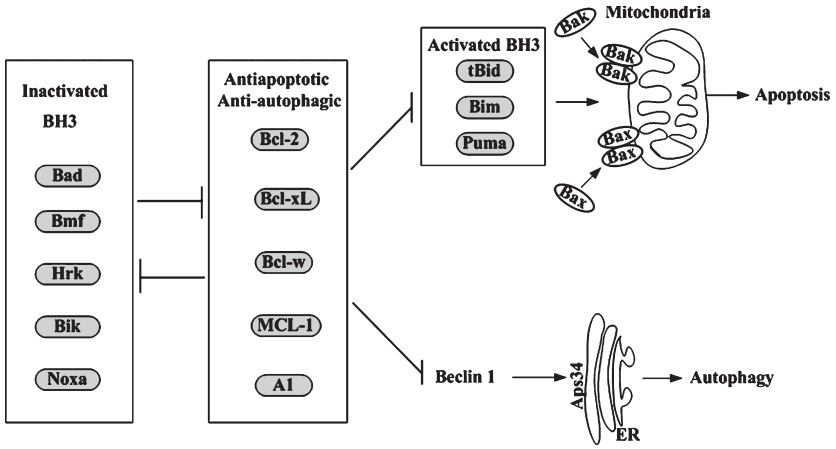

Fig. 1. Regulation of Bcl-2 family members between apoptosis and autophagy. Depending on their specificity and preferential subcellular localization, BH3-only proteins can activate apoptosis or autophagy.

conversion of Bcl-2 from a protector to a killer was revealed in 1997 by Cheng *et al.*, who showed that the loop domain of Bcl-2 is cleaved at Asp34 by caspase 3 in cells overexpressing caspase 3 and subjected to Fas ligation and interleukin-3 withdrawal. The C-terminal Bcl-2 cleavage product triggered cell death and accelerated Sindbis virus-induced apoptosis, which was dependent on the BH3 and transmembrane domains of Bcl-2 [33]. Lin *et al.* [34] discovered another mechanism for conversion of Bcl-2 into a killer in HEK293T cells and human peripheral blood lymphocytes, through the N-terminal loop region interaction with orphan nuclear receptor Nur77/TR3 on the mitochondria to induce the conformational change in Bcl-2. Later, Bivona *et al.* [35] revealed a similar mechanism for Bcl-xL, showing that protein kinase C regulation of K-Ras can promote its association with Bcl-xL on mitochondria and induce apoptosis. Thus, depending on the proteins that interact with Bcl-2 and Bcl-xL, their function can be converted from antiapoptotic to proapoptotic. Recent work by Schwartz *et al.* [36] showed superior cytotoxic activity in Bcl-2/Bcl-xL-overexpressing cells than in control cells, using either murine TAMH hepatocyte cells or rat INS-1 cells, treated with 2-methoxyantimycin A, providing a potential explanation for why high levels of Bcl-2 expression are sometimes associated with better patient prognosis [37].

Bcl-2, Bcl-xL and autophagy

Briefly, the initial step of autophagy is regulated by class I and class III phosphoinositide 3-kinases (PI3Ks). The PI3Ks generate lipid 'second messengers' that mediate signal transduction, and have been divided into four classes, referred to as I_A, I_B, II and III, in view of their structural characteristics and substrate specificity (Fig. 2).

Activation of class I PI3K inhibits autophagy through activation of protein kinase B (Akt) and mammalian target of rapamycin (mTOR). In contrast, activation of class III PI3K in a complex with the autophagy-associated protein Beclin 1 promotes autophagy [38]. These two pathways play an important role upstream of autophagy and are induced by growth factor withdrawal and stress situations, including hypoxia and oxidative stress [39–41]. Recent studies have indicated that activation of Beclin 1 and inhibition of the Akt–mTOR pathway have consistently been associated with induction of autophagy in cancer cells [42,43].

Bcl-2 and Bcl-xL inhibit Beclin 1-dependent autophagy

Bcl-2, by interacting with the evolutionarily conserved autophagy protein Beclin 1, inhibits Beclin 1-dependent autophagy in yeast and mammalian cells [4].

Beclin 1, the mammalian ortholog of yeast Atg6/Vps30, was originally discovered in a yeast two-hybrid screen as a Bcl-2-interacting protein, and was the first human protein shown to be indispensable for autophagy [44]. The interaction between Beclin 1 and its binding partners regulates the initial steps of autophagy. Beclin 1 also possesses a so-called BH3 domain (amino acids 114–123) that mediates the interaction with Bcl-2 and other close Bcl-2 homologs, such as Bcl-xL and Mcl-1 [45]. Mutation of the BH3 domain of Beclin 1 or of the BH3 receptor domain of Bcl-2/Bcl-xL abolishes their capacity to inhibit Beclin 1-dependent autophagy [46].

Class III PI3Ks, such as hVps34, are significant regulators in the initial steps of autophagy [47]. In mammals, hVps34 activated by Beclin 1 and depended on

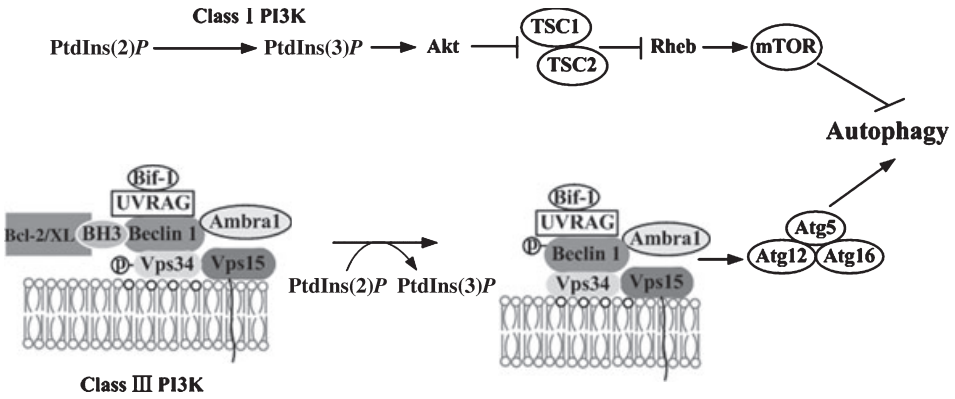

Fig. 2. Model of class I PI3K and class III PI3K in autophagy regulation. Class I PI3K activates the Akt–mTOR signaling pathway to inhibit autophagy. Class III PI3Ks liberate Beclin 1 to induce autophagy. Proteins that contain BH3 domains or small molecules that mimic BH3 domains can bind to the BH3 receptor domain of Bcl-2 or Bcl-xl, to disrupt the interaction between Bcl-2 or Bcl-xl and Beclin 1. In addition, Bcl-2/Bcl-xL phosphorylation results in Bcl-2/Bcl-xL dissociation from Beclin 1. This probably leads to activation of VPS34, thereby provoking the production of phosphatidylinositol 3-phosphate [PtdIns(3)*P*]. PtdIns(2)*P*, phosphatidylinositol 3-phosphate.

the UVRAG, Ambra-1 and Bif-1 (also called endophilin B1) participation in autophagy [46,48]. Beclin 1 can be present in two different complexes, one that stimulates autophagy and involves an interaction with hVps34, and another that inhibits autophagy and involves an interaction with Bcl-2 and Bcl-xL. Accordingly, overexpression of Bcl-2 and Bcl-xL disrupts the hVps34–Beclin 1 interaction, suggesting that Bcl-2/Bcl-xL inhibit autophagy by sequestering Beclin 1 away from hVps34 [4]. Beclin 1 forms a dimer in solution via its coiled-coil domain both *in vivo* and *in vitro* [49]. Viral Bcl-2 binds independently to two sites on the Beclin 1 dimer, one with high affinity and one with lower affinity, whereas human Bcl-xL binds both sites equally, with relatively low affinity. Both Bcl-2-like proteins reduce the affinity of UVRAG for Beclin 1, suggesting that they stabilize the Beclin 1 dimer [49]. Thus, Bcl-2 and Bcl-xL inhibit autophagy in two different ways: (a) by sequestering Beclin 1 away from hVps34; and (b) by reducing the affinity of UVRAG for Beclin 1 and stabilizing the Beclin 1 dimer (Fig. 2).

Rapid induction of autophagy regardless of Bcl-2 and Bcl-xL expression

Autophagy can provide nutrients to support essential basal metabolism in growth factor-withdrawn cells, but antiapoptotic Bcl-2 family proteins can suppress autophagy in some settings. However, Altman *et al.* [50] showed that autophagy was rapidly induced in hematopoietic cells upon growth factor withdrawal, regardless of Bcl-2 or Bcl-xL expression. In particular, they observed regulation of BH3-only Bim in a chop-dependent manner in cells after growth factor withdrawal might have sufficiently disrupted the Bcl-2/Bcl-xL–Beclin-1 interaction to allow for autophagy induction [50]. Similar to those results, autophagy induction has been observed in the presence of overexpressed Bcl-2 or Bcl-xL after ischemia [51] or DNA damage in tumor cells [52].

Bcl-2-mediated autophagy through both Beclin 1 and Akt–mTOR signaling

It has been reported that H_2O_2 induces autophagy through PI3K–Beclin 1 activation and PI3K–Akt–mTOR inhibition in human U251 glioma cells. Overexpression of cellular Bcl-2 partially inhibited autophagy through both the Beclin 1 and the Akt–mTOR pathways [53].

As described above, being part of the class III PI3K complex, Beclin 1 participates in autophagosome formation and is important in mediating the localization of other autophagic proteins to pre-autophagosomal membranes [54]. Bcl-2 interacts with Beclin 1 and downregulates Beclin 1-dependent autophagy by inhibiting the formation of the Beclin 1–hVps34 PI3K complex and Beclin 1-associated class III PI3K activity.

Beyond the Beclin 1–Bcl-2 complex, Bcl-2 is also a regulator of PI3K–Akt signaling [55]. Bcl-2 can be a strict mediator downstream of PI3K–Akt signaling, positively regulating the mTOR signaling pathway, which can inhibit cell autophagic activity [56].

Subcellular localization of the Bcl-2 family

Bcl-2 family proteins were found to have diverse subcellular locations, to respond to various intrinsic and

extrinsic stimuli. BH3-only proteins are primarily localized in the cytosol, whereas other Bcl-2 family members are anchored to intracellular membranes [57]. Bcl-2 and Bcl-xL are localized to the membrane surface of mitochondria, the endoplasmic reticulum (ER) and the nucleus by a hydrophobic C-terminal membrane-spanning domain [58–60]. In contrast, inactive Bax is a cytosolic monomeric protein, because its C-terminal anchor domain is internalized within a hydrophobic pocket formed by the BH1–3 domains [61]. Following an apoptotic stimulus, Bax changes conformation, leading to the exposure of the C-terminal tail and the translocation of active Bax to the mitochondrial membrane [21].

The principal site of action of apoptosis regulation by Bcl-2 family proteins is probably the mitochondrial membrane. Antiapoptotic multidomain proteins (Bcl-2, Bcl-xL, Bcl-w, and Mcl-1) mainly reside in mitochondria, protecting against mitochondrial membrane permeabilization (MMP), one of the rate-limiting events of apoptosis induction [62]. However, recent work has revealed that certain members of the Bcl-2 family are present on the ER, where they seem to have more extensive functions. It has also been found that the anti-autophagic function of Bcl-2/Bcl-xl is dissociated from the mitochondrial location. Whereas the autophagy-inhibitory effects of Bcl-2 or Bcl-xl depend on their subcellular localization, only ER-localized (but not mitochondrial) Bcl-2 or Bcl-xl inhibits autophagy [4].

Regulation of crosstalk between autophagy and apoptosis by Bcl-2 and Bcl-xL

Many signaling pathways involved in the regulation of autophagy also regulate apoptosis. The molecular regulators of both pathways are interconnected; numerous death stimuli are capable of activating either pathway, and the pathways share several genes that are critical for their respective functions [63,64].

The interplay between Atg5 and Bcl-2/Bcl-xL in apoptosis and autophagy

Atg5 is a critical protein required for autophagy at the stage of the synthesis of autophagosome precursor, an important mediator of apoptosis. Atg5 can be cleaved following death stimuli, and appears to promote mitochondria-mediated apoptosis. It cooperates with Bcl-2 and Bcl-xL to regulate both apoptosis and autophagy [15,17].

During autophagy regulation, the Atg12–Atg5 conjugate localizes to autophagosome precursors and dissociates just before or after completion of autophagic vacuole formation. Its deletion in yeast or mammalian cells/mice effectively blocks autophagy [65,66].

Atg5 is also important during apoptosis regulation. The key finding of Yousefi *et al.* was the identification of a 24-kDa truncated form of Atg5 (comprising residues 1–193) that participates in apoptosis regulation, either in human neutrophils following withdrawal of granulocyte–macrophage colony-stimulating factor, or in Jurkat cells in response to antibody against CD95, a Fas ligand mimic. Their subsequent studies confirmed that Atg5 was cleaved by calpains 1 and 2 to form this 1–193 cleavage product. Intriguingly, truncated Atg5 translocated from the cytosol to mitochondria, to trigger cytochrome *c* release and caspase activation [17].

The 24-kDa Atg5 fragment, but not full-length Atg5, binds to Bcl-xL, displacing Bcl-xL–Bax complexes, to inactivate Bcl-xL antiapoptotic activity, thereby promoting Bax–Bax complex formation. Bcl-2 could block the cell death induced by this Atg5 fragment. The death-inducing activity of the truncated form of Atg5 (1–193) was also observed in the absence of autophagy. These results suggest that Atg5 may be an independent key player in both apoptosis and autophagy. It is possible that the low levels of Atg5 cleavage product may have significant effects on apoptosis, but not the intact Atg5 that participates in autophagy [17].

Regulation of Ca^{2+} signals by Bcl-2 as common mediators of both apoptosis and autophagy

Hoyer-Hansen *et al.* emphasized the important role of Ca^{2+} in formation of the autophagosome, and Ca^{2+} homeostasis and signaling were modulated by Bcl-2 in macro-autophagy [18].

In earlier work, they discovered that cytoplasmic Ca^{2+} elevation mediates autophagy in MCF-7 breast cancer cells treated with 1,25-dihydroxyvitamin D_3 (vitamin D) and its analog EB1089, or other agents that mobilize intracellular Ca^{2+} [67], were dependent upon Beclin 1. In their current work, a signaling cascade that mediated autophagy in response to elevated Ca^{2+} had been identified. The suggested cascade involves sequential activation of calcium/calmodulin-dependent kinase kinase-β (CAMKK-β) and AMP-activated protein kinase (AMPK), leading to autophagy through repression of mTOR [18].

The elevated Ca^{2+}-mediated autophagy occurs via a signaling pathway involving CaMKK-β, AMPK, and mTOR, and it has been shown that ER-located Bcl-2 effectively inhibits this pathway [18]. Bcl-2 inhibits autophagy by reducing the amount of agonist-induced

Ca^{2+} release from the ER to the cytosol, through increasing the Ca^{2+} permeability of the ER membrane [68–70]. There are two main mechanisms by which Bcl-2 and Bcl-xL could augment ER ionic homeostasis. One early proposal was direct release of ER Ca^{2+} through Bcl-2 and Bcl-xL 'ion channels', based on the discovery that the crystal structure of Bcl-xL bore similarities to the pore-forming domains of the bacterial toxins colicins and diphtheria toxin [21,71]. Moreover, Bcl-2 and Bcl-xL were shown to be capable of forming ion-conductive channels in synthetic lipid membranes [72–74]. Consistent with this view that Bcl-2 functions as an ion channel or a modulator of an ion channel, Bcl-2 reduced the steady-state ER [Ca^{2+}] in MCF-7 cells [18].

Ca^{2+} is a major intracellular second messenger in mediating apoptosis [75]; but when Ca^{2+} is induced, how do the cells decide whether to undergo apoptosis, autophagy, or both? The Jaattela group reported that vitamin D compounds induced both autophagy and apoptosis in MCF-7 cells [67], but apoptosis was not evident in their study, even though the stimulus is well known to induce apoptosis. In addition, when apoptosis is blocked in cancer cells, autophagy can also take over [51]. Future studies will be required to understand the balance between apoptosis and autophagy, and the regulatory mechnisms of the common regulatory factors.

Dual role of c-Jun N-terminal kinase (JNK)1-mediated phosphorylation of Bcl-2 in autophagy and apoptosis regulation

In recent study, Wei *et al*. [76] found that, upon nutrient withdrawal, JNK1 was activated and induced phosphorylation at multiple residues (Thr69, Ser70, and Ser87) in the nonstructured loop of Bcl-2, located between the BH4 and BH3 domains. Autophagy and apoptosis are fundamental cellular pathways, and are both regulated by JNK-mediated Bcl-2 phosphorylation [77]. Wei *et al*. found that, during nutrient starvation in HeLa cells, rapid Bcl-2 phosphorylation could occur initially to promote cell survival by disrupting the Bcl-2–Beclin 1 complex, inducing autophagy (4 h). After 16 h, when autophagy was no longer able to keep the cell alive, Bcl-2 phosphorylation could then turn to disrupt the Bcl-2–Bax complex, and to active caspase 3 dependent pathway [78]. This model can be used to understand the interrelationship between autophagy and apoptosis regulation by JNK1-mediated Bcl-2 phosphorylation [78]. Thus, Bcl-2 phosphorylation may not only be a mechanism for regulating autophagy and a mechanism for regulating apoptosis, but, perhaps, also a mechanism for regulating the switch between the two pathways.

Regulation of apoptosis and autophagy by the BH3 domain and its mimetic ABT-737

BH3-only proteins can either promote autophagy or abolish the antiapoptotic ability of Bcl-2/Bcl-xL. ABT-737, a small-molecule BH3 domain mimetic that functions as a Bcl-2/Bcl-xL inhibitor, has been shown to bind with high affinity to Bcl-2 and Bcl-xL (Fig. 3). It can either free Beclin 1 to trigger autophagy, or free Bax or Bak to trigger MMP and caspase-3 activation and, subsequently, cell apoptosis [79,80].

The fact that Beclin 1 binds to Bcl-2 and Bcl-xL through a BH3–BH3 receptor interaction has important functional consequences. BH3-only proteins stimulate autophagy by competitively disrupting the interaction between Beclin 1 and Bcl-2/Bcl-xL, hence liberating Beclin 1 from its inhibition [45]. The pharmacological BH3 mimetic ABT-737 acts in the same way to induce autophagy. Overexpression of Bad stimulates the autophagy-associated formation of punctuate green fluorescent protein–LC3, and this effect is lost when the BH3 domain of Bad is disrupted [81]. Taken together, these findings show that BH3-only proteins (or BH3 mimetics) could trigger autophagy by competitively interacting with Bcl-2/Bcl-xL to free Beclin 1 in the ER but not in mitochondria.

BH3-only proteins can exert their proapoptotic action by at least two different mechanisms. Some BH3-only proteins (prototypes: Bad and Noxa) preferentially interact with antiapoptotic Bcl-2 proteins (Bad with Bcl-2 and Bcl-xL; Noxa with Mcl-1) to free Bax/Bak-like proteins, which in turn mediate MMP. Others (prototype: t-Bid) may directly activate Bax/Bak-like proteins to initiate MMP [79,82]. The fact that Beclin 1 possesses a BH3 domain is counterintuitive, because the so-called BH3-only proteins are generally known to be proapoptotic. However, overexpression of

Fig. 3. Hypothetical mechanism of ABT-737-stimulated autophagy. ABT-737 disrupts the interaction between Beclin 1 and Bcl-2/Bcl-xL, liberating Beclin 1 from an inhibitory complex.

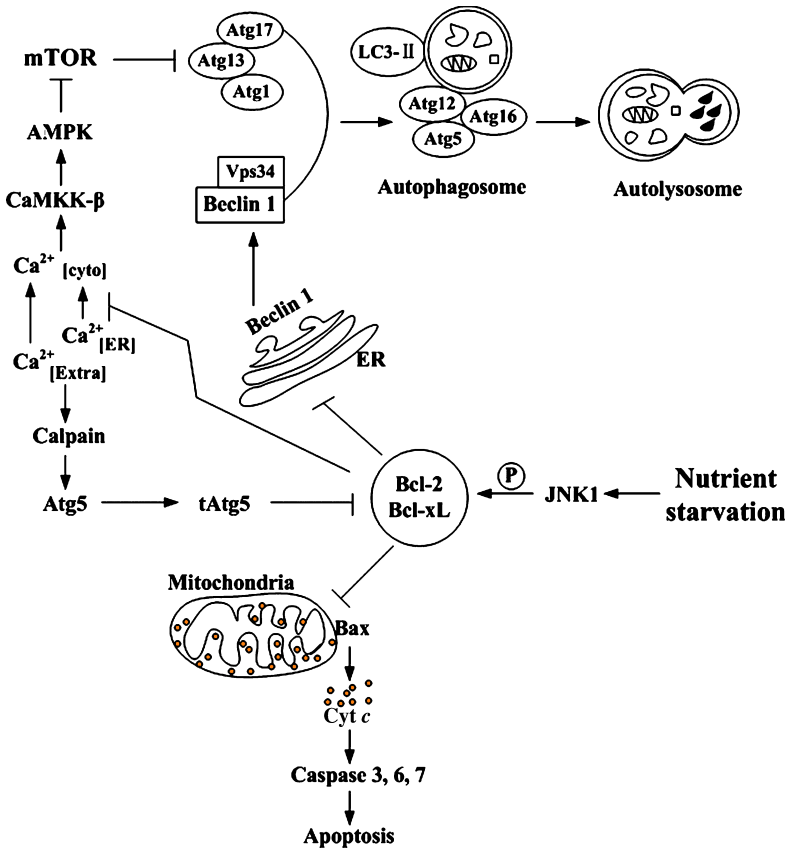

Fig. 4. Regulation between autophagy and apoptosis. Induction of autophagy requires the activity of Beclin 1 and its interacting partner, a class III PI3K, also known as hVps34. By contrast, autophagy is negatively regulated by a class I PI3K through mTOR. The elongation and shape of autophagosomes are controlled by two protein (and lipid) conjugation systems, namely the Atg 12 pathway and the microtubule-associated protein 1 light chain 3 (LC3)–phosphatidylethanolamine pathway. Bcl-2/Bcl-xL can bind to Beclin 1 and inhibit autophagy. Atg5 is cleaved by calpains 1 and 2 to form a 1–193 cleavage product. Truncated Atg5 is translocated from the cytosol to the mitochondria, is associated with Bcl-xL, and triggers cytochrome *c* release and caspase activation. Ca^{2+}-induced autophagy occurs via a signaling pathway involving CaMKK-β, AMPK, and mTOR. Bcl-2 inhibits autophagy by repressing Ca^{2+} signals. JNK1, but not JNK2, mediates stress-induced Bcl-2/Bcl-xL phosphorylation, Bcl-2/Bcl-xL dissociation from Beclin 1, and autophagy activation. BH3-only proteins (or BH3 mimetics) would trigger autophagy by liberating Beclin 1 from its inhibition by Bcl-2/Bcl-xL, presumably at the level of the ER. BH3-only proteins (or BH3 mimetics) preferentially interact with Bcl-2/Bcl-xL, dissociating them from Bax/Bak-like proteins, presumably at the level of the mitochondria.

Beclin 1 clearly does not cause apoptosis [83]. This contrasts with the apoptosis-inducing potential of a Beclin 1-derived peptide that contains the BH3 domain. At the same time, other studies made the intriguing finding that Bcl-2, as it interacted with Beclin 1, did not lose its antiapoptotic potential [84].

These findings may have far-reaching implications for understanding the crosstalk between apoptosis and autophagy. Unlike the cell death pathway of apoptosis, autophagy is a complex cellular process with a dual role. It may serve as a mechanism for adaptation to stress, in special circumstances such as a route to cell death [85,86]. How BH3-only proteins switch between autophagy and apoptosis is very uncertain. We can understand the interrelationship between them by the mitochondria, which may function as a switch between apoptosis and autophagy. MMP triggered in response to low-intensity stress leads to the induction of autophagy, which selectively removes damaged mitochondria as a cytoprotective mechanism [87]. BH3-only proteins can stimulate mitochondrial autophagy by competitively disrupting the interaction between Beclin 1 and Bcl-2/Bcl-xL. With increasing stress or at a certain point, proapoptotic factors are released from mitochondria and promote apoptosis through BH3-only proteins interacting with antiapoptotic Bcl-2 proteins and dissociating them from Bax/Bak-like proteins, which in turn mediate MMP.

Conclusion

Although much research has focused on Bcl-2 and Bcl-xL, they have numerous unclarified interaction partners that regulate their activities and link them to a wide variety of cellular pathways. Bcl-2 and Bcl-xL operate as critical nodes in complex networks to integrate information and make ultimate life/death decisions. At the molecular level, the crosstalk between apoptosis and autophagy is manifested by the numerous genes that are shared by both pathways (Fig. 4). Nonetheless, it remains an ongoing conundrum how the cells 'decide' to respond to similar stimuli by preferentially undergoing autophagy or apoptosis. In-depth studies on the interplay between autophagy and apoptosis are necessary and likely to have important implications for the understanding of both processes in development, normal physiology, and disease.

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