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ORIGINAL PAPER

Autophagy and apoptosis: where do they meet?

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Abstract Autophagy and apoptosis are two important cellular processes with complex and intersecting protein networks; as such, they have been the subjects of intense investigation. Recent advances have elucidated the key players and their molecular circuitry. For instance, the discovery of Beclin-1's interacting partners has resulted in the identification of Bcl-2 as a central regulator of autophagy and apoptosis, which functions by interacting with both Beclin-1 and Bax/Bak respectively. When localized to the endoplasmic reticulum and mitochondria, Bcl-2 inhibits autophagy. Cellular stress causes the displacement of Bcl-2 from Beclin-1 and Bax, thereby triggering autophagy and apoptosis, respectively. The induction of autophagy or apoptosis results in disruption of complexes by BH3-only proteins and through post-translational modification. The mechanisms linking autophagy and apoptosis are not fully defined; however, recent discoveries have revealed that several apoptotic proteins (e.g., PUMA, Noxa, Nix, Bax, XIAP, and Bim) modulate autophagy. Moreover, autophagic proteins that control nucleation and elongation regulate intrinsic apoptosis through calpain- and caspase-mediated cleavage of autophagy-related proteins, which switches the cellular program from autophagy to apoptosis. Similarly, several autophagic proteins are implicated in extrinsic apoptosis. This highlights a dual cellular role for autophagy. On one hand, autophagy degrades damaged mitochondria and caspases, and on the other hand, it provides a membrane-based intracellular platform for caspase processing in the regulation of apoptosis. In this review, we highlight the crucial factors governing the crosstalk between autophagy and apoptosis and describe the mechanisms controlling cell survival and cell death.

Keywords Autophagy · Apoptosis · Crosstalk · Bcl-2 · Beclin-1 · BH3-only proteins

Introduction

Autophagy and apoptosis play major roles in determining cellular fate. Accordingly, they participate in development, cellular homeostasis, and both physiological as well as pathological processes. Apoptosis and autophagy are discrete cellular processes that are mediated by distinct groups of regulatory and executioner molecules [1, 2]. Apoptosis is type I form of programmed cell death that is executed by activated caspases, which are specific enzymes that participate in signalling cascades that culminate in the rapid removal of organelles and other cellular structures [3, 4]. Autophagy is a highly conserved cytoprotective process whereby cytoplasmic contents are sequestered, transported via double-membrane autophagosomes to lysosomes, and degraded. This process allows cells to mitigate various types of cellular stress. There is a basal level of autophagy, which allows for the physiological turnover of damaged organelles, long-lived proteins, and cytoplasmic contents. Autophagy has been characterized as both a unique cell-death pathway and an adaptation to stress that promotes cell survival. Autophagy ensures the delivery of metabolic substrates to cells in order to fulfill their energy demand during stress, thus supporting cell growth and survival [5–7].

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The crosstalk between autophagy and apoptosis is complex, and studies have yielded conflicting results. Nevertheless, this crosstalk is critical to cellular fate. Under certain cellular conditions, autophagy can promote cell survival and avert apoptosis [7]. Under other conditions, autophagy may culminate in cell death either in concert with apoptosis or independently in the event of apoptotic failure. It remains uncertain whether autophagy represents a mechanism for preventing apoptosis or for enacting non-apoptotic programmed cell death.

Apoptosis: the cell-death machinery

The apoptotic-signalling cascade is divided into two major pathways, extrinsic and intrinsic, which gets triggered by soluble molecules that bind to plasma-membrane receptors or by various mitochondrial stimuli, respectively.

The extrinsic apoptotic pathway is activated by death receptors (DR), which are cell-surface receptors that bind specific ligands and transmit apoptotic signals. Such ligands include soluble molecules of the tumor necrosis factor (TNF) family, which are secreted as homotrimers and bind to members of the TNF-receptor (TNF-R) family, including TNFR-1, Fas/CD95, and TRAIL receptors DR-4 and DR-5. Ligand binding causes receptor trimerization and subsequent activation [8, 9]. TNF-Rs possess a death domain (DD), which recruits other DD-containing proteins, such as TNF-R type 1-associated death domain protein (TRADD) and Fas-associated protein with death domain (FADD). These proteins bind to initiator caspases-8 and -10, thus enabling homodimerization and subsequent activation of death-inducing signalling complex (DISC) [10–12]. Following the activation of caspases-8 and -10, the effector caspases-3, -6, and -7 are cleaved, leading to cellular degradation in the final stage of apoptosis [13].

The intrinsic apoptotic pathway is initiated by various intracellular stimuli, including oxidative stress, DNA damage, hypoxia, and growth-factor deprivation, which induce outer mitochondrial membrane permeabilization [14]. Mitochondrial integrity can be controlled by various members of the Bcl-2 superfamily, of which there are two subcategories: pro-apoptotic and anti-apoptotic. Bax, Bid, Bak, Bad, Noxa, and PUMA are pro-apoptotic family members, while Bcl-xL, Bcl-2, Mcl-1, and A1 are anti-apoptotic family members [15]. During apoptosis, pro-apoptotic Bax and Bak undergo dimerization and insert into the outer mitochondrial membrane, triggering the intrinsic apoptotic pathway. After mitochondrial permeabilization, cytochrome c is released into the cytosol, where it binds to apoptotic protein activating factor-1 (Apaf-1), which initiates the formation of the apoptosome. The apoptosome is a multi-protein platform comprising a seven-spoked wheel-shaped complex and is essential for the recruitment and subsequent activation of caspase-9 [16]. Once activated, caspase-9 activates caspase-3, thereby promoting the execution of apoptosis. Caspases are controlled by specific cellular inhibitors called inhibitor of apoptotic proteins (IAP), which bind to and inhibit caspases. To escape IAP's inhibitory control, Smac/DIABLO and HtrA2/Omi are released from the mitochondria and bind to or cleave IAPs, respectively [14, 17].

Autophagy: the self-degradative mechanism

Autophagy is a catabolically driven process whereby stressed cells form cytoplasmic, double-layered, crescent-shaped membranes known as phagophores, which mature into complete autophagosomes. The autophagosomes engulf long-lived proteins and damaged cytoplasmic organelles to provide cellular energy and building blocks for biosynthesis. A major advancement in our understanding of the molecules regulating autophagy came from genetic analysis in yeast, which identified 35 *Atg* (*Au*tophag*y*-related) genes [18]. In the first step of phagophore nucleation, cellular stress inactivates a stress sensor, mammalian target of rapamycin (mTOR), resulting in hypophosphorylation of Atg13, which then binds to Atg1 (mammalian homologue of Ulk1) with the help of Atg17. Subsequently, Atg1 actively recruits Atg9, which extracts lipids from various sources, including endosomes, Golgi bodies, the nucleus, and endoplasmic reticulum (ER). After nucleation, the nascent phagophore is elongated and matured through the action of class III phosphoinositide (PI) 3-kinases, such as vesicular protein sorting 34 (Vps34), which interacts with Beclin-1 (mammalian homologue of yeast Atg6) and harvests phosphatidylinositol-3-phosphate (PI3P) [19]. PI3P acts as a vital localization stimulus that facilitates fusion during the final step of double-membrane autophagosome synthesis. The interaction between Vps34 and Beclin-1 is promoted by activating molecule in Beclin1-regulated autophagy protein-1 (Ambra-1), ultraviolet radiation resistance-associated gene (UVRAG), and Bax interacting factor-1 (Bif-1). In contrast, Bcl-2, Bcl-xL, and Run domain Beclin-1 interacting cysteine-rich containing protein (Rubicon) inhibit their interaction [20].

Two ubiquitin-like conjugation systems are associated with autophagosome formation and contribute to vesicle elongation. In a cascade process, Atg12 binds to Atg7 (E1 ubiquitin-like activating enzyme) in an ATP-dependent manner; afterwards, Atg12 non-covalently binds to Atg10 (E2-like ubiquitin carrier), which links Atg12 to Atg5; next, Atg16 dimers conjugate with this complex and aid the formation of the expanding phagophore. The Atg5-Atg12-Atg16 complex helps to develop the membranes at the

growing edges of the phagophore. This trimeric assembly disassociates when the phagophore develops into the autophagosome [21]. The ubiquitin-like system then processes microtubule-associated light chain 3 (LC3) (mammalian homologue of Atg8). Next, a cysteine proteinase, Atg4 (also referred as autophagin), cleaves LC3 to give rise to LC3I and conjugates E1-like Atg7 through an ATP-dependent mechanism, thereby stimulating LC3I. Thereafter, E2-like carrier Atg3 associates with activated LC3I, culminating in lipidation, which gives rise to LC3I-phosphatidylethanolamine (PE)-conjugate or LC3II [22].

The growing phagophore membrane interacts selectively with protein aggregates and organelles. Interestingly, LC3-II plays an important role as a receptor at the phagophore membrane and interacts with adaptor moieties present on protein aggregates and damaged mitochondria, thereby promoting their selective uptake and degradation. The best-identified molecule in this process is the multi-adaptor molecule p62/SQSTM1, which is associated with ubiquitinated proteins and interacts with Atg8/LC3 on the phagophore for engulfment [5, 6]. Similarly, in yeast, Atg32 is a protein that enables the selective uptake of mitochondria, a process known as mitophagy [23]. Mature autophagosomes are anchored to lysosomes and undergo membrane fusion, producing a hybrid structure known as the autolysosome, where the acidic lysosomal compartment degrades cargo and supplies energy to cells to counteract stressors [6, 24]. Initially, the autophagic pathway functions as an adaptive response to stress. However, in the face of extreme or protracted stress, cells are committed to undergo autophagic cell death [5, 6].

Connection between autophagy and apoptosis

Bcl-2: an apoptotic and autophagic protein

The Bcl-2 protein was discovered by analysis of a chromosomal translocation in B cell follicular lymphoma, which led to the subsequent identification of other Bcl-2 family proteins [25]. All proteins in the Bcl-2 family contain at least one of four conserved α-helical motifs, which are known as Bcl-2 homology (BH) domains (BH1–BH4). Based on their apoptotic properties, the family members are classified into three groups. One group inhibits apoptosis and contains all four BH domains; these proteins include Bcl-2, Bcl-xL, Bcl-w, Bcl-B, Mcl-1, and A1. There are two classes of pro-apoptotic proteins: the multi-domain proteins (Bax, Bak, and Bok), which contain three BH domains, and the BH3-only proteins (Bad, Bid, Bim, Bmf, Bik, Hrk, Noxa, and PUMA), which contain a conserved BH3 domain and interact with the anti-apoptotic Bcl-2 protein family to promote apoptosis [15, 26, 27, 80]. The anti-apoptotic proteins Bcl-2 and Bcl-xL possess a hydrophobic BH3-binding pocket formed by the BH1, BH2, and BH3 domains. The BH3-binding pocket accommodates the BH3 domains of pro-apoptotic members of the Bcl-2 protein family, activating BH123 proteins and/or neutralizing BH1234 proteins [28, 29]. In a yeast two-hybrid system, Beclin-1 was discovered as a binding partner of Bcl-2. Accordingly, the Bcl-2-Beclin-1 complex may regulate the crosstalk between apoptotic and autophagic signalling pathways [30]. Subsequently, Beclin-1's BH3 domain interacts with Bcl-2, Bcl-xL, and Mcl-1 but not with the pro-apoptotic BH3-only domain of the Bcl-2 family members, which inhibits Beclin-1-dependent autophagy. Although Beclin-1 contains the same BH3 domain as pro-apoptotic Bcl-2 family members, it does not contain a hydrophobic amino acid at position 119 and instead contains a polar Thr. This lowers Beclin-1's affinity for Bcl-2 as compared with other BH3-containing proteins [31, 32].

The anti-apoptotic proteins Bcl-2 and Bcl-xL are anchored through their C-termini to membrane surfaces of the mitochondria, ER, and nucleus; in contrast, other Bcl-2 family proteins are restricted to the cytoplasm [3, 28]. Bcl-2 proteins primarily regulate apoptosis at the mitochondrial membrane, where they sequester Bax, thus preventing mitochondrial membrane permeability and cytochrome c release. Likewise, Bcl-2 localized to the ER prevents Beclin-1-mediated starvation-regulated autophagy by binding to nutrient-deprivation autophagy factor-1 (NAF-1), which stabilizes the interaction between Bcl-2 and Beclin-1 at the ER surface [33–35]. In cells deficient in NAF-1, the interaction between Bcl-2 and Beclin-1 is disrupted, and initiation of autophagy is impaired, suggesting that Bcl-2 family members inhibit Beclin-1-mediated autophagy [35]. In addition, mitochondrial Bcl-2 has been shown to indirectly inhibit autophagy by sequestering the Beclin-1-activating protein Ambra-1 from Beclin-1 [36].

Under conditions of stress, Bcl-2 must be displaced from Beclin-1 and Bax to induce autophagy and apoptosis, respectively (Fig. 1; Table 1). When autophagy is induced, the Bcl-2-Beclin-1 complex is disrupted by pro-apoptotic BH3-only proteins (described in next section). These proteins bind to the BH3-binding pocket of Bcl-2 or Bcl-xL and disrupt the interaction between Bcl-2/Bcl-xL and Beclin-1 to induce autophagy [33, 34]. Interestingly, the Bcl-2/Bcl-xL-Beclin-1 interaction does not affect Bcl-2's anti-apoptotic function. Although the detailed mechanism is not yet fully elucidated, it is thought that Bcl-2 has a relatively weak affinity for Beclin-1 as compared with its affinity for BH3-only proteins [32].

In addition to regulation by BH3-only proteins, the interaction of Beclin-1 and Bax with Bcl-2/Bcl-xL can be

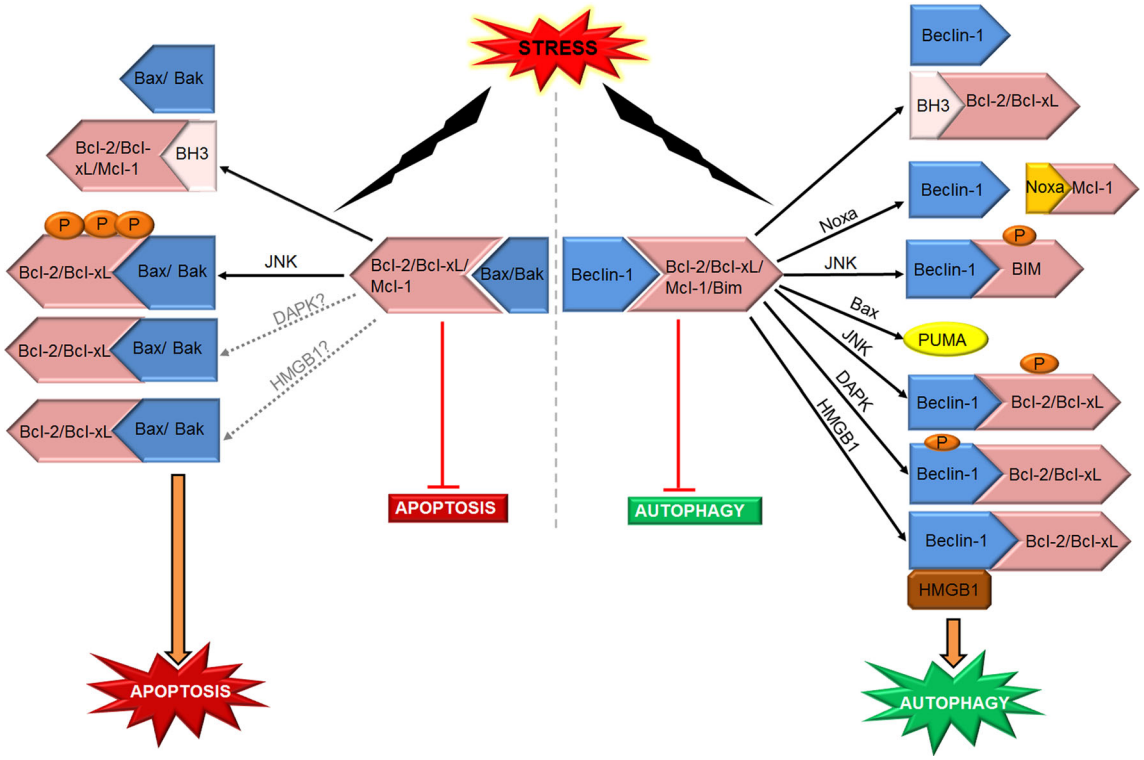

Fig. 1 Induction of autophagy and apoptosis through disruption of the Bcl-2/Bcl-xL-Beclin-1 and Bcl-2/Bcl-xL-Bax/Bak interaction, and post-translational modifications: When nutrients are sufficient, Beclin-1 and Bax/Bak binds with Bcl-2 or Bcl-xL preventing initiation of autophagy and apoptosis respectively. During stress conditions, several mechanisms mediate the disruption of this interaction to allow induction of autophagy and apoptosis. These mechanisms include competitive pro-apoptotic BH3-only protein interactions, DAPK-mediated phosphorylation of the BH3 domain of Beclin-1, JNK-mediated phosphorylation of the non-structured loop of Bcl-2, binding of the DAMP molecule HMGB-1 to Beclin-1. However detailed role of DAMP and HMGB-1 in apoptosis is not known

regulated by post-translational modification to stimulate both autophagy and apoptosis (Fig. 1). Conditions of stress (e.g., serum starvation) activate JNK1, which phosphorylates multiple residues (Ser87, Ser70, and Thr69) of Bcl-2's regulatory loop located between the BH3 and BH4 domains. As a result, Bcl-2 dissociates from Beclin-1, thus promoting autophagy [36]. Cells with mutations in these phosphorylation sites or a deficiency in JNK1 are defective in initiating starvation-induced autophagy. Moreover, expression of a constitutively active JNK1 prevents the initiation of autophagy even under conditions of sufficient nutrients in cells expressing wild-type Bcl-2 but not a non-phosphorylatable Bcl-2 mutant. Under conditions of long-term starvation, phosphorylated Bcl-2 binds to the pro-apoptotic protein Bax and inhibits apoptosis. In contrast, under conditions of extreme starvation, JNK1 induces hyper-phosphorylation of Bcl-2, causing its dissociation from Bax, which promotes apoptosis through caspase 3-dependent pathways. Thus, JNK1-mediated Bcl-2 phosphorylation represents an important link between autophagy and apoptosis [37]. In addition, following the initiation of autophagy, death-associated protein kinase (DAPK) phosphorylates Thr119 of Beclin-1, promoting its dissociation from Bcl-2 [38]. Accordingly, cells expressing Thr 119-phosphorylated Beclin-1 within the BH3 domain exhibit decreased Bcl-xL-Beclin-1 binding and enhanced autophagosome formation. Conversely, cells expressing a non-phosphorylatable Beclin-1 exhibit increased binding of Beclin-1 to Bcl-xL. DAPK has been implicated in apoptosis through inhibiting integrin and focal adhesion kinase [39]. Another stress-related signal that promotes the dissociation of Bcl-2/Bcl-xL and Beclin-1 is the cytosolic translocation of high mobility group box 1 (HMGB1), which is a member of the damage-associated molecular pattern (DAMP) protein family. Cytosolic translocation of HMGB-1 displaces Bcl-2 through direct interaction of the intramolecular disulphide bridge (C23/45) of HMGB1 with Beclin-1. Inhibition of HMGB-1's cytosolic translocation decreases starvation-induced autophagy [40, 41].

Table 1 Proteins with dual role in autophagy and apoptosis

Protein	Role in autophagy	Role in apoptosis
Autophagic proteins		
mTOR	Inactive form involves in initiation	mTOR regulates apoptosis
Beclin-1	Autophagosome nucleation	Cleaved C-fragment induces mitochondrial apoptosis
UVRAG	Upregulates Vps34–Beclin1 interaction	Antiapoptotic, inhibits Bax translocation from cytosol to mitochondria
AMBRA	Upregulates Vps34–Beclin1 interaction	Regulate mitochondrial apoptosis; cleaved by caspases and calpains
Atg3	Conjugates with Atg12	Regulates mitochondrial cell death
Atg5	Conjugates with Atg12, autophagosome elongation	Interacts with FADD to inhibit apoptosis, cleaved N-fragment induces mitochondrial apoptosis
Atg12	Autophagosome elongation	Stimulates mitochondrial apoptosis by inactivating Bcl-2 and Mcl-1
Atg4D	LC3 processing	Cleaved Atg4D localize to mitochondria and induces apoptosis
p62	Binds with LC3, promotes degradation of polyubiquitinated protein aggregates	Caspase-8 processing and activation
Apoptotic proteins		
Bcl-2, Bcl-xL	Interacts with Beclin-1 and inhibit autophagy	Antiapoptotic
Bad, Bak, BNIP3, Nix	Proautophagic, disrupting Beclin-1/Bcl-2 interaction	Proapoptotic
Bax, PUMA	Proautphagic, noncanonical type	Proapoptotic
p53	Inhibits by cytoplasmic p53	Proapoptotic
	Induces by nuclear p53 through DRAM	Proapoptotic
Noxa	Induces autophagy by disrupting Mcl-1/Beclin-1 interaction	Proapoptotic
Bim	Sequesters Beclin-1, inhibits autophagy	Proapoptotic
XIAP	Inhibits by Mdm2-p53 signalling	Inhibits caspase 3,7
cFLIP	Prevent interaction between Atg3 and LC3	Inhibits caspase 8

In contrast, oxidized HMGB-1 enhances the cytotoxicity of anti-cancer agents and induces apoptosis through the intrinsic pathway [42]. The mechanism by which Bcl-2 regulates HMGB-1-mediated apoptosis remains unknown.

Apoptosis-regulating proteins in the modulation of autophagy

Recently, several proteins known to regulate apoptosis have also been identified as inducers of autophagy (Fig. 1; Table 1). The first pro-apoptotic protein identified as an inducer of autophagy was the BH3-only protein Bad, which disrupts the interaction between Bcl-2 and Beclin-1 to induce autophagy [34, 43]. Several other pro-apoptotic proteins, including Bak, BNIP3, and Nix, have been identified as regulators of autophagy by disrupting the interaction between Bcl-2 and Beclin-1 [44–47]. In addition, during apoptosis, Bax promotes caspase-mediated cleavage of Beclin-1 at the D149 position, which prevents autophagy. Subsequently, both the C- and N-termini of Beclin-1 change conformation and cannot interact normally with Vps34, which is necessary for the induction of autophagy. Interestingly, cleavage of Beclin-1 by cellular caspases is critical to the inhibition of autophagy, as expression of non-cleavable Beclin-1 restores autophagy [44]. Through a different mechanism, Noxa induces autophagy by displacing Beclin-1 from Mcl-1, another Bcl-2 family member. Oncogenic H-RasV12 promotes autophagy through promoting the expression of the BH3-only proteins Noxa and Beclin-1 [48].

In the nucleus, the p53 tumor suppressor triggers the extrinsic apoptotic pathway through increasing the expression of Fas receptor, TRAIL receptor. Conversely, when in the cytoplasm, p53 triggers the intrinsic apoptotic pathway through increasing the expression of pro-apoptotic proteins, such as PUMA, Bax, Bid, and Noxa, resulting in cytochrome c release and apoptosis. p53 also activates Apaf-1 of the apoptosome [1, 49]. In addition to apoptosis, p53 plays a significant role in autophagy [1, 2]. Genotoxic stress triggers nuclear-p53-mediated autophagy through transcriptional activation of damage-regulated autophagy modulator (DRAM), whose signalling cascade stimulates autolysosome formation [50]. However, cytoplasmic p53 represses autophagy through inactivation of AMP kinase, which activates mTOR signalling [51]. In this network, PUMA, which is a p53-inducible BH3-only protein, triggers mitochondria-specific autophagy, resulting in the degradation of mitochondria [52]. This function of PUMA depends on Bax/Bak and does not affect the interaction between Bcl-2/Bcl-xL and Beclin-1. Moreover, PUMA/Bax induces the non-canonical autophagy pathway regulated via Atg5, Atg10, and Atg7. PUMA's initiation of autophagy promotes cytochrome c release, which then leads to apoptosis. Interestingly, inhibition of PUMA- or Bax-mediated autophagy reduces apoptotic activity, suggesting that selective engulfment of mitochondria through autophagy enhances apoptosis.

Unlike other BH3-only proteins, Bim inhibits autophagy beyond its role in apoptosis [53]. Bim, which is associated with dynein light chain 1 (DYNLL1, also known as LC8) on microtubules, inhibits autophagosome formation by sequestering Beclin-1 to DYNLL1 from presumed site of autophagic activity at ER. However, under conditions of stress, Bim is phosphorylated by MAPK8/JNK and dissociates from DYNLL1, which releases Beclin-1 from microtubules, allowing it to translocate to the ER and induce autophagy. Moreover, Bim dissociates from DYNLL1 and binds to Bcl-2 proteins to activate Bax-Bak, which induces mitochondrial apoptosis. Recently, several IAPs, including XIAP and survivin, have been shown to regulate autophagy. For example, XIAP suppresses autophagy via Mdm2 (P53 E3 Ubiquitin Protein Ligase), a negative regulator of p53 [54]. Under nutrient-rich conditions, activated Akt induces XIAP phosphorylation, promoting its interaction with Mdm2, which triggers rapid degradation of XIAP and prevents p53-mediated autophagy. Conversely, serum starvation results in Akt-mediated XIAP dephosphorylation, causing XIAP to dissociate from Mdm2, which promotes p53 degradation and serum-starvation-induced autophagy.

Autophagic proteins in intrinsic apoptosis

In addition to the role that apoptosis-related proteins play in autophagy, numerous autophagic proteins also have apoptotic potential (Fig. 2; Table 1). These autophagic proteins positively or negatively regulate mitochondrial apoptosis either directly or indirectly via their protease-cleavage products [33, 55]. In addition to its role in autophagy, mTOR has multiple effects on apoptosis that depend on specific cellular conditions and downstream targets, including Bad, Bcl-2, and p53 [2, 56]. Two mTOR-binding proteins, the proline-rich Akt substrate of 40 kDa (PRAS40) and the protein Q6MZQ0/FLJ14213/CAE45978, complex with mTOR and promote apoptosis, thereby connecting apoptosis with autophagy [57]. Notably, the mTOR inhibitor everolimus induces apoptosis rather than autophagic cell death in human nasopharyngeal carcinoma [58]. Moreover, the PI3K/Akt/mTOR pathway has been identified as a major inducer of hypervascularization in carcinoma. Inhibition of this pathway with a dual PI3K/mTOR inhibitor induces both autophagy and apoptosis [59]. Beclin-1 and other partners that promote autophagosome initiation, including Ambra-1 and UVRAG, also regulate mitochondrial apoptosis. Caspases generated during apoptosis cleave and inactivate Beclin-1, thus inhibiting autophagy and consequently promoting apoptosis [60–62]. Beclin-1 cleavage disrupts its interaction with the autophagy-initiation complex, and its N- and C-terminal cleavage products are found in the nucleus and mitochondria, respectively. Interestingly, Beclin-1's C-terminus lacks the BH3 domain, resulting in release of cytochrome c and HtrA2/Om, which then amplify the apoptotic signal. Ambra-1, a key molecule that promotes the initial steps of autophagy, is irreversibly cleaved by both calpains and caspases. This represents a potential molecular switch between autophagy and apoptosis [63, 64]. In addition to its pro-autophagic activity, UVRAG has been shown to have anti-apoptotic activity during tumor treatment through interacting with Bax. Specifically, UVRAG prevents Bax translocation from the cytoplasm to the mitochondria during chemotherapy- and UV-induced apoptosis of human cancer cells [65]. Moreover, deletion of the C2 domain of UVRAG prevents Bax binding and subsequent anti-apoptotic activity.

Proteins regulating the elongation process of autophagy have also been shown to participate in mitochondrial apoptosis. For instance, apoptosis-associated calpain-mediated cleavage of Atg5 contributes to spontaneous apoptosis in human neutrophils. In various cell types, the N-terminal cleavage product of Atg5 translocates to the mitochondria, where it associates with Bcl-xL to induce cytochrome c release [66]. Moreover, ectopic expression of this N-terminal Atg5 cleavage fragment promotes nuclear fragmentation and prevents LC3-II accumulation. These findings confirm the ability of cleaved Atg5 to directly induce apoptosis but not autophagy. In our study, we used melanoma differentiation-associated gene 7 (mda-7)/interleukin-24 (IL-24) to promote apoptosis and found that apoptosis-associated calpain-cleaved Atg5 switches the cellular program from autophagy to apoptosis [67]. Similarly, caspase 3-induced cleavage of the autophagy-related endopeptidase Atg4D is downregulated in apoptotic cells. The cleaved form of Atg4D cleaves and delipidates the Atg8 paralogue γ-aminobutyric acid receptor-associated protein like 1 (GABARAP-L1), resulting in decreased formation of GABARAP-L1 mediated autophagosomes. Interestingly, cleaved Atg4D localizes to the mitochondria and triggers apoptosis. Atg4D-mediated apoptosis is facilitated by a putative C-terminal BH3 domain, which is preceded by caspase-independent recruitment of Atg4D to mitochondria through a mitochondrial-targeting sequence [68, 69].

Autophagy and apoptosis are also connected through proteins with dual functions, including the autophagic protein Atg12 that participates in both processes. Atg12 positively regulates mitochondrial apoptosis by directly binding to and inactivating Mcl-1 and Bcl-2 through a unique BH3-like motif. Knockdown of Atg12 inhibits Bax activation and release of cytochrome c, while ectopic expression of Atg12 supresses the anti-apoptotic activity of Mcl-1 [70]. Conjugation of Atg12 with Atg3 controls

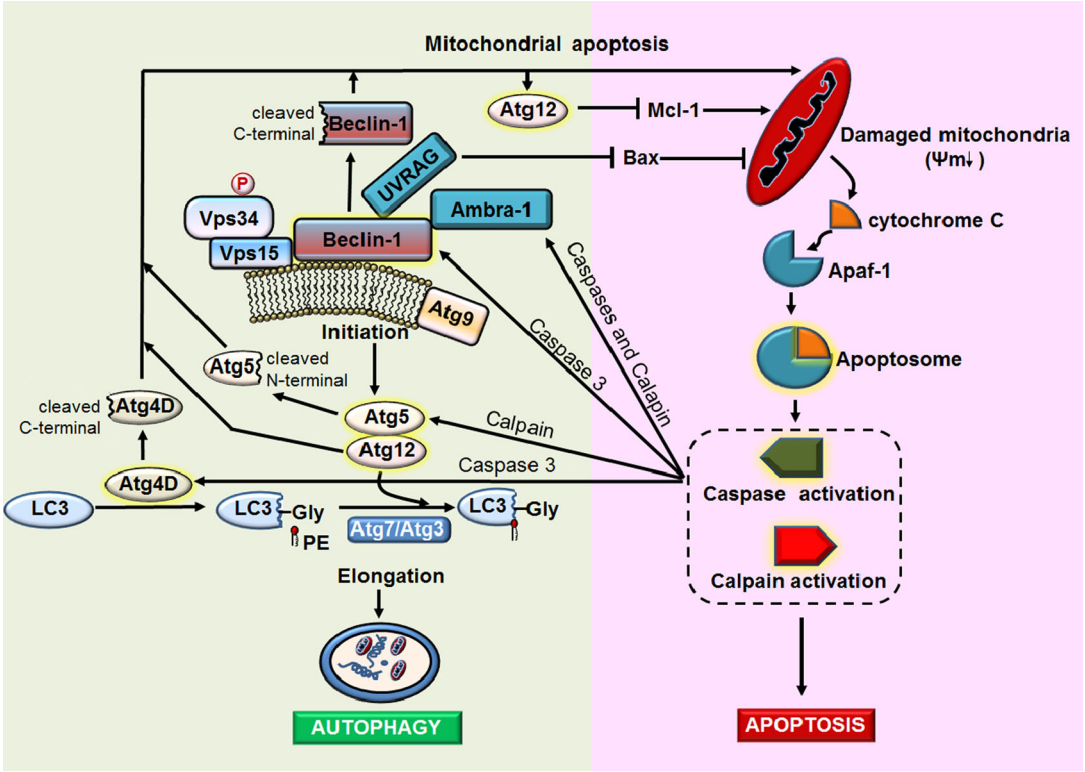

Fig. 2 Autophagic proteins in intrinsic apoptosis: Autophagic proteins involved in initiation and elongation process regulate mitochondrial apoptosis. Beclin-1 and Atg4D are cleaved by caspases, and Atg5 is cleaved by calpain-1 and calpain-2. These cleavage events serve not only to inhibit autophagy, but also to enhance apoptosis, as a C-terminal Beclin-1 and Atg4D cleavage product (CT) and an N-terminal Atg5 cleavage product (NT) are each targeted to the mitochondria, where they directly induce the release of cytochrome c. Ambra-1 is cleaved by both caspases and calpain and inhibit autophagy. Moreover, Atg12 directly activates mitochondrial apoptosis by sequestering Mcl-1. On other hand, UVRAG has anti-apoptotic activity through inhibition of Bax translocation from the cytosol to mitochondria

mitochondrial homeostasis and cell death. The Atg12-Atg3 complex sensitizes cells to intrinsic apoptosis without affecting the extrinsic apoptotic pathway. Interestingly, disrupting this conjugation results in increased mitochondrial mass, disintegration of the mitochondrial network, and ultimately, resistance to intrinsic apoptosis [71].

Connection between autophagy and extrinsic apoptosis

Several autophagic proteins are involved in the extrinsic apoptotic pathway (Fig. 3; Table 1). The connection between autophagy and extrinsic apoptosis is supported by the discovery of extensive autophagy in cells deficient in caspase-8 and FADD [72]. Additionally, Atg5 directly interacts with FADD through the DD, which prevents apoptosis without affecting autophagosome formation [73]. Crosstalk between autophagy and extrinsic apoptosis is further supported by the identification of the caspase-8 inhibitory protein, FLICE-like inhibitory protein (FLIP), which inhibits autophagy [74]. The underlying molecular mechanism involves Atg3's interaction with FLIP, which prevents its interaction with LC3 and the induction of autophagy. Autophagy is also inhibited during extrinsic apoptosis, and cleavage of Atg3 by caspase-8 is a major pathway by which autophagy is inhibited by death-receptor activation [75]. This pathway is inhibited by a caspase-8-specific inhibitor, zIETD, and a pan-caspase inhibitor, zVAD. Interestingly, overexpression of caspase-8 results in the degradation of Atg3, and mutation of the caspase-8 cleavage site on Atg3 prevents its cleavage both in vivo and in vitro, confirming that Atg3 is a direct target of caspase-8. In addition, inhibition of autophagy also promotes p62 and increases the level of active caspase-8, thus triggering apoptosis in the presence of H_2O_2 in U87MG cells [76]. Polyubiquitination can regulate the extrinsic apoptotic signalling, and this process is mediated by the ubiquitin-binding protein p62, which promotes the aggregation of cullin3-modified caspase-8 in a p62-dependent manner, ultimately committing cells to apoptosis [77]. Further, upregulation of p62 increases caspase-8 aggregation and activation by the BH3-mimetic agent ABT-263 on

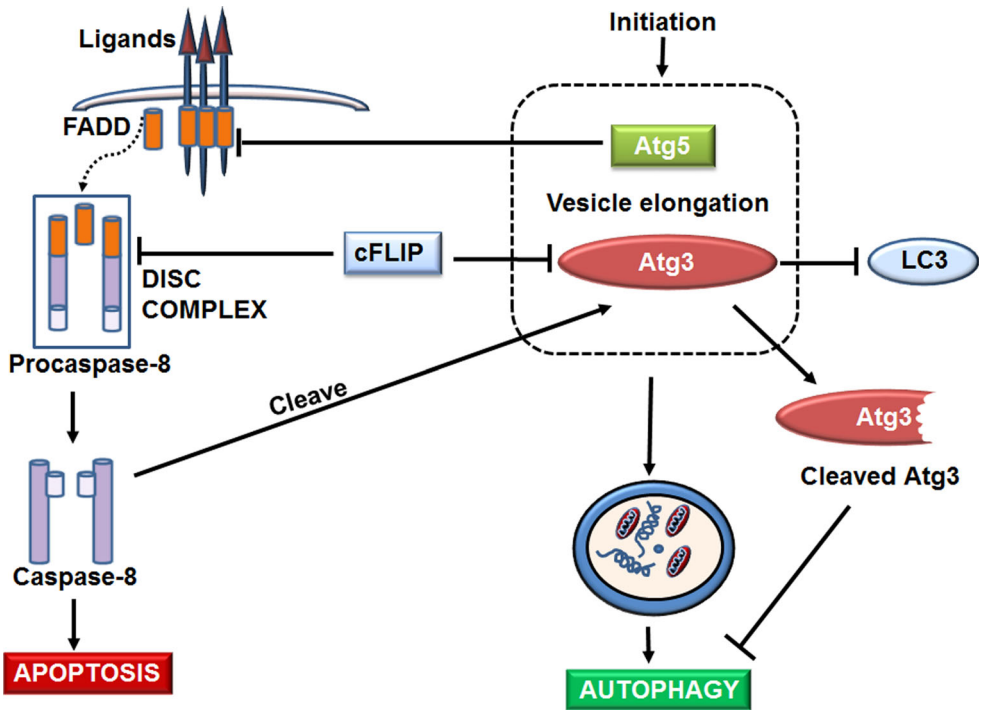

Fig. 3 Connection between autophagy and extrinsic apoptosis: Extrinsic apoptosis is initiated with the binding of a death receptor to its ligand which results in interaction with the adapter protein FADD to the DISC and release of active caspase 8, which mediates apoptosis. The caspase 8 inhibitory proteins c-FLIP interacts with Atg3, thereby preventing interaction between Atg3 and LC3 and inhibiting autophagy. During apoptosis Atg3 is cleaved by caspase 8 and cleaved Atg3 inhibits autophagy. In addition, FADD directly interacts with Atg5; however, this interaction appears to inhibit apoptosis rather than autophagy

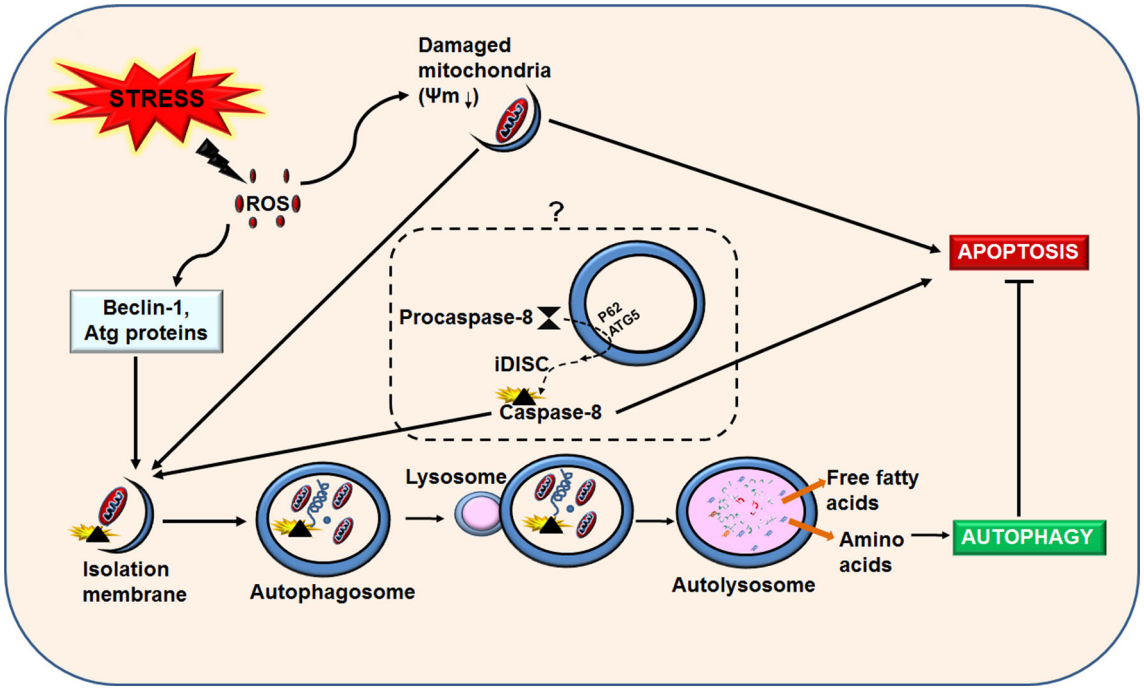

Fig. 4 The autophagosome in apoptosis regulation: Stress stimuli elicit the induction of autophagy and apoptosis, and final outcome as death or survival depends on the complex network of molecular interactions occurring between them. Autophagy inhibits apoptosis by engulfing proapoptotic caspases, such as caspase 8, and damaged reactive mitochondria to prevent the release of cytochrome c. Moreover, autophagosomal membrane functions as platform for intracellular death-inducing signaling complex (iDISC)-mediated caspase-8 activation and promote apoptosis. The complete mechanism how autophagy decides for processing and degradation of caspases is not known

the autophagosome. This establishes p62 as a mediator of the crosstalk between autophagy and apoptosis [78].

The autophagosome in apoptosis

In addition to the role that autophagic proteins play in apoptosis, the autophagosome also regulates apoptosis (Fig. 4). The autophagosome engulfs damaged mitochondria and apoptotic proteins to promote cellular survival under conditions of stress and anti-cancer therapy. Specifically, autophagy protects cells from serum starvation by sequestering reactive oxygen species (ROS)-producing mitochondria through autophagosome formation [79]. Inhibition of autophagy by 3-MA increases the production of ROS and enhances apoptosis. During TRAIL-induced autophagy, active caspase-8 is degraded, which silences apoptotic activity [80]. In contrast, the autophagosomal membrane functions as a platform for processing apoptotic proteins [81]. For example, there is autophagy-dependent caspase-8 activation and induction of the apoptotic cascade in response to SKI-I, a pan-sphingosine kinase inhibitor, and bortezomib, a proteasome inhibitor. The FADD associated with caspase-8 is recruited to the expanding autophagosomal membrane through interacting with Atg5. The autoactivation of caspase-8 and its association with the autophagosomal membrane occur in a p62-dependent manner for formation of an intracellular pro-apoptotic signalling complex.

Connection between autophagy and apoptosis under pathological conditions

There is a complex interaction between autophagy and apoptosis, which controls cell fate under both physiological and pathological conditions [1, 2, 6, 82]. Although the molecular pathways connecting autophagy and apoptosis remain poorly characterized, they have physiological and pathological significance. For instance, the connection between autophagy and apoptosis has been extensively studied in cancer aetiology and treatment [5, 6]. Apoptosis is tumor suppressive, while autophagy's role in cancer is more complicated, as its function changes throughout tumorigenesis. Autophagy plays an essential role in tumor initiation and development, and autophagy-associated cell death may act as a tumor suppressor. Cancer cells are often defective in autophagy, and autophagy-related genes are deleted in various cancers; this supports a role for autophagic cell death in tumor suppression. Moreover, induction of autophagic death could be a useful therapeutic approach for the treatment of apoptosis-resistant cancer cells [1, 5]. Cancer cells derived from mouth, breast, liver, kidney, and pancreas show enhanced autophagic cell death in response to different anti-cancer therapies. Indeed, autophagic cell death is triggered by anti-cancer therapies, such as chemotherapeutic drugs (e.g., alkylating agents and actinomycin D), radiation therapy, photodynamic therapy, cytokines (IFN-γ), hormonal therapies (e.g., tamoxifen and vitamin D analogues), gene therapies (e.g., p53 and mda-7/IL-24), and natural compounds (e.g., resveratrol and plant lectins), in various cancer models [6, 31, 83, 84].

During tumor progression, cancer cells are exposed to different types of stress, including nutrient deprivation, metabolic stress, and hypoxia, especially in the central area of the tumor. Autophagy's cytoprotective role sustains tumor cell viability and enhances survival, which prevents cancer cells from undergoing apoptosis [5, 6]. In this connection, autophagy inhibitors will be most effective when used in combination with cytotoxic drugs that activate a protective autophagy to permit cancer cell survival upon treatment. For example, melanoma differentiation associated gene-7/Interleukin-24 (MDA-7/IL-24) is a cytokine that has anti-cancer potential both in vitro and in vivo through inducing cancer-specific apoptosis [85]. Our group demonstrated that the apoptotic potential of MDA-7/IL-24 is increased by inhibiting autophagy with 3-methyladenine (3-MA) in prostate cancer [67]. Similarly, suppression of autophagy sensitizes chemotherapy-resistant cells to TRAIL-mediated apoptosis in apoptotically dysfunctional leukemic and colon cancer cell lines [86]. Autophagy also serves as a protective response to chemotherapy and promotes chemoresistance-associated tumor regrowth with recurrence. Furthermore, oral cancer stem cells that are highly resistant to apoptosis have an increased level of autophagy, which promotes survival [87]. Treatment of oestrogen-receptor-positive breast carcinoma cells with the anti-oestrogen tamoxifen combined with a histone deacetylase inhibitor sustains a subpopulation of apoptosis-resistant cells that display an elevated level of autophagy. Nevertheless, inhibition of autophagy promotes apoptosis in apoptosis-resistant cells [88].

Emerging cancer therapies that inhibit autophagy may sensitize cancer cells to metabolic stress. For instance, promising targeted therapies, including inhibitors of angiogenesis, growth factors, and growth factor receptors, synergize with autophagy inhibitors to kill cancer cells [89–91]. Moreover, autophagy removes potentially damaged organelles, which suggests that organelle-damaging drugs, including sigma-2 receptor agonists, may be combined with an autophagy inhibitor as a novel therapeutic approach [92]. Lysosome-mediated protein turnover through autophagy is integrated with and augments ubiquitin proteasome protein degradation. Subsequently, targeting both proteasome- and autophagy-mediated protein degradation is a powerful approach for targeting

metabolically active tumor cells [93]. The combined use of an autophagy inhibitor, chloroquine, and alkylating agents is an effective anti-cancer treatment for metastatic tumor cells in mice and humans [90, 94].

In addition to cancer, the connection between autophagy and apoptosis plays an important role in other cellular settings. As such, the disturbance of their delicate balance could promote various diseases [95–97]. For example, the crosstalk between autophagy and apoptosis has been implicated in various heart diseases, where autophagy plays a protective role. Specifically, a deficiency of cardiac-specific Atg5 leads to the accumulation of abnormal proteins and organelles, promoting ER stress and apoptosis, which contribute to cardiac hypertrophy, left ventricular dilatation, and contractile dysfunction [96].

The crosstalk between autophagy and apoptosis has also associated with trauma (e.g., haemorrhage and sepsis), Alzheimer's disease, HIV infection, and neural damage [97–100]. Although apoptosis is the major mechanism of cell death in these disease processes, autophagy plays dual roles by mediating cytoprotection and cell death. Elucidating the mechanisms underlying the connection between autophagy and apoptosis in these diseases may be useful for the development of targeted treatments.

Conclusion

A connection between apoptosis and autophagy has long been suggested. Recently, the molecular link has been elucidated by the discovery that several genes are shared by both pathways. Despite progress in elucidating the mechanism of autophagy and its interaction with apoptosis, its role in cancer and other diseases remains a topic of debate. In particular, it is difficult to define a single function for autophagy, as the process has different effects depending on cell type, stimuli, and escape from apoptosis (e.g., in response to drug treatment). Nonetheless, it remains a promising challenge to understand how a cell responds to similar stimuli by undergoing apoptosis versus autophagy. In contrast to the initial descriptions of autophagic cell death, accumulating evidence points to antagonistic roles for apoptosis and autophagy. Future studies investigating the interplay between apoptosis and autophagy will have significant implications for our understanding of both processes under physiological and pathological conditions.

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Conflict of interest The authors declare that they have no conflict of interest.

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