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Review

The cellular decision between apoptosis and autophagy

Yong-Jun Fan and Wei-Xing Zong

Abstract

Apoptosis and autophagy are important molecular processes that maintain organismal and cellular homeostasis, respectively. While apoptosis fulfills its role through dismantling damaged or unwanted cells, autophagy maintains cellular homeostasis through recycling selective intracellular organelles and molecules. Yet in some conditions, autophagy can lead to cell death. Apoptosis and autophagy can be stimulated by the same stresses. Emerging evidence indicates an interplay between the core proteins in both pathways, which underlies the molecular mechanism of the crosstalk between apoptosis and autophagy. This review summarizes recent literature on molecules that regulate both the apoptotic and autophagic processes.

Key words Apoptosis, autophagy, cell death, cancer

In the view of the cancer researchers in the cell death field, the only good cancer cells are dead cells. Most front-line cancer therapeutics induce cytotoxic stress on cancer cells that ultimately trigger programmed cell death. Programmed cell death was originally described as a cell self-destruction process that plays a crucial role during the development of metazoans. The term *apoptosis* was invented to describe a type of programmed cell death that exhibits specific microscopic features such as chromatin condensation, nuclear fragmentation, and plasma membrane blebbing[1]. For a long time, the terms *apoptosis* and *programmed cell death* were used synonymously, until *autophagic cell death* and *programmed necrosis* were brought to attention of the research community.

Like apoptosis, autophagic cell death was initially characterized by the microscopic features of dying/dead cells. Autophagic cells often lack the typical features of apoptotic cells and almost always contain multi-layer or double-layer intracellular membrane structures enclosing a bulk of cytoplasmic materials or subcellular organelles. These structures, termed autophagosomes, are also observed in yeast cells upon nutrient starvation. Autophagosomes fuse with lysosomes to form autolysosomes, and the contents enclosed in autolysosomes are digested by lysosomal proteases. This process, which allows recycling of intracellular organelles and proteins, benefits cells by (1) removing damaged or unwanted organelles and macromolecules and (2) providing an energy source and building blocks for cellular functions and *de novo* biosynthesis. These functions clearly have a positive effect on cell survival. Yeast strains harboring mutations in autophagy genes die rapidly during starvation, suggesting that autophagy serves as a survival mechanism[2,3]. The survival-promoting function of autophagy in yeast is contradictory to the theory that autophagy is a death mechanism. This raises the question of whether autophagy can really serve as an active form of cell death or if it is a cell-responsive event whose features are observed when cells die of irreparable damage[4]. Indeed, the role of autophagy in multicellular organisms is much more complicated than that in yeast, which lacks well-defined apoptosis machinery as seen in metazoans. It is becoming increasingly apparent that apoptosis and autophagy may not be mutually exclusive but rather closely linked, with complementary pathways involved in the activation and inhibition of both processes. Morphological features characteristic of both autophagy and apoptosis have been observed to occur simultaneously in the same tissue. Furthermore, the ability of autophagy to elicit a cell-survival or cell-death response seems to be significantly impacted by whether or not apoptotic machinery is functional. The interplay between autophagy and apoptosis is a seemingly complex process whose outcome depends on cell type and environmental conditions. Situations have been described in which autophagy acts to antagonize

Authors' Affiliation: Department of Molecular Genetics & Microbiology, Stony Brook University, Stony Brook, NY 11794, USA.

Corresponding Author: Wei-Xing Zong, Department of Molecular Genetics & Microbiology, 216A Life Sciences Building, Stony Brook University, Stony Brook, NY 11794-5222, USA. Tel: +1-631-632-4104; Fax: +1-631-632-9797; Email: weixing.zong@stongbrook.edu.

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apoptosis, whereas in other cases, autophagy acts as an agonist of apoptosis. Still yet, there are cases where autophagy seemingly induces cell death irrespective of apoptosis. A number of molecules can affect both autophagy and apoptosis, suggesting that they may act as molecular switches of these two cellular processes in response to cell damage signaling.

Core Proteins of Autophagy

Autophagy-related genes (ATGs) are involved in various stages of autophagy, including initiation, nucleation, maturation of autophagic vesicles, and fusion of autophagic vesicles with lysosomes. These proteins can be viewed as the core players of autophagy. Notably, several of these autophagy core players have been implicated in apoptosis.

Atg3 is a noncanonical ubiquitination E2 enzyme that mediates the conjugation of the ubiquitin-like protein Atg8 (also termed microtubule-associated protein light chain 3, LC3) to phosphatidylethanolamine (PE) during autophagy. In addition to its effects on Atg8, recent studies have shown that Atg3, like Atg5, can be covalently conjugated to Atg12[5]. Atg3-Atg12 complex is localized to the mitochondrial outer membrane and may regulate mitochondrial homeostasis by mediating mitochondrial fusion. Disturbing the formation of this complex does not affect nonselective autophagy, whereas selective mitochondrial autophagy (mitophagy) is severely reduced. Atg12 conjugation to Atg3 sensitizes cells to mitochondria-mediated apoptosis but has no effect on death receptor–mediated apoptosis. However, the mechanism of this sensitization remains unclear.

Atg4 is a unique cysteine protease responsible for the cleavage of the C-terminus of LC3, a step that primes newly synthesized Atg8 for covalent attachment to PE. Atg4 can also delipidate Atg8 at the lysosomal fusion step[6]. One study found that a human Atg4 member Atg4D is cleaved at DEVD(63)K by caspase-3 during apoptosis, generating two fragments[6]. The N-terminal fragment gains protease activity and can prime/delipidate GABA (A) receptor-associated protein like 1 (GABARAP-L1), an Atg8 family member. The C-terminal fragment contains a putative BH3 domain and can be transiently recruited to the mitochondrial matrix, resulting in enhanced cell death by regulating ROS generation[7].

Atg5 participates in autophagy initiation through a ubiqiutin-like conjugation system[8]. Reports indicate that a truncated Atg5, generated through calpain-mediated proteolysis, interacts with Bcl-x_L, translocates to mitochondria, and hence, activates apoptosis[9].

Atg6/Beclin1 has a key role in the initiation of autophagy. Several reports have provided evidence that Beclin1 is a substrate of caspase-3[10-12]. When caspase-3 is activated, it cleaves Beclin1 at two potential sites: TDVD(133) and DQLD(149). Cleaved Beclin1 loses its ability to promote autophagy, and more importantly, the C-terminal fragment can localize to mitochondria and sensitize cells to apoptosis. Interestingly, this Beclin1-C fragment does not include the putative BH3 domain in full-length Beclin1. The mechanism of how the Beclin1-C fragment mediates mitochondrial damage requires further research.

LC3 is a ubiquitin-like protein with only one known target: PE. LC3-conjugated PE is recruited to the phagophore and is involved in the elongation and enclosure of the autophagosome. LC3 can directly interact with the ubiquitin-binding protein SQSTM1/p62 and facilitate the clearance of polyubiquitinated protein aggregates. Two recent studies suggest that LC3 can serve as the platform to facilitate apoptosis by mediating caspase-8 activation[13,14]. Inhibition of autophagic protein degradation leads to the accumulation of LC3 and SQSTM1/p62. Because p62 interacts with caspase-8, the accumulation of LC3 and p62 leads to caspase-8 aggregation and autoactivation, resulting in apoptosis. Besides functioning as the docking site for caspase-8, LC3 can also regulate extrinsic apoptosis in cigarette-smoke–induced emphysema[15] and hyperoxia-induced epithelial cell death[16]. LC3 has been found to interact with the death-associated ligand Fas in lipid rafts via caveolin-1 (Cav-1). However, the outcome of the LC3/Fas interaction in cell death regulation is different in these two systems. In cigarette-smoke–induced cell death, siRNA-mediated silencing of LC3 inhibits apoptosis by increasing Cav-1–dependent Fas sequestration[15]. In hyperoxia-induced cell death, silencing LC3 promotes cell death while overexpressing LC3 confers cytoprotection[16]. How the interaction among LC3, Cav-1, and Fas generates different outcomes is not clear. The distinct binding sites and/or different binding affinity between LC3 and Cav-1 may contribute to this divergence.

Atg12 is a ubiquitin-like protein that can covalently conjugate with Atg3 and Atg5 and regulate apoptosis and autophagy, respectively. A recent study revealed that the free form of Atg12 can function as a potent apoptogenic factor in response to various cell death stimuli[17]. A putative BH3-like motif has been identified in Atg12. Atg12 can interact with anti-apoptotic Bcl-2 family members through its BH3-like motif, thereby regulating Bax activation and mitochondrial outer membrane permeabilization (MOMP). Atg12 deficiency blocks intrinsic but not extrinsic apoptosis, consistent with its role as a BH3-only protein that regulates mitochondrial cell death. It is worth noting that the BH3 domain of Atg12 does not structurally resemble a classic BH3 domain.

UV radiation resistance-associated gene protein (UVRAG), a regulator of autophagy that interacts with the Vps34/Beclin1 complex[18], has been reported to be able to inhibit apoptosis by direct association with Bax. This association blocks Bax activation and its translocation to mitochondria [19]. Another autophagy-related protein that has a role in apoptosis is AMBRA1 (activating molecule in Beclin1-regulated autophagy), which is a Beclin1 binding partner and stimulates autophagy[20,21]. Like Beclin-1, AMBRA1 can be cleaved by caspases and calpains, which facilitates apoptosis by inhibiting the pro-survival function of autophagy[22].

Bcl-2 Family of Proteins

The Bcl-2 family of proteins is critical in controlling mitochondrial membrane permeabilization and apoptosis. These proteins are distinguished by the presence of up to four Bcl-2 homology domains (BH1–4 domains) and are usually grouped into three distinct subfamilies based on their function and the BH domains: (1) anti-apoptotic proteins, including Bcl-2, Bcl-x_L, Mcl-1, Bcl-W and A1/Bfl-1; (2) "multidomain" pro-apoptotic proteins, including Bax, Bak, and Bok; and (3) "BH3-only" proteins, such as Bad, Bim, Noxa, Puma, Bmf, and Bnip3. The multidomain pro-apoptotic proteins Bax and Bak are the key molecules necessary for inducing apoptosis. In response to apoptotic stimuli, these proteins undergo conformational changes leading to their oligomerization on the mitochondrial outer membrane, followed by cytochrome *c* release and activation of the caspase cascade. Anti-apoptotic Bcl-2 proteins block this process by interacting with Bax and Bak. BH3-only proteins respond to different cell death stimuli. All BH3-only proteins identified so far are pro-apoptotic and function by either activating Bax and Bak or inhibiting the anti-apoptotic Bcl-2 proteins[23].

Anti-apoptotic Bcl-2 proteins

The anti-apoptotic Bcl-2 family proteins Bcl-2 and Bcl-x_L can inhibit autophagy via interaction with the autophagy essential factor Beclin1. In fact, Beclin1 was discovered in a yeast two-hybrid screen for Bcl-2–interacting proteins[24]. A similar interaction was found in *C. elegans* between Beclin1 and the Bcl-2 homolog ced-9 [25]. Other anti-apoptotic Bcl-2 family members, including Bcl-x_L, Mcl-1, and the herpesvirus-encoded Bcl-2 homolog v-Bcl-2, were also found to interact with Beclin1[26,27]. These anti-apoptotic Bcl-2 proteins suppress starvation-induced autophagy by inhibiting the formation of the complex between Beclin1 and the class III phosphatidylinositol 3-kinase (PI3K) Vps34 [28]. The subcellular localization of Bcl-2 appears to affect its function as an autophagy regulator. While the majority of cellular Bcl-2 is localized to the mitochondria, a portion of Bcl-2 is localized to the ER membrane[29]. ER-targeted Bcl-2, but not mitochondrial Bcl-2, inhibits autophagy[28]. A possible explanation for this is that Bcl-2 may require factors such as inositol triphosphate (IP3) receptors on the ER membrane to function as an autophagy modulator.

Another potential mechanism for the inhibitory effect of Bcl-2 on autophagy is modulation of intracellular Ca^{2+} homeostasis. An increase in free cytosolic Ca^{2+} can induce autophagy. This may result from inhibition of the target of rapamycin (TOR) signaling pathway by calmodulin-dependent kinase kinase-β (CaMKKβ) and 5' adenosine monophosphate–activated protein kinase (AMPK) [30]. Bcl-2 can reduce the steady-state level of ER Ca^{2+}, preventing the stress-induced increase of cytosolic Ca^{2+}[31,32] and thereby suppressing the induction of autophagy[30].

Contrary to the above conditions, where Bcl-2 functions to inhibit autophagy, Bcl-2 and Bcl-x_L have also been found to enhance autophagy under certain conditions, such as in response to treatment with etoposide and staurosporine [33]. The underlying mechanism whereby Bcl-2 and Bcl-x_L can promote autophagy is currently unclear. It is possible that distinct stress signals—for example, starvation or genotoxins—may cause Bcl-2 family proteins to localize to different subcellular compartments or to interact with different sets of molecules, thus imposing distinctive effects on autophagy induction.

Bax and Bak

Bax and Bak are key molecules in mediating the intrinsic apoptosis pathway. Cells deficient in both Bax and Bak are resistant to a wide range of apoptotic stimuli [34-36]. Nevertheless, these cells can still undergo non-apoptotic cell death, namely autophagic and necrotic cell death [33,37]. Long-term treatment of *bax*$^{-/-}$*bak*$^{-/-}$ cells with the genotoxin etoposide led to a decrease in cell viability, which could be blocked by the inhibition of autophagy, suggesting that autophagy is critical in this Bax/Bak-independent cell death [33]. Similar results were found in irradiated *bax*$^{-/-}$*bak*$^{-/-}$ cells[38,39]. Because Bax and Bak directly interact with the anti-apoptotic Bcl-2 proteins, it is reasonable to consider that Bax and Bak may displace Bcl-2 or Bcl-x_L from Beclin1, thereby affecting autophagy. Another hypothesis is that Bax and Bak play a role in keeping phosphatase and tensin homolog (PTEN) activity low, which can result in constitutively active Akt-mTOR signaling and autophagy inhibition[40]. However, there is no direct evidence to support these theories. Indeed, in certain settings, Bax and Bak proteins do not seem to affect the induction of

autophagy[41]. It is more likely that the deficiency in apoptosis due to the absence of Bax and Bak enables autophagy to progress to an extent that the cells die of damage resulting from excessive autophagy.

BH3-only proteins

The pro-apoptotic BH3-only proteins are categorized into two types. One includes Bim and Bid, which contain an α-helical BH3 domain that can directly induce oligomerization of Bax and Bak on the mitochondrial outer membrane, leading to cytochrome c release. The other includes Bad and Bik, whose BH3 domain binds and antagonizes anti-apoptotic Bcl-2 proteins and sensitizes cells to apoptosis. In both cases, the BH3 peptides interact with a hydrophobic groove formed by the BH1, BH2, and BH3 domains and upon doing so, induce apoptosis either by activating Bax and Bak or by neutralizing Bcl-2 and Bcl-x_L[42]. A small molecule compound that mimics the BH3 peptide has been shown to be able to induce apoptosis in cancer cells and can be explored as a potential anticancer drug[43].

In addition to their pro-apoptotic function, BH3-only proteins may also regulate autophagy. Bcl-2/adenovirus E1B 19kDa interacting protein 3 (Bnip3), a hypoxia-inducible, BH3 domain containing protein, leads to accumulation of autophagosomes and non-apoptotic cell death when overexpressed[44]. Arsenic trioxide (As_2O_3), which can kill hematological tumor cells via apoptosis, induces autophagic cell death in solid tumor cells by up-regulating the expression of Bnip3[45].

Bad, egl-1 (the *C. elegans* BH3-only homolog), and the chemical BH3 peptide mimetic ABT737 have been found to enhance basal level and starvation-induced autophagy. This autophagy enhancement occurs through the competitive interaction of BH3 peptides with Bcl-2/Bcl-x_L, which displaces Bcl-2/Bcl-x_L from Beclin1[26]. Thus, BH3-only proteins may have dual functions, serving as inducers for both apoptosis and autophagy. It remains to be determined whether the Bid/Bim-like and Bad/Bik-like BH3-only proteins have the same or different effects in regulating autophagy. Nevertheless, in the above settings, autophagy acts as a survival mechanism, as the inhibition of autophagy enhanced apoptosis induced by BH3 mimetics and BH3-only proteins[26]. Moreover, while the Beclin1/Bcl-2 interaction requires the BH1 and BH2 domains in Bcl-2[28], a putative BH3 domain has been found in Beclin1[46], suggesting a retrospective role for Beclin1 in regulating apoptosis.

Calpains

Calpains are a family of Ca^{2+}-dependent cysteine proteases. There are at least 14 members, and each can be categorized into one of two subfamilies: μ-calpains or m-calpains. The names of these subfamilies reflect their requirements for Ca^{2+}. μ-calpains are activated by micromolar concentrations of Ca^{2+}, whereas m-calpains are activated by millimolar[47,48]. The two subfamilies share a common 30-kDa regulatory subunit and contain a distinct ~80-kDa catalytic subunit[49]. Calpains reside in the cytosol in an inactive form. In response to increased levels of cytosolic Ca^{2+}, calpains translocate to the intracellular membrane and are activated by autocatalytic hydrolysis[48]. Calpains are involved in both apoptotic and necrotic cell death. Apoptosis is promoted by calpain-mediated cleavage of anti-apoptotic proteins such as Bcl-2 or by the cleavage of certain pro-caspases into their active form[50-52]. Calpain-mediated cleavage of the Na^+/Ca^{2+} exchanger in the plasma membrane results in sustained secondary intracellular Ca^{2+} overload and subsequent necrotic cell death[53]. Calpains also causes lysosomal membrane permeability (LMP), which leads to the release of lysosomal enzymes such as cathepsins and cell death[54].

Calpains have been reported to play a role in autophagy. These proteins are activated by several stimuli that trigger apoptosis and autophagy. One recent report showed that calpains are required for autophagy induced by these conditions[55]. Genetic deletion or RNAi-mediated knockdown of the calpain small 1 regulatory subunit (CAPNS1), which is required for the activation of both μ-calpain and m-calpain, results in a block of autophagy induced by rapamycin, starvation, aceramide, and etoposide[55]. Inhibition of autophagy due to calpain deprivation makes cells more sensitive to apoptosis, suggesting that calpain-mediated autophagy plays a protective role against apoptosis. Another report, however, showed that in response to similar insults, Atg5 is cleaved by calpain at its C-terminus, generating a 24-kDa fragment from its 33-kDa proform. This 24-kDa fragment translocates to mitochondria, where it antagonizes Bcl-x_L and thereby induces apoptosis[9]. Thus, in this setting, calpain activation switches cells from autophagy to apoptosis by cleaving Atg5. These reports indicate that calpains are an important regulator of apoptosis and autophagy, although the discrepancy between these reports needs to be further investigated.

p53

p53 is a major cellular stress-sensing molecule, which, upon activation, triggers cell cycle arrest or apoptosis[56,57]. In addition, p53 also regulates autophagy. Treatment with genotoxins including etoposide, doxorubicin, and actinomycin D resulted in p53-dependent induction of autophagy[4,58]. One mechanism for p53-mediated autophagy is through the activation of AMPK,

subsequently leading to activation of the tuberous sclerosis protein kinases (TSC1 and TSC2) and inhibition of mTOR)[4]. In addition to this rapidly induced inhibition of mTOR, p53 activation also leads to the up-regulation of PTEN and TSC2 at the transcriptional level, which may contribute to long-term suppression of mTOR[4]. Another mechanism for p53-induced autophagy is through transcriptional activation of damage-regulated autophagy modulator (DRAM), a p53 transcriptional target[58]. While DRAM is required for p53-mediated autophagy, it is not required for autophagy induced by amino acid starvation. This DRAM-dependent autophagy appears to facilitate p53-dependent apoptosis. While DRAM is a known lysosomal protein, how it activates autophagy remains unclear.

p53 is well recognized as a DNA-damage–responsive factor; yet its ability to activate AMPK and inhibit mTOR signifies that it is also capable of signaling to the nutrient sensing pathway. Additional support for this role of p53 is illustrated by its activation in response to nutrient starvation. Indeed, glucose starvation leads to AMPK-mediated phosphorylation of serine 15 on human p53 (serine 18 in mouse)[59], indicating p53 activation. In normal cells, phosphorylation of Ser15 is quickly reversed by a phosphatase composed of the α4 regulatory subunit and the protein phosphatase 2 (PP2A) catalytic subunit, which is in turn activated via phosphorylation by TOR kinase[60]. Thus, TOR forms a negative feedback loop in the phosphorylation of Ser15 of p53, while AMPK positively acts on Ser15 and activates p53.

However, as a multifaceted protein, p53 has also been reported to have inhibitory effects on autophagy. Genetic deletion, depletion, or pharmacological inhibition of p53 leads to induction of autophagy. Expression of wild type p53 or mutant p53 predominantly localized in the cytosol in p53-deficient cells inhibits basal level autophagy. When a classic autophagy inducer stimulates autophagy, the process is associated with p53 degradation. Blocking the degradation of p53 prevents autophagy induction in response to various autophagic stimuli[61,62]. While the mechanism of how cytosolic p53 inhibits autophagy has yet to be determined, studies have found that the autophagy suppression activity of p53 is strictly cell cycle-dependent (i.e., p53 inhibition triggers autophagy mostly in G_1 phase and less so in S but never in G_2/M phases)[62].

$p27^{Kip1}$

$p27^{Kip1}$, a member of the Cip/Kip family of cyclin-dependent kinase (Cdk) inhibitors, binds to Cdk2 (and other Cdks) and potently inhibits Cdk2 kinase activity[63]. $p27^{Kip1}$ overexpression in human cells leads to cell cycle arrest in G_1 phase. $p27^{Kip1}$ has been reported to mediate the decision to enter autophagy or apoptosis when cells are under metabolic stress[27]. The stability and function of $p27^{Kip1}$ is regulated by different kinases through phosphorylation. Activation of the liver kinase B1 (LKB1)–AMPK pathway results in the phosphorylation of Thr198 of $p27^{Kip1}$, which promotes $p27^{Kip1}$ stability. Overexpression of a stable $p27^{Kip1}$ phosphomimetic mutant (Thr198Asp) induced autophagy even in the presence of serum. In serum-starved cells, both Thr198 phosphorylation and $p27^{Kip1}$ stability increased, concurrent with autophagy induction, suggesting that $p27^{Kip1}$ plays a role in serum deprivation-induced autophagy. While serum deprivation, inhibition of PI3K by LY294002, or inhibition of mTOR by rapamycin induced autophagy in cells with normal $p27^{Kip1}$ levels, these treatments induced apoptosis in cells in which $p27^{Kip1}$ had been silenced through RNAi[27]. Thus, $p27^{Kip1}$ can function as a determinant of whether quiescent cells enter the autophagy cell survival pathway or undergo apoptosis. Since tumor cells often encounter metabolic stress imposed by their microenvironment and certain chemotherapeutic treatments, $p27^{Kip1}$ may function to promote tumor cell survival through the maintenance of autophagy.

smArf

Mouse $p19^{Arf}$ ($p14^{ARF}$ in humans) is a tumor suppressor protein that activates p53 by antagonizing the p53 inhibitor Mdm2 (HDM2 in humans)[64], thereby initiating p53-dependent cell cycle arrest and enhancing apoptosis. However, in mice lacking functional Mdm2 and p53, deficiency in $p19^{Arf}$ makes these animals more tumor prone, suggesting an Mdm2- and p53-independent tumor suppressive function of $p19^{Arf}$[65]. While several explanations have been proposed for the p53-independent effects of $p19^{Arf}$[66], a recent study showed that smArf, a short form of Arf, can induce autophagic cell death in a p53-independent manner. This may also contribute to its p53-independent tumor suppressor function[67].

Translation of the smArf protein is initiated from the single internal methionine codon in both the mouse and human Arf mRNA (Met45 in mouse $p19^{Arf}$ and Met48 in human $p14^{Arf}$). Mutation of the canonical $p19^{Arf}$ initiation codon (Met1) or deletion of sequences encoding the 40 N-terminal amino acids of Arf leads to increased production of smArf. Unlike full-length Arf, smArf is localized to mitochondria, where it causes morphological changes, loss of mitochondrial membrane potential, and eventually cell death. These effects were observed in mouse embryo fibroblasts (MEFs) lacking functional p53 or the pro-apoptotic proteins Bax and Bak, and they

could not be circumvented by the anti-apoptotic factors Bcl-2 and Bcl-x_L. Moreover, smArf does not trigger cytochrome c release or caspase activation, all pointing to a non-apoptotic form of cell death. Indeed, smArf was found to stimulate the accumulation of autophagic vesicles. RNAi-mediated knockdown of Atg5 and Beclin1 attenuated smArf-induced cell death. Thus it appears that smARF can induce autophagy as a mechanism to promote cell death[67]. Therefore, there may be different tumor suppressing mechanisms mediated by the two Arf isoforms. More specifically, full-length $p19^{Arf}$ triggers a rapid, p53-dependent nuclear response, whereas the short form, smArf, activates a slowly evolving, mitochondria-based autophagy program that gains primacy when p53 is dysfunctional.

Death-Associated Protein Kinase (DAPK)

DAPK is a calcium/calmodulin-regulated serine/threonine kinase, initially isolated as a mediator of apoptosis induced by interferon-γ[68]. Further studies revealed DAPK as the first member of a protein kinase family that includes two other proteins, Zipper-interacting protein kinase (ZIPK) and dynamin-related protein-1 (DRP-1). These proteins share a high degree of homology in their kinase catalytic domains and function to promote cell death triggered by various death stimuli, including interferon-γ, cell death receptors, TGF-β, oncogene expression, and anoikis (loss of adherence to extracellular matrix)[69,70]. Interestingly, the cell death type induced by ectopic expression of the DAPK family members depends on cellular and experimental conditions. In primary fibroblasts, overexpression of DAPKs leads to apoptosis, whereas in tumor cell lines, such as HeLa, MCF-7, or 293T cells, overexpression of DAPKs leads to cell death with autophagic features[71]. Dominant-negative DRP-1 reduced the level of starvation and tamoxifen-induced autophagy in MCF-7 breast carcinoma cells, whereas reduction of DAPK expression by antisense RNA attenuated interferon-γ–induced autophagy in HeLa cells[71]. This suggests that these kinases may be necessary for autophagy. Interestingly, DRP-1 is localized to the lumen of autophagic vesicles, which raises the possibility that DRP-1 may have a direct role in autophagic vesicle formation, possibly by phosphorylating factors involved in this process[71]. Indeed, DAPK can phosphorylate Thr119 in the BH3 domain of Beclin1, promoting its dissociation from Bcl-2/Bcl-x_L[72]. DAPK has also been reported as a mediator of ER stress-induced caspase activation and autophagic cell death in primary fibroblasts[73]. Deletion of DAPK attenuated the accumulation of the active form of caspase-3, as well as the accumulation of LC3-II. Consequently, cell death was partially blocked. Depletion of DAPK did not affect ER stress intensity or the unfolded protein response (UPR) signaling pathway, which includes CCAAT/enhancer-binding protein (C/EBP) homologous protein (CHOP) induction, caspase-12 activation, and c-Jun N-terminal kinase (JNK) phosphorylation. These findings suggest that DAPK works downstream of the ER stress response to mediate apoptosis and autophagy[73].

DNA-PK

DNA-protein kinase (PK) is a nuclear serine/threonine kinase and a member of the PI3K-like family. It consists of a catalytic subunit (DNA-PKcs) and a DNA binding component (Ku70/Ku80)[74]. DNA-PK activity is stimulated by double-stranded DNA ends. DNA-PK plays a central role in the non-homologous end joining (NHEJ) pathway for double strand break repair and in V(D)J recombination. The phosphorylation targets of DNA-PKcs include DNA-PKcs[75], both Ku subunits[76], XRCC4[77], p53[78], Mdm2[79], and c-ABL[80]. Phosphorylation of DNA-PKcs, Ku subunits, and XRCC4 is associated with DNA repair, whereas that of p53, MDM2, and c-ABL induces apoptosis. The distinct functions of these targets are, therefore, consistent with the notion that DNA-PK has dual roles in responding to DNA damage: one is to sense DNA damage and facilitate the repair processes, whereas the other is to induce apoptosis[81]. DNA damage does not induce apoptosis in DNA-$PKcs^{-/-}$ thymocytes[78,82]. Further studies found that when treated with γ-irradiation, DNA-PKcs–deficient M059J glioblastoma cells die with autophagic features. Inhibition of DNA-PKcs induced autophagy and sensitized malignant glioma cells to γ-irradiation[83]. Thus, DNA-PKcs may also multitask by inducing apoptosis while inhibiting autophagy.

c-Jun N-terminal Kinases (JNK)

The molecular mechanism of JNK regulating apoptosis and autophagy was first described in nutrient-starvation–induced autophagy and apoptosis[84]. Upon nutrient starvation, JNK is activated and phosphorylates Bcl-2 at multiple sites, which decreases the ability of Bcl-2 to bind BH3 domain-containing proteins, such as Bax and Beclin1. Due to the weak binding of the Beclin1 to Bcl-2, the Bcl-2/Beclin1 complex disassembles and the freed Beclin1 can induce autophagy. Upon prolonged starvation, JNK activity is sustained at a high level, resulting in increased Bcl-2 phosphorylation and dissociation from Bax, which leads to Bax-mediated apoptosis[84]. JNK has also been reported to up-regulate Beclin1 expression at the transcription level through its

bona fide substrate c-Jun. However, in most instances, JNK activation and Beclin1 expression is associated with autophagic cell death induced by triggers such as ceramide or stimulation of the human death receptor 5 (DR5) in cancer cell lines[85,86].

Closing Remarks

Autophagy, as a cellular response to nutrient deprivation, is evolutionarily a more ancient event than apoptosis. As unicellular organisms like yeast developed a complex molecular pathway to regulate autophagy, apoptosis appeared later in metazoans to ensure tissue and organismal homeostasis. Both processes play critical roles in controlling cell death and survival and, thus, need to be delicately regulated. It is therefore not surprising that certain canonical apoptotic molecules can regulate autophagy, and vice versa. Multitasking by certain molecules offers advantages to regulate these important biological events more rapidly, efficiently, and precisely. Autophagy clearly has both pro-survival and pro-death functions, and this is largely dependent on the cell's ability to die by apoptosis. In response to stress or damage, autophagy is induced to shut down the anabolic processes to keep cells viable and metabolically inert, allowing cellular repair and escape from further damage. When cellular damage is too severe, apoptosis kicks in to eliminate irreparable cells to maintain tissue homeostasis. In cells with defective apoptosis, autophagy may serve as a backup strategy for cellular demise or as a mechanism to promote other forms of cell death, such as necrosis. This is of significant interest in cancer cells because the apoptosis machinery is often mutated during tumorigenesis. Thus, targeting autophagy should be an important consideration in developing novel strategies for cancer therapy.

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References

[1] Kerr JF, Wyllie AH, Currie AR. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer, 1972,26:239–257.
[2] Tsukada M, Ohsumi Y. Isolation and characterization of autophagy-defective mutants of Saccharomyces cerevisiae. FEBS Lett, 1993,333:169–174.
[3] Otto GP, Wu MY, Kazgan N, et al. Macroautophagy is required for multicellular development of the social amoeba Dictyostelium discoideum. J Biol Chem, 2003,278:17636–17645.
[4] Feng Z, Zhang H, Levine AJ, et al. The coordinate regulation of the p53 and mTOR pathways in cells. Proc Natl Acad Sci U S A, 2005,102:8204–8209.
[5] Radoshevich L, Murrow L, Chen N, et al. ATG12 conjugation to ATG3 regulates mitochondrial homeostasis and cell death. Cell, 2010,142:590–600.
[6] Betin VM, Lane JD. Caspase cleavage of Atg4D stimulates GABARAP-L1 processing and triggers mitochondrial targeting and apoptosis. J Cell Sci, 2009,122:2554–2566.
[7] Betin VM, Macvicar TD, Parsons SF, et al. A cryptic mitochondrial targeting motif in Atg4D links caspase cleavage with mitochondrial import and oxidative stress. Autophagy, 2012,8:664–676.
[8] Maiuri MC, Zalckvar E, Kimchi A, et al. Self-eating and self-killing: crosstalk between autophagy and apoptosis. Nat Rev Mol Cell Biol, 2007,8:741–752.
[9] Yousefi S, Perozzo R, Schmid I, et al. Calpain-mediated cleavage of Atg5 switches autophagy to apoptosis. Nat Cell Biol, 2006,8:1124–1132.
[10] Cho DH, Jo YK, Hwang JJ, et al. Caspase-mediated cleavage of ATG6/Beclin-1 links apoptosis to autophagy in HeLa cells. Cancer Lett, 2009,274:95–100.
[11] Luo S, Rubinsztein DC. Apoptosis blocks Beclin 1–dependent autophagosome synthesis: an effect rescued by Bcl-x_L. Cell Death Differ, 2010,17:268–277.
[12] Wirawan E, Vande Walle L, Kersse K, et al. Caspase-mediated cleavage of Beclin-1 inactivates Beclin-1–induced autophagy and enhances apoptosis by promoting the release of proapoptotic factors from mitochondria. Cell Death Dis, 2010,1:e18.
[13] Pan JA, Ullman E, Dou Z, et al. Inhibition of protein degradation induces apoptosis through a microtubule-associated protein 1 light chain 3-mediated activation of caspase-8 at intracellular membranes. Mol Cell Biol, 2011,31:3158–3170.
[14] Young MM, Takahashi Y, Khan O, et al. Autophagosomal membrane serves as platform for intracellular death-inducing signaling complex (iDISC)–mediated caspase-8 activation and apoptosis. J Biol Chem, 2012,287:12455–12468.
[15] Chen ZH, Lam HC, Jin Y, et al. Autophagy protein microtubule-associated protein 1 light chain-3B (LC3B) activates extrinsic apoptosis during cigarette smoke–induced emphysema. Proc Natl Acad Sci U S A, 2010,107:18880–18885.
[16] Tanaka A, Jin Y, Lee SJ, et al. Hyperoxia-induced LC3B interacts with the Fas apoptotic pathway in epithelial cell death. Am J Respir Cell Mol Biol, 2012,46:507–514.
[17] Rubinstein AD, Eisenstein M, Ber Y, et al. The autophagy protein Atg12 associates with antiapoptotic Bcl-2 family members to promote mitochondrial apoptosis. Mol Cell, 2011,44:698–709.
[18] Liang C, Feng P, Ku B, et al. UVRAG: a new player in autophagy and tumor cell growth. Autophagy, 2007,3:69–71.
[19] Yin X, Cao L, Kang R, et al. UV irradiation resistance-associated gene suppresses apoptosis by interfering with BAX activation. EMBO Rep, 2011,12:727–734.
[20] Fimia GM, Stoykova A, Romagnoli A, et al. Ambra1 regulates autophagy and development of the nervous system. Nature, 2007,447:1121–1125.
[21] Strappazzon F, Vietri-Rudan M, Campello S, et al. Mitochondrial BCL-2 inhibits AMBRA1-induced autophagy. EMBO J, 2011,30:1195–1208.
[22] Pagliarini V, Wirawan E, Romagnoli A, et al. Proteolysis of Ambra1 during apoptosis has a role in the inhibition of the autophagic pro-survival response. Cell Death Differ, 2012,19:1495–1504.
[23] Danial NN, Korsmeyer SJ. Cell death: critical control points.

Cell, 2004,116:205–219.
[24] Liang XH, Kleeman LK, Jiang HH, et al. Protection against fatal Sindbis virus encephalitis by beclin, a novel Bcl-2–interacting protein. J Virol, 1998,72:8586–8596.
[25] Takacs-Vellai K, Vellai T, Puoti A, et al. Inactivation of the autophagy gene bec-1 triggers apoptotic cell death in C. elegans. Curr Biol, 2005,15:1513–1517.
[26] Maiuri MC, Le Toumelin G, Criollo A, et al. Functional and physical interaction between Bcl-X(L) and a BH3-like domain in Beclin-1. EMBO J, 2007,26:2527–2539.
[27] Jia K, Hart AC, Levine B. Autophagy genes protect against disease caused by polyglutamine expansion proteins in Caenorhabditis elegans. Autophagy, 2007,3:21–25.
[28] Furuya N, Yu J, Byfield M, et al. The evolutionarily conserved domain of Beclin 1 is required for Vps34 binding, autophagy and tumor suppressor function. Autophagy, 2005,1:46–52.
[29] Ferri KF, Kroemer G. Organelle-specific initiation of cell death pathways. Nat Cell Biol, 2001,3:E255–E263.
[30] Hoyer-Hansen M, Bastholm L, Szyniarowski P, et al. Control of macroautophagy by calcium, calmodulin-dependent kinase kinase-beta, and Bcl-2. Mol Cell, 2007,25:193–205.
[31] Pinton P, Ferrari D, Magalhaes P, et al. Reduced loading of intracellular Ca(2+) stores and downregulation of capacitative Ca(2+) influx in Bcl-2-overexpressing cells. J Cell Biol, 2000, 148:857–862.
[32] Foyouzi-Youssefi R, Arnaudeau S, Borner C, et al. Bcl-2 decreases the free Ca^{2+} concentration within the endoplasmic reticulum. Proc Natl Acad Sci U S A, 2000,97:5723–5728.
[33] Shimizu S, Kanaseki T, Mizushima N, et al. Role of Bcl-2 family proteins in a non-apoptotic programmed cell death dependent on autophagy genes. Nat Cell Biol, 2004,6:1221–1228.
[34] Wei MC, Zong WX, Cheng EH, et al. Proapoptotic BAX and BAK: a requisite gateway to mitochondrial dysfunction and death. Science, 2001,292:727–730.
[35] Zong WX, Lindsten T, Ross AJ, et al. BH3-only proteins that bind pro-survival Bcl-2 family members fail to induce apoptosis in the absence of Bax and Bak. Genes Dev, 2001,15:1481–1486.
[36] Cheng EH, Wei MC, Weiler S, et al. BCL-2, BCL-X(L) sequester BH3 domain-only molecules preventing BAX- and BAK-mediated mitochondrial apoptosis. Mol Cell, 2001,8:705–711.
[37] Zong WX, Ditsworth D, Bauer DE, et al. Alkylating DNA damage stimulates a regulated form of necrotic cell death. Genes Dev, 2004,18:1272–1282.
[38] Kim KW, Mutter RW, Cao C, et al. Autophagy for cancer therapy through inhibition of pro-apoptotic proteins and mammalian target of rapamycin signaling. J Biol Chem, 2006,281:36883–36890.
[39] Buytaert E, Callewaert G, Hendrickx N, et al. Role of endoplasmic reticulum depletion and multidomain proapoptotic BAX and BAK proteins in shaping cell death after hypericin-mediated photodynamic therapy. FASEB J, 2006,20:756–758.
[40] Moretti L, Attia A, Kim KW, et al. Crosstalk between Bak/Bax and mTOR signaling regulates radiation-induced autophagy. Autophagy, 2007,3:142–144.
[41] Ullman E, Fan Y, Stawowczyk M, et al. Autophagy promotes necrosis in apoptosis-deficient cells in response to ER stress. Cell Death Differ, 2008,15:422–425.
[42] Letai A, Bassik MC, Walensky LD, et al. Distinct BH3 domains either sensitize or activate mitochondrial apoptosis, serving as prototype cancer therapeutics. Cancer Cell, 2002,2:183–192.
[43] Oltersdorf T, Elmore SW, Shoemaker AR, et al. An inhibitor of Bcl-2 family proteins induces regression of solid tumours. Nature, 2005,435:677–681.
[44] Vande Velde C, Cizeau J, Dubik D, et al. BNIP3 and genetic control of necrosis-like cell death through the mitochondrial permeability transition pore. Mol Cell Biol, 2000,20:5454–5468.
[45] Kanzawa T, Zhang L, Xiao L, et al. Arsenic trioxide induces autophagic cell death in malignant glioma cells by upregulation of mitochondrial cell death protein BNIP3. Oncogene, 2005,24:980–991.
[46] Oberstein A, Jeffrey P, Shi Y. Crystal structure of the Bcl-X_L-Beclin 1 peptide complex: Beclin 1 is a novel BH3-only protein. J Biol Chem, 2007,282:13123–13132.
[47] Suzuki K, Sorimachi H. A novel aspect of calpain activation. FEBS Lett, 1998,433:1–4.
[48] Goll DE, Thompson VF, Li H, et al. The calpain system. Physiol Rev, 2003,83:731–801.
[49] Suzuki K, Hata S, Kawabata Y, et al. Structure, activation, and biology of calpain. Diabetes, 2004,53 Suppl 1:S12–S18.
[50] Chen M, He H, Zhan S, et al. Bid is cleaved by calpain to an active fragment *in vitro* and during myocardial ischemia/reperfusion. J Biol Chem, 2001,276:30724–30728.
[51] Nakagawa T, Yuan J. Cross-talk between two cysteine protease families. Activation of caspase-12 by calpain in apoptosis. J Cell Biol, 2000,150:887–894.
[52] Wood DE, Thomas A, Devi LA, et al. Bax cleavage is mediated by calpain during drug-induced apoptosis. Oncogene, 1998,17:1069–1078.
[53] Bano D, Young KW, Guerin CJ, et al. Cleavage of the plasma membrane Na(+)/Ca(2+) exchanger in excitotoxicity. Cell, 2005, 120:275–285.
[54] Yamashima T. Ca^{2+}-dependent proteases in ischemic neuronal death: a conserved "calpain-cathepsin cascade" from nematodes to primates. Cell Calcium, 2004,36:285–293.
[55] Demarchi F, Bertoli C, Copetti T, et al. Calpain is required for macroautophagy in mammalian cells. J Cell Biol, 2006,175:595–605.
[56] Kastan MB, Zhan Q, el-Deiry WS, et al. A mammalian cell cycle checkpoint pathway utilizing p53 and GADD45 is defective in ataxia-telangiectasia. Cell, 1992,71:587–597.
[57] Lowe SW, Ruley HE, Jacks T, et al. p53-dependent apoptosis modulates the cytotoxicity of anticancer agents. Cell, 1993,74:957–967.
[58] Crighton D, Wilkinson S, O'Prey J, et al. DRAM, a p53-induced modulator of autophagy, is critical for apoptosis. Cell, 2006,126:121–134.
[59] Jones RG, Plas DR, Kubek S, et al. AMP-activated protein kinase induces a p53-dependent metabolic checkpoint. Mol Cell, 2005,18:283–293.
[60] Kong M, Fox CJ, Mu J, et al. The PP2A-associated protein alpha4 is an essential inhibitor of apoptosis. Science, 2004,306:695–698.
[61] Tasdemir E, Maiuri MC, Galluzzi L, et al. Regulation of autophagy by cytoplasmic p53. Nat Cell Biol, 2008,10:676–687.
[62] Morselli E, Tasdemir E, Maiuri MC, et al. Mutant p53 protein localized in the cytoplasm inhibits autophagy. Cell Cycle, 2008,7:3056–3061.
[63] Sherr CJ, Roberts JM. CDK inhibitors: positive and negative regulators of G1-phase progression. Genes Dev, 1999,13:1501–1512.
[64] Kamijo T, Weber JD, Zambetti G, et al. Functional and physical interactions of the ARF tumor suppressor with p53 and Mdm2. Proc Natl Acad Sci U S A, 1998,95:8292–8297.
[65] Weber JD, Jeffers JR, Rehg JE, et al. p53-independent functions of the p19 (ARF) tumor suppressor. Genes Dev, 2000,14:2358–2365.
[66] Sherr CJ, Bertwistle D, DEN Besten W, et al. p53-Dependent and -independent functions of the Arf tumor suppressor. Cold Spring Harb Symp Quant Biol, 2005,70:129–137.
[67] Reef S, Zalckvar E, Shifman O, et al. A short mitochondrial

form of p19ARF induces autophagy and caspase-independent cell death. Mol Cell, 2006,22:463–475.
[68] Deiss LP, Feinstein E, Berissi H, et al. Identification of a novel serine/threonine kinase and a novel 15-kD protein as potential mediators of the gamma interferon-induced cell death. Genes Dev, 1995,9:15–30.
[69] Cohen O, Kimchi A. DAP-kinase: from functional gene cloning to establishment of its role in apoptosis and cancer. Cell Death Differ, 2001,8:6–15.
[70] Jang CW, Chen CH, Chen CC, et al. TGF-beta induces apoptosis through Smad-mediated expression of DAP-kinase. Nat Cell Biol, 2002,4:51–58.
[71] Inbal B, Bialik S, Sabanay I, et al. DAP kinase and DRP-1 mediate membrane blebbing and the formation of autophagic vesicles during programmed cell death. J Cell Biol, 2002,157: 455–468.
[72] Zalckvar E, Berissi H, Mizrachy L, et al. DAP-kinase–mediated phosphorylation on the BH3 domain of beclin 1 promotes dissociation of beclin 1 from Bcl-XL and induction of autophagy. EMBO Rep, 2009,10:285–292.
[73] Gozuacik D, Bialik S, Raveh T, et al. DAP-kinase is a mediator of endoplasmic reticulum stress-induced caspase activation and autophagic cell death. Cell Death Differ, 2008,15:1875–1886.
[74] Smith GC, Jackson SP. The DNA-dependent protein kinase. Genes Dev, 1999,13:916–934.
[75] Douglas P, Sapkota GP, Morrice N, et al. Identification of in vitro and in vivo phosphorylation sites in the catalytic subunit of the DNA-dependent protein kinase. Biochem J, 2002,368:243–251.
[76] Chan DW, Ye R, Veillette CJ, et al. DNA-dependent protein kinase phosphorylation sites in Ku 70/80 heterodimer. Biochemistry, 1999,38:1819–1828.
[77] Qu X, Yu J, Bhagat G, et al. Promotion of tumorigenesis by heterozygous disruption of the beclin 1 autophagy gene. J Clin Invest, 2003,112:1809–1820.
[78] Woo, Jack MT, Xu Y, et al. DNA damage–induced apoptosis requires the DNA-dependent protein kinase, and is mediated by the latent population of p53. EMBO J, 2002,21:3000–3008.
[79] Mayo LD, Turchi JJ, Berberich SJ. Mdm-2 phosphorylation by DNA-dependent protein kinase prevents interaction with p53. Cancer Res, 1997,57:5013–5016.
[80] Kharbanda S, Pandey P, Jin S, et al. Functional interaction between DNA-PK and c-Abl in response to DNA damage. Nature, 1997,386:732–735.
[81] Bernstein C, Bernstein H, Payne CM, et al. DNA repair/pro-apoptotic dual-role proteins in five major DNA repair pathways: fail-safe protection against carcinogenesis. Mutat Res, 2002,511:145–178.
[82] Wang S, Guo M, Ouyang H, et al. The catalytic subunit of DNA-dependent protein kinase selectively regulates p53-dependent apoptosis but not cell-cycle arrest. Proc Natl Acad Sci U S A, 2000,97:1584–1588.
[83] Daido S, Yamamoto A, Fujiwara K, et al. Inhibition of the DNA-dependent protein kinase catalytic subunit radiosensitizes malignant glioma cells by inducing autophagy. Cancer Res, 2005,65:4368–4375.
[84] Wei Y, Pattingre S, Sinha S, et al. JNK1-mediated phosphorylation of Bcl-2 regulates starvation-induced autophagy. Mol Cell, 2008,30:678–688.
[85] Park KJ, Lee SH, Lee CH, et al. Upregulation of Beclin-1 expression and phosphorylation of Bcl-2 and p53 are involved in the JNK-mediated autophagic cell death. Biochem Biophys Res Commun, 2009,382:726–729.
[86] Li DD, Wang LL, Deng R, et al. The pivotal role of c-Jun NH2-terminal kinase–mediated Beclin 1 expression during anticancer agents-induced autophagy in cancer cells. Oncogene, 2009,28: 886–898.