

Self-eating and self-killing: crosstalk between autophagy and apoptosis

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Abstract | The functional relationship between apoptosis ('self-killing') and autophagy ('self-eating') is complex in the sense that, under certain circumstances, autophagy constitutes a stress adaptation that avoids cell death (and suppresses apoptosis), whereas in other cellular settings, it constitutes an alternative cell-death pathway. Autophagy and apoptosis may be triggered by common upstream signals, and sometimes this results in combined autophagy and apoptosis; in other instances, the cell switches between the two responses in a mutually exclusive manner. On a molecular level, this means that the apoptotic and autophagic response machineries share common pathways that either link or polarize the cellular responses.

Autophagy

In this context, autophagy is used synonymously with macroautophagy, a process in which a portion of the cytoplasm is engulfed by a specific membrane and later digested by lysosomal enzymes.

Programmed cell death

A genetically controlled cell-death process that is turned on in response to external or internal signals.

Two self-destructive processes, autophagy ('self-eating') (BOX 1) and apoptosis ('self-killing') (BOX 2), have captivated the imagination of scientists. Apoptosis is the best-described form of programmed cell death and involves the activation of catabolic enzymes — in particular proteases — in signalling cascades, which leads to the rapid demolition of cellular structures and organelles^{1,2}. When this process culminates in cellular shrinkage with nuclear chromatin condensation (pyknosis) and nuclear fragmentation (karyorrhexis), it complies with the morphological definition of apoptosis (also known as type I cell death)³.

During macroautophagy (hereafter referred to as autophagy), parts of the cytoplasm and intracellular organelles are sequestered within characteristic double- or multi-membraned autophagic vacuoles (named autophagosomes) and are finally delivered to lysosomes for bulk degradation. Autophagy is a highly regulated process that can either be involved in the turnover of long-lived proteins and whole organelles in a generalized fashion or can specifically target distinct organelles (for example, mitochondria in mitophagy and the endoplasmic reticulum (ER) in reticulophagy), thereby eliminating superfluous or damaged organelles^{4,5}. Thus, apoptosis and autophagy constitute the two processes through which superfluous, damaged or aged cells or organelles are eliminated. Beyond this homeostatic function, autophagy is also a process by which cells adapt their metabolism to starvation, imposed by decreased extracellular nutrients or by decreased intracellular metabolite concentrations that result from the loss of growth-factor signalling (which often governs the uptake of nutrients). By the

catabolism of macromolecules, autophagy generates metabolic substrates that meet the bioenergetic needs of cells and thereby allows for adaptive protein synthesis⁶.

The functional relationship between apoptosis and autophagy is complex in the sense that, in several scenarios, autophagy constitutes a stress adaptation that avoids cell death (and *de facto* suppresses apoptosis), whereas in other cellular settings, autophagy constitutes an alternative pathway to cellular demise that is called autophagic cell death (or type II cell death)^{7–12}. In general terms, it appears that similar stimuli can induce either apoptosis or autophagy. Whereas a mixed phenotype of autophagy and apoptosis can sometimes be detected in response to these common stimuli, in many other instances, autophagy and apoptosis develop in a mutually exclusive manner, perhaps as a result of variable thresholds for both processes, or as a result of a cellular 'decision' between the two responses that may be linked to a mutual inhibition of the two phenomena (FIG. 1). Recently, several pathways that link the apoptotic and autophagic machineries or that polarize the cellular response between autophagy and apoptosis have been deciphered at the molecular level. Here, we discuss which circumstances and which molecular pathways dictate the choice between autophagy and apoptosis. Moreover, we review the literature that links autophagy to either cytoprotection or cell death.

Autophagy when apoptosis is inhibited

BAX and **BAK** are two multidomain protein members of the BCL2 family that are required for mitochondrial outer membrane permeabilization (MOMP), which is

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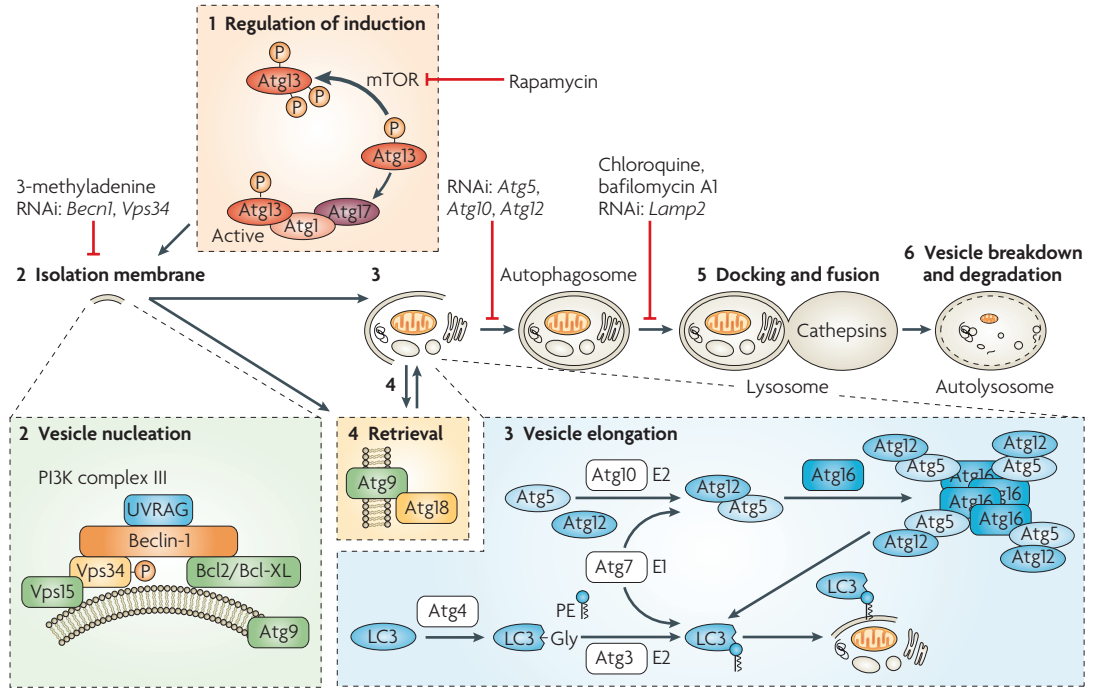
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Box 1 | Autophagy and its inhibitors



Lysosome

A membrane-surrounded sac that contains >50 acid hydrolases (phosphatases, nucleases, glycosidases, proteases, peptidases, sulphatases and lipases), which can digest all of the major macromolecules of the cell to breakdown products that are then available for metabolic reuse.

Catabolism

A metabolic state that leads to the degradation of macromolecules to yield small molecules and/or ATP, usually as a consequence of nutrient deprivation. Catabolic reactions include glycogenolysis, β -oxidation of fatty acids and autophagy.

BCL2 family

A family of proteins that contain at least one BCL2 homology (BH) region. The family is divided into anti-apoptotic multidomain proteins (such as BCL2, BCL-XL and MCL1), which contain four BH domains (BH1, BH2, BH3, BH4), pro-apoptotic multidomain proteins (for example, BAX and BAK), which contain BH1, BH2 and BH3, and the pro-apoptotic BH3-only protein family.

Mitochondrial outer membrane permeabilization (MOMP)

An apoptosis-associated process that results in apoptosis-inducing proteins (such as cytochrome *c*, apoptosis-inducing factor, Smac/Diablo) that are normally retained in the mitochondrial intermembrane space being released through the outer membrane into the cytosol.

Autolysosome

Also called autophagolysosome. A vesicle that is formed by the fusion of an autophagosome (or an amphisome) with a lysosome.

Autophagy starts with the stepwise engulfment of cytoplasmic material (cytosol and/or organelles) by the phagophore (also called isolation membrane), which sequesters material in double-membraned vesicles named autophagosomes (also called autophagic vacuoles). In many cellular settings, the first regulatory process (see figure, step 1) involves the de-repression of the mTOR Ser/Thr kinase, which inhibits autophagy by phosphorylating autophagy protein-13 (Atg13). This phosphorylation leads to the dissociation of Atg13 from a protein complex that contains Atg1 kinase and Atg17, and thus attenuates the Atg1 kinase activity. When mTOR is inhibited, re-association of dephosphorylated Atg13 with Atg1 stimulates its catalytic activity and induces autophagy. Notably, the mammalian orthologue of the yeast Atg13 has not been identified to date. Among the initial steps of vesicle nucleation is the activation of mammalian Vps34, a class III phosphatidylinositol 3-kinase (PI3K), to generate phosphatidylinositol-3-phosphate (PtdIns3P). Vps34 activation depends on the formation of a multiprotein complex in which beclin-1 (Becn1; the mammalian orthologue of Atg6), UVRAG (UV irradiation resistance-associated tumour suppressor gene) and a myristylated kinase (Vps15, or p150 in humans) participate.

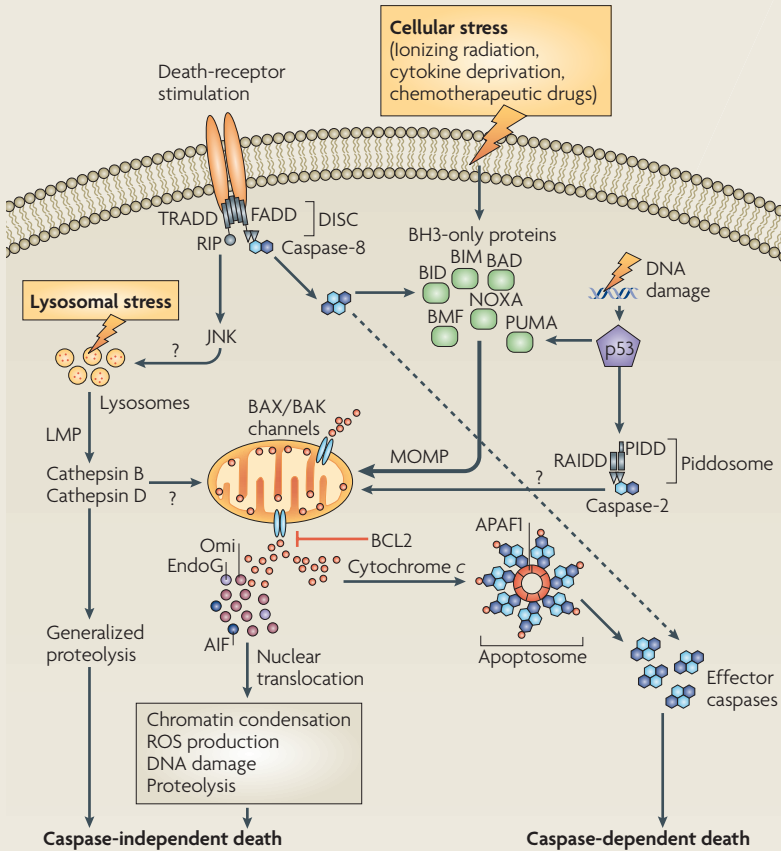
Two ubiquitin-like conjugation systems are part of the vesicle elongation process (step 3). One pathway involves the covalent conjugation of Atg12 to Atg5, with the help of the E1-like enzyme Atg7 and the E2-like enzyme Atg10. The second pathway involves the conjugation of phosphatidylethanolamine (PE) to LC3/Atg8 (LC3 is one of the mammalian homologues of Atg8) by the sequential action of the protease Atg4, the E1-like enzyme Atg7 and the E2-like enzyme Atg3. Lipid conjugation leads to the conversion of the soluble form of LC3 (named LC3-I) to the autophagosome-associated form (LC3-II). LC3-II is used as a marker of autophagy because its lipidation and specific recruitment to autophagosomes provides a shift from diffuse to punctate staining of the protein and increases its electrophoretic mobility on gels compared with LC3-I. Moreover, green fluorescent protein-LC3 fusion proteins can be used to visualize autophagosomes by fluorescence videomicroscopy⁹¹. The mechanism of retrieval in which the Atg9 complex participates is poorly studied (step 4).

Autophagosomes undergo maturation by fusion with lysosomes to create autolysosomes (steps 5 and 6). In the autolysosomes, the inner membrane as well as the luminal content of the autophagic vacuoles is degraded by lysosomal enzymes that act optimally within this acidic compartment^{4,92}. Pharmacological inhibitors and small interfering RNAs that are capable of inhibiting distinct steps of this process are shown (red blocking arrows). Bcl2 and Bcl-XL are regulators of beclin-1. Lamp2, lysosome-associated membrane glycoprotein-2.

one of the decisive steps in apoptotic cell death (BOX 2). As a result, mouse embryonic fibroblasts (MEFs) from double-knockout *Bax*^{-/-} *Bak*^{-/-} mice are resistant to a range of apoptosis inducers. When treated with DNA-damaging agents such as etoposide (a topoisomerase-2 inhibitor), *Bax*^{-/-} *Bak*^{-/-} MEFs failed to undergo apoptosis and instead manifested massive autophagy followed by delayed cell death. Knockdown of the essential

autophagy gene (Atg) products beclin-1 (*BECN1*; Atg6 in yeast) and *ATG5* reduced the etoposide-induced cell death of *Bax*^{-/-} *Bak*^{-/-} MEFs¹³. In this cellular setting, no autophagy was induced when apoptosis was inhibited by other perturbations, such as *Apaf1*^{-/-} and caspase-9^{-/-} MEFs or addition of the caspase inhibitor z-Val-Ala-Asp(OMe)-fluoromethylketone (Z-VAD-FMK) to wild-type MEFs. This suggests that the autophagic cell

Box 2 | Caspase-dependent and caspase-independent routes to cell death



Two main pathways lead to caspase-dependent apoptosis (see figure). The extrinsic pathway involves stimulation of members of the tumour necrosis factor receptor (TNFR) superfamily, such as CD95/Fas, TNFR or TRAILR (death receptors). The intrinsic pathway is characterized by mitochondrial outer membrane permeabilization (MOMP) and the release of mitochondrial cytochrome *c*, which results in assembly of a caspase-activating complex between caspase-9 and APAF1 (the apoptosome). Death-receptor stimulation typically results in the recruitment and activation of caspase-8 by the Fas-associated via death domain (FADD)/TNFR1-associated death domain protein (TRADD) to form a death-inducing signalling complex (DISC) that can further propagate death signals in three ways: via proteolysis of the BCL2 homology-3 (BH3)-only protein BID, which provokes translocation of truncated BID to mitochondria and consequent MOMP; by direct proteolytic activation of downstream effector caspases; or via activation of the kinase RIP⁹³. In the intrinsic pathway, a range of BH3-only proteins act as sentinels for cell stress, organelle-specific damage or infection, and can be mobilized via post-translational modification (such as proteolysis or phosphorylation) or subcellular relocalization to initiate MOMP. The BH3-only proteins stimulate MOMP by triggering the oligomerization of BAX and/or BAK in the outer mitochondrial membrane, thereby forming channels that permit the escape of multiple proteins from the mitochondrial intermembrane space^{26,56,57}. In the context of DNA damage, stabilization of the p53 tumour-suppressor protein can result in transcriptional activation of the BH3-only proteins (such as PUMA and NOXA) that can promote MOMP via the BAX/BAK channel⁵². Alternatively, DNA damage can activate caspase-2 in a multinuclear complex that involves the p53-induced protein with a death domain (PIDD) and the RIP-associated protein with a death domain (RAIDD) (together known as the piddosome)⁹⁴. Caspase-2 may then induce MOMP and/or direct caspase activation. Several factors among the mitochondrial proteins that are released as a result of MOMP (apoptosis-inducing factor (AIF), Omi, EndoG) can promote caspase-independent cell death⁹⁵, which can also result from stimuli that cause lysosomal membrane permeabilization (LMP), resulting in the release of cathepsin proteases into the cytosol⁹. Such cathepsins can also trigger MOMP, thereby stimulating the mitochondrial pathway of apoptosis. JNK, c-Jun N-terminal kinase; LMP, lysosomal membrane permeabilization; ROS, reactive oxygen species.

death of *Bax*^{-/-} *Bak*^{-/-} MEFs is specifically triggered by the absence of BAX and BAK¹³. This could imply that BCL2-family proteins such as BAX and BAK may have direct or indirect roles in regulating autophagy that are independent from their role in modulating apoptosis (for a cautionary note, see BOX 3).

In other cellular settings, other types of perturbations in the apoptotic machinery, such as caspase inhibition, have also been reported to induce autophagic cell death. Chemical inhibition of cysteine proteases, for example with Z-VAD-FMK, can cause autophagic cell death in selected cell types (such as the L929 mouse fibrosarcoma and the human Jurkat T cell lymphoma). This effect is likely to involve the combined inhibition of one or several caspases, including caspase-8, as well as that of a protease from another class of cysteine proteases, most probably a calpain¹⁴. Also, in U937 monocytoid cells and macrophages that were treated with lipopolysaccharide, the inhibition of cysteine proteases with Z-VAD-FMK induced autophagic cell death, which was attenuated by the RNA interference (RNAi)-mediated knockdown of beclin-1 (REF. 15). Interestingly, cell death induced by Z-VAD-FMK is associated with the autophagy-mediated depletion of the reactive oxygen species (ROS)-detoxifying enzyme catalase and is inhibited by depletion of *ATG7* and beclin-1 (REFS 16,17).

Notably, when cells are exposed to stress signals that cause decreased intracellular metabolite concentrations (resulting from the loss of growth-factor signalling)⁶, the autophagy that arises from the inhibition of apoptosis actually protects cells from cell death. In this respect, it was elegantly shown that immortalized interleukin-3 (IL3)-dependent cell lines generated from the bone marrow of *Bax*^{-/-} *Bak*^{-/-} mice failed to undergo apoptosis upon IL3 withdrawal (which is the default pathway of wild-type cells) and entered a month-long autophagic process that caused a severe reduction in cell size with the removal of most of the cytoplasm. Inhibition of autophagy by knockdown of *Atg5* or *Atg7* or by the addition of 3-methyladenine (an inhibitor of the phosphatidylinositol 3-kinase *Vps34*) or chloroquine (an inhibitor of lysosomal acidification, which, in turn, is required for fusion between autophagosomes and lysosomes; BOX 1) killed *Bax*^{-/-} *Bak*^{-/-} cells in this setting, which indicates that autophagy functions as a survival mechanism^{6,18}. Autophagy in this cellular setting is the catabolic process that mobilizes nutrients by macromolecular degradation, thus replenishing the vanishing energy reserves of the starving cell and, hence, preventing a bioenergetic catastrophe that would otherwise culminate in cell death¹⁸.

Altogether, these data suggest that the removal or functional inhibition of essential proteins from the apoptotic machinery can de-inhibit autophagy or switch a cellular stress response from the apoptotic default pathway to a state of massively increased autophagy. However, there are also many examples in which apoptosis only develops when autophagy is inhibited (see below), which underscores the notion that both catabolic phenomena can inhibit each other.

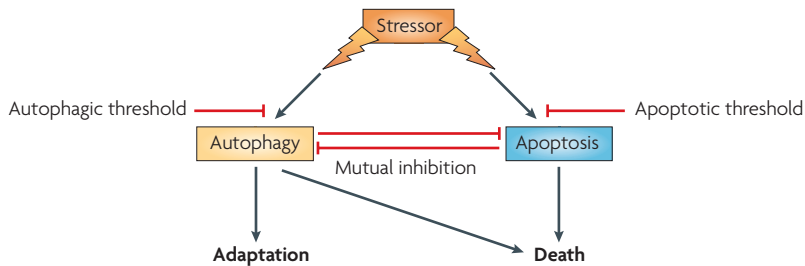


Figure 1 | The relationship between apoptosis and autophagy. Similar stressors can induce either apoptosis or autophagy in a context-dependent fashion. It is possible that different sensitivity thresholds, the exact nature of which remain to be determined, can dictate whether autophagy or apoptosis will develop. Alternatively, the choice between apoptosis and autophagy is influenced by the fact that the two catabolic processes exhibit some degree of mutual inhibition. In some cases, a mixed phenotype of apoptosis and autophagy can be detected at the single-cell level. Although autophagy mostly allows cells to adapt to stress, massive autophagy can also kill cells.

Apoptosis due to inhibited autophagy

When HeLa or HCT116 cancer cells are cultured in the absence of nutrients, they rapidly induce autophagy in order to recycle essential metabolites, such as lipids and amino acids, for fuelling the bioenergetic machinery. In these circumstances, the inhibition of autophagy results in an accelerated cell death that manifests hallmarks of apoptosis including chromatin condensation, MOMP and activation of caspases¹⁹. This cell death can be postponed to some extent by depletion of BAX and BAK or by the inhibition of caspases, indicating that it is indeed apoptotic¹⁹. Notably, the phenotype of the cells (as observed before they undergo apoptosis) is profoundly influenced by the stage at which autophagy is inhibited. When autophagy is blocked at an early stage by depletion of beclin-1, ATG5, ATG10, ATG12 or VPS34, no autophagic vacuoles are found in the cells, which undergo a typical type I cell death. By contrast, when the fusion of autophagosomes and lysosomes is blocked by the addition of lysosomal inhibitors (such as chloroquine or the vacuolar ATPase inhibitor bafilomycin A) or by depletion of the lysosomal protein LAMP2, autophagic vacuoles accumulate and the cells manifest a mixed type I–type II morphology before the cells succumb to death^{19,20}. This result shows that the inhibition of autophagy, either at early or late stages of the process, may lead to apoptosis as a result of the failure to adapt to starvation. Moreover, it illustrates that autophagic vacuolization is not always the result of enhanced autophagy but may also result from the failed elimination of autophagic debris.

In vivo experiments confirmed that the inactivation of Atg genes can cause cell death. The neuron-specific knockout of *Atg5* or *Atg7* causes neurodegeneration, accumulation of cytoplasmic inclusion bodies and apoptotic death of neurons^{21,22}. T-cell-specific knockout of *Atg5* also results in the increased apoptosis of mature T cells in peripheral organs, which correlates with an enhanced susceptibility to cell death induced by stimulation of the T-cell receptor²³. Similarly, depletion of beclin-1 during *Caenorhabditis elegans* development increased caspase-dependent apoptosis and the number

of apoptotic cell corpses²⁴. The mechanisms through which the inhibition of autophagy may favour cell death (Supplementary information S1 (table)) are not entirely clear. It is possible that the inhibition of autophagy results in a bioenergetic shortage that triggers apoptosis^{6,9}. Thus, the absence of redox equivalents may favour oxidative reactions that trigger apoptosis, presumably through a direct effect on mitochondria, and the absence of NADPH₂ and ATP may de-inhibit the activation of caspase-2 or MOMP^{25,26}. In a more subtle fashion, inhibition of autophagy may subvert the capacity of cells to remove damaged organelles or to remove misfolded proteins⁵, which, in turn, would favour apoptosis.

It should be noted that inhibition of autophagy does not always induce cell death by apoptosis, and that other types of cell death may also result from autophagy inhibition²⁷. For example, malignant glioma cells that are treated with DNA-damaging agents (such as etoposide and temozolomide) exhibit a surge in autophagy that, when inhibited, causes non-apoptotic cell death with micronucleation²⁸. Similarly, cancer cells in which both the apoptotic and the autophagic programs are suppressed die from necrosis²⁹.

Autophagy-induced cytoprotection

Autophagy-induced cytoprotection is the result of the basic cellular functions of autophagy in eukaryotic cells on the one hand, and the inhibitory effects that autophagy exerts on apoptosis under stress conditions on the other hand.

Autophagy-mediated removal of protein aggregates.

Basal autophagy in non-stressed cells might be essential for neurons because these cells do not grow and cannot reduce the per-cell amount of waste, such as protein aggregates, by cell division. Indeed, mutant mice in which ATG5 or ATG7 is depleted develop neurodegeneration with cytoplasmic inclusion bodies that contain protein aggregates^{21,22}. Intracellular protein substrates that are cleared by autophagy include mutant huntingtin (which causes *Huntington's disease*), proteins that are mutated in certain spinocerebellar ataxias, forms of α -synuclein that are mutated in familial forms of *Parkinson's disease*, and tau mutants that cause frontotemporal dementia or tauopathy. Accordingly, stimulation of autophagy by rapamycin or its analogue CCI-779 (two inhibitors of mTOR; BOX 1) reduced the levels of soluble and aggregated huntingtin and attenuated its toxicity in cells, as well as in transgenic *Drosophila melanogaster* and mouse models of Huntington's disease^{30,31}. Similarly, treatment with lithium, which stimulates mTOR-independent autophagy, promoted aggregate clearance in cells and in *D. melanogaster* models of Huntington's disease³².

It is noteworthy that mutant huntingtin can recruit beclin-1 and impair the beclin-1-mediated long-lived protein turnover in neuronal cells³³. This implies that, as humans age and beclin-1 levels decline while the levels of aggregate-prone proteins accumulate, the system of autophagic homeostasis must collapse at a given point, leading to irreversible dysfunction and/or death of neurons^{5,34}.

Caspase

One of a family of cysteine proteases that cleave after aspartate residues in substrate proteins. Initiator caspases are typically activated in response to a specific triggering event (for example, caspase-8 upon death-receptor ligation, caspase-9 upon apoptosome activation, caspase-2 upon DNA damage), whereas effector caspases (mainly caspase-3, -6 and -7) are important for the ordered dismantling of vital cellular structures.

Reactive oxygen species (ROS). Reduced derivatives of molecular oxygen (O₂), including classical oxygen radicals and peroxides, which are formed within cells.

Death receptor

One of a family of cell-surface receptors that mediate cell death upon ligand-induced trimerization. The best-studied members include tumour necrosis factor receptor-1 (TNFR1), FAS (or CD95, which binds FAS ligand) and two receptors for TNF-related apoptosis-inducing ligand (TRAILR1 and -R2).

Cytochrome c

A haem protein that is normally confined to the mitochondrial intermembrane space. Upon induction of apoptosis, cytochrome c is released from mitochondria and triggers the formation of the apoptosome, a caspase activation complex.

Apoptosome

A complex that forms when cytochrome *c* is released from mitochondria and interacts with the cytosolic protein APAF1, which, in turn, recruits pro-caspase-9. In the presence of ATP, this interaction results in the allosteric activation of caspase-9 and in the subsequent activation of the effector caspase-3.

Apoptosis-inducing factor

(AIF). A flavoprotein that is normally present in the mitochondrial intermembrane space. Following apoptosis induction, AIF translocates to the nucleus where it activates a molecular complex that causes large-scale DNA fragmentation, presumably in a caspase-independent fashion.

Lysosomal membrane permeabilization

(LMP). A perturbation of lysosomal membrane function, leading to the translocation of lysosomal hydrolases (including cathepsins) from the lysosomal lumen to the rest of the cell. LMP can be induced by endogenous signal transducers (such as reactive oxygen species and sphingosine) as well as by lysosomotropic drugs.

Autophagy-mediated removal of harmful organelles.

Induction of autophagy may have a general apoptosis-inhibitory effect beyond the removal of potentially harmful (and hence apoptogenic) protein aggregates. For example, pre-treatment of cells with rapamycin (which induces autophagy) can cause a decrease in the mitochondrial mass by ~50% while reducing the susceptibility of cells to MOMP-dependent apoptotic stimuli (BOX 2). In this system, inhibition of autophagy by depletion of ATG7 suppressed this cytoprotective action of rapamycin *in vitro*, in human cells³⁵. Similar data have been recapitulated *in vivo* in *D. melanogaster*. Whereas pre-treatment with rapamycin reduced the lethal effect of the mitochondrial ROS generator paraquat in wild-type *D. melanogaster*, presumably by reducing the mitochondrial mass, a loss-of-function mutation of ATG1 abolished the life-rescuing effect of rapamycin³⁵.

A recent report suggests that mitophagy may be particularly important for inhibiting cell death (see below). Indeed, mitochondria that have undergone MOMP (and, in that respect, appear to be irreversibly damaged) can be removed by mitophagy in cells that have been stressed by agents, such as actinomycin D or staurosporin, in the presence of caspase inhibitors. One factor that stimulates mitophagy in this context is glyceraldehyde phosphate dehydrogenase (GAPDH)³⁶. When overexpressed, GAPDH can rescue cells from death in the context of failed caspase activation (either due to the addition of caspase inhibitors or as a result of *APAF1* knockout) and with several different lethal stimuli, including transient treatment with staurosporin, actinomycin D or etoposide, or transfection with the human adenovirus protein E1A plus the small GTPase Ras³⁶. To mediate this effect, GAPDH must be enzymatically active in glycolysis and, in addition, must mediate the transcriptional activation of ATG12, thereby increasing mitophagy and causing a transient decline of mitochondrial mass³⁶. Whether GAPDH

functions as a regulator of cell death and autophagy in a (patho)physiological context, perhaps as a sensor of glycolytic intermediates, remains an open conundrum for future investigation.

Autophagy and cell-cycle arrest in cell-death inhibition.

Recently, the cyclin-dependent kinase inhibitor p27 has been shown to be a critical link between induction of autophagy and inhibition of apoptosis in response to nutrient depletion. Metabolic stress results in the decline of ATP:ADP ratios with a subsequent increase in AMP concentrations, which stimulates the activity of the AMP-dependent kinase (AMPK), a Ser/Thr kinase that is also activated by the *Peutz-Jeghers syndrome* gene product LKB1. The LKB1-AMPK pathway-dependent phosphorylation of p27 at Thr198 stabilizes the cyclin-dependent kinase inhibitor p27, which permits cells to survive growth-factor withdrawal and metabolic stress through autophagy instead of undergoing apoptosis³⁷. These findings point to an important link between cell cycle and autophagy regulation that awaits further study.

Altogether, these examples demonstrate how autophagy can prevent apoptosis and show the metabolic pathways through which cytoprotective autophagy can be stimulated.

Autophagy-mediated cellular suicide

Autophagy can protect cells against death, but it can also mediate cellular demise, depending on the specific circumstances. How does autophagy kill a cell? The cytotoxic effects of autophagy may be explained by the direct self-destructive potential of massive autophagy (type II cell death) or, alternatively, by hardwiring of the autophagic process to pro-apoptotic signals (type I cell death).

Autophagic killing. In the first scenario, autophagy destroys large proportions of the cytosol and organelles that, beyond a certain threshold, would cause irreversible cellular atrophy with a consequent collapse of vital cellular functions. Indeed, during extensive autophagy, the volume that is occupied by autophagic vacuoles and dense bodies may be roughly equal to, or greater than, that of 'free' cytosol and organelles^{7,18,38}. It is therefore plausible that such a degree of cellular destruction could lead to cellular demise.

Notably, cell killing through autophagy has also been documented in some *in vivo* model systems. In the salivary glands of *D. melanogaster*, a striking autophagic phenotype is followed by apoptotic DNA fragmentation. In this model, expression of the caspase inhibitor p35 or loss-of-function mutations of a caspase (*drice*) or APAF1 (*ark*) partially prevents cell loss. This further suggests that, even though apoptosis is part of the mechanisms through which the cells are finally destroyed, autophagy can also mediate cell death as a genuine effector mechanism^{7,39}. In another recent study, transgenic overexpression of ATG1 in the *D. melanogaster* system caused a reduction of cell size and final cell loss, which was blocked by deletion of ATG3 or ATG8a. In this system, cell loss was accompanied by an apoptotic morphology and was delayed by expression of p35 (REF. 40).

Box 3 | One protein, multiple functions: against molecular reductionism

Many cell-death researchers assumed that the sole function of a whole series of proteins — including the BCL2-family proteins, caspases and their activators — was the regulation of apoptosis. However, recent work has revealed that caspases regulate normal activation and differentiation processes^{96,97} and that BCL2 proteins may regulate neural plasticity⁹⁸. Even in *Caenorhabditis elegans*, it was found that the sole BCL2 homology-3 (BH3)-only protein EGL-1, which is required for developmental cell death, is also required for non-lethal autophagy induced by starvation⁵⁸. Similarly, an essential component of the caspase activation complex, CED-4 (the nematode equivalent of APAF1), has a non-apoptotic role in the DNA-damage checkpoint⁹⁹.

Some of the Atg proteins, which are required for autophagy, may also have unsuspected roles outside of the autophagic process. One prominent example is ATG5, which, under certain circumstances, is proteolytically activated to become a pro-apoptotic molecule that translocates to mitochondria and triggers mitochondrial outer membrane permeabilization (MOMP)⁵¹. Another example is beclin-1, which bears a BH3 domain^{58,62} that might favour apoptosis, at least in some circumstances. As a result, it may be an oversimplification to assume that the inhibition of cell death in response to DNA damage or the activation of apoptosis/necrosis during nutrient deprivation (which both result from the knockout or knockdown of a single Atg gene) is due to suppression of the autophagic process. Several genes from different modules of autophagy should be knocked down independently to find out whether the resulting phenotype is similar before final conclusions are drawn. The same holds for experiments in which apoptotic pathways have been interrupted by knocking out or knocking down single genes.

Autophagy as a trigger of apoptosis or necrosis. In the second scenario, autophagy develops as a primary response to stress stimuli and then triggers either apoptosis or necrotic cell death that kills the cell. For example, CD4/CXCR4-expressing T cells that interact with cells expressing HIV-1-encoded envelope glycoproteins first manifest autophagy and then apoptosis. In this model, depletion of beclin-1 or ATG7 inhibits apoptosis⁴¹.

Induction of autophagy may also cause necrotic cell death. Lenardo and co-workers showed that catalase, a key enzyme of the cellular antioxidant defence mechanism, was selectively eliminated during autophagic cell death and that catalase depletion caused necrotic cell death, which could be prevented by autophagy inhibition as well as antioxidants¹⁷. Thus, catalase may exemplify an essential cytoprotector, the elimination of which by autophagy causes irreversible cellular damage and hence death. Interestingly, in the same system, serum-starvation-induced autophagy did not lead to the elimination of catalase nor to cell death, indicating that catalase degradation is strongly tied to cell death and depends on the upstream signal causing autophagy¹⁷. Discovery of additional cytoprotective autophagy targets may clarify how autophagic catabolism can constitute a lethal event.

Autophagy as a guardian of the genome? The discovery that beclin-1 is a haploinsufficient tumour-suppressor gene^{42,43} does not necessarily imply that the relevant mechanism is lack of cell death due to impaired autophagy. Rather, the tumour-suppressive function of beclin-1 may be linked to the ability of autophagy to protect cells from DNA damage. Along these lines, reduced autophagy might trigger oncogenic events such as chromosomal aberrations and mutations by deregulating the turnover of centrosomes (which results in multipolar division and chromosomal instability) or by compromising the quality control of mitochondria (which increases ROS production and ROS-mediated DNA damage). Indeed, deletion of one *BECN1* allele or knockout of *ATG5* causes a failure to resolve DNA-damage foci after metabolic stress, and results in numerical and structural chromosomal instability provided that the cells are transfected with BCL2 (REF. 44). So, tumour promotion by reduction or inactivation of an autophagic gene may, paradoxically, result from the breakdown of the cytoprotective mechanisms of autophagy.

The concept that invalidation of the autophagic process would cause resistance to cell death is currently not supported by genetic experiments in mammalian systems. The field requires the development of mouse model systems that use a conditional knockout approach to delete autophagic genes specifically in the tissues that display autophagy-mediated cellular atrophy¹¹.

Common upstream triggers

Several signal transduction pathways that are triggered by common cellular stress as well as more specific signalling pathways can elicit both autophagy and apoptosis.

General stress mediators: ROS, ceramide and Ca²⁺. ROS can favour pro-apoptotic MOMP as well as stimulate the proteolytic activity of ATG4, thereby stimulating autophagy⁴⁵. The sphingolipid ceramide is a prominent apoptosis inducer that acts through the intrinsic pathway, but it can also stimulate autophagy⁴⁶. Interestingly, another sphingolipid, sphingosine-1 phosphate, is a potent inhibitor of ceramide-induced apoptosis⁴⁷ but also induces autophagy⁴⁶, which indicates how the intracellular milieu can contribute to ‘deciding’ between the two processes.

Increases in free Ca²⁺ ion concentrations in the cytosol ([Ca²⁺]_c), Ca²⁺ efflux from the ER and Ca²⁺ overload of mitochondria all constitute prominent pro-apoptotic signals⁴⁸. In addition, [Ca²⁺]_c increases can trigger autophagy, presumably by activating calmodulin-dependent kinase kinase-β. This activates AMPK, which then inhibits mTOR, thereby de-inhibiting autophagy⁴⁹. However, this is not the only pathway that links increases in [Ca²⁺]_c to catabolism. [Ca²⁺]_c is a strong stimulator of calpains, which might contribute to autophagy⁵⁰ or apoptosis⁵¹. These findings underscore the extent to which the apoptotic cell death- and autophagy-inducing pathways can be intermingled by intracellular signals.

The role of p53. The transcription factor p53 is a quintessential tumour suppressor and apoptosis inducer. However, accumulating evidence suggests that p53 has a positive role in cell survival in response to physiological (as opposed to genotoxic) stress; for example, by stimulating antioxidant pathways⁵² and autophagy⁵³. In one study, p53 was linked to autophagy through mTOR inhibition via AMPK and the tumour suppressors tuberous sclerosis-1 (TSC1) and TSC2 (REF. 54). In addition, p53 has been shown to transactivate DRAM (damage-regulated autophagy modulator), a lysosomal protein that can stimulate the accumulation of autophagic vacuoles⁵⁵ (FIG. 2). Knockdown of *DRAM* abolishes the accumulation of autophagic vacuoles induced by p53 and also reduces the induction of apoptosis⁵⁵.

BH3-only proteins and BH3 mimetics. BCL2 homology-3 (BH3) domains are present in all members of the BCL2 family. They can bind to BH3 receptors (which are present in the multidomain members of the BCL2 family), thereby inhibiting the anti-apoptotic BCL2-family members (such as BCL2 and BCL-XL) or activating the pro-apoptotic BCL2-family members (such as BAX and BAK)^{56,57}.

Pharmacological BH3 mimetics such as ABT737 (REF. 58) and HA14-1 (REF. 59) can induce autophagy in cells that do not undergo apoptosis or before they undergo apoptosis. In this context, inhibition of autophagy by knockdown of *BECN1* promotes apoptosis; conversely, inhibition of caspases promotes autophagy^{58,59}. The autophagy induced by ABT737 specifically concerns mitochondria and not the ER⁵⁸. Endogenous BH3-only proteins also induce autophagy. The BH3-only protein BAD, which is known to be activated by the withdrawal of obligate growth factors (presumably by dephosphorylation⁵⁷), is involved in the induction of autophagy

Cathepsin

A protease that localizes mainly to lysosomes and lysosome-like organelles. Cathepsin proteases can be divided into three subgroups according to their active-site amino acid: cysteine (cathepsins B, C, H, F, K, L, O, S, V, W and X/Z), aspartate (cathepsins D and E) and serine (cathepsin G).

Haploinsufficient

A gene that requires biallelic expression to produce the amount of protein that is sufficient to guarantee a normal biological function.

Tumour-suppressor gene

A gene that, when eliminated or inactivated, is permissive for the development of cancers. These genes often determine cell-cycle checkpoints or facilitate the induction of programmed cell death.

p53

A transcription factor that is activated by numerous genotoxic insults to induce cell-cycle arrest, cellular senescence or apoptosis. p53 is frequently mutated or functionally inactivated in cancer.

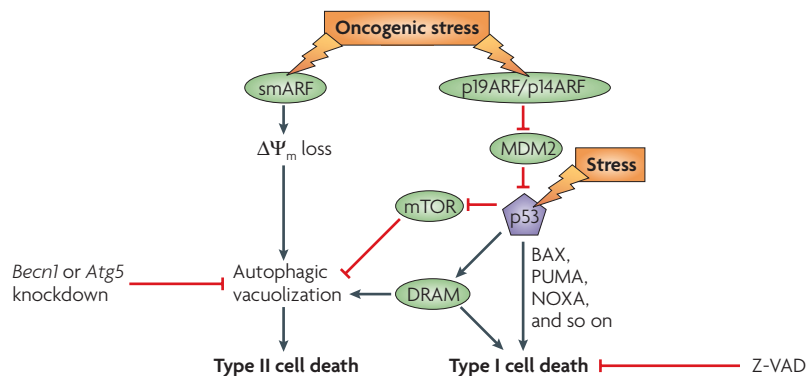


Figure 2 | Regulation of autophagy and apoptosis by p53 and p19ARF/p14ARF. Oncogenic stress can activate two different isoforms of murine p19ARF (and of the human orthologue p14ARF). One isoform corresponds to the full-length nucleolar p19ARF/p14ARF and the second one corresponds to a shorter form, smARF, which results from internal initiation of translation. Whereas smARF translocates to mitochondria, reduces the mitochondrial transmembrane potential ($\Delta\Psi_m$) and stimulates autophagy and caspase-independent cell death, the nucleolar p19ARF/p14ARF activates p53 by binding and inhibiting the negative regulator of p53, MDM2. p53 induces caspase-dependent apoptotic cell death via the induction of multiple pro-apoptotic proteins, most of which function in the intrinsic pathway. Whereas the nucleolar pathway is inhibited by Z-VAD, smARF-induced cell death is blocked by knocking down beclin-1 (*Becn1*) or *Atg5*. In some cellular settings, activated p53 induces a transcriptional programme that favours the accumulation of autophagic vacuoles via the induction of the lysosomal protein DRAM (damaged-regulated autophagy modulator). p53-elicited DRAM may be essential, both for autophagic vacuolization and for apoptosis.

by serum and nutrient depletion because knockout or knockdown of *BAD* blunts the autophagic response of cells that are placed into nutrient-free culture conditions⁵⁸. Another BH3-only protein, BNIP3, can induce autophagy in malignant glioma cells that have been treated with ceramide⁶⁰ and in cardiomyocytes after ischaemia reperfusion⁶¹.

How are these autophagy-inducing effects achieved? BH3-only peptides trigger apoptosis either by activating the MOMP-triggering activity of BAX or BAK, or by inhibiting the anti-apoptotic activity of BCL2, BCL-XL or their homologues^{56,57}. Beclin-1 has recently been identified as a novel BH3-only protein^{58,62}. Like other BH3-only proteins, beclin-1 interacts with anti-apoptotic multidomain proteins of the BCL2 family (in particular BCL2 and its homologue BCL-XL) via its BH3 domain, an amphipathic α -helix that binds to the hydrophobic cleft of BCL2 or BCL-XL^{58,62}. The BH3 domains of other BH3-only proteins such as BAD, as well as BH3-mimetic compounds such as ABT737, competitively disrupt the interaction between beclin-1 and BCL2 or BCL-XL^{58,63}, thus liberating beclin-1 from an inhibitory complex and allowing it to allosterically activate VPS34, a lipid kinase that stimulates autophagy⁶⁴ (FIG. 3).

Death-associated protein kinase (DAPK) family. DAPK (also named DAPK1) is a calcium/calmodulin-regulated Ser/Thr death-promoting kinase⁶⁵. DAPK1 controls either type I apoptosis or type II autophagic cell death in response to various stimuli including interferon- γ , activation of Fas/CD95 receptors, tumour necrosis factor- α (TNF α), transforming growth factor- β (TGF β),

detachment from the extracellular matrix and oncogenes⁶⁶⁻⁷⁰. DAPK1 is a prominent tumour suppressor⁶⁶ and its expression is lost in many tumours, mainly owing to DNA methylation⁶⁵. In addition, a germ-line predisposition to chronic lymphatic leukaemia caused by a mutation in the promoter region of DAPK1 has recently been discovered⁷¹. DAPK1 can induce apoptosis by several pathways: activation of p19ARF (which inhibits the negative regulator of p53, MDM2) and hence p53 in MEFs⁶⁶; inhibition of integrin-mediated adhesion in NIH/3T3 cells⁶⁷; and stimulation of p53-independent, mitochondrion-dependent apoptosis in Hep3B hepatoma cells⁶⁸.

The DAPK1 relatives DAPK-related protein kinase-1 (DRP1; also known as DAPK2) and ZIP-kinase (or DAPK3) can also participate in controlling both apoptosis and autophagic cell death^{69,70}. Physical interactions and a complex cascade-like signalling connection that amplifies both processes exist between the members of DAPK family⁷⁰. Notably, these kinases differ in their intracellular localization; whereas DAPK1 is mainly associated with the actin cytoskeleton, DRP1 is highly concentrated in the lumen of autophagic vesicles upon its ectopic expression. So, the DAPK family of proteins seems to consist of stress-activated kinases that link different cellular stresses to type I and type II cell death⁶⁵.

p19ARF/p14ARF and their shorter isoforms. The murine nucleolar p19ARF and its human orthologue p14ARF are potent apoptotic inducers that bind to and inhibit MDM2, thereby activating the death-promoting functions of p53. A shorter isoform of p19ARF/p14ARF localizes to mitochondria. This isoform, smARF, is generated by internal translation at Met45, which removes the nucleolar functional domains. When ectopically expressed, smARF can provoke the dissipation of the mitochondrial transmembrane potential ($\Delta\Psi_m$) without MOMP, followed by autophagy and caspase-independent cell death that is partially inhibited by knockdown of *Atg5* and *Becn1* (REF. 72) (FIG. 2).

Organellar stress in apoptosis or autophagy

Autophagy can mediate the specific removal of stressed or damaged cytoplasmic organelles, including mitochondria and the ER.

Mitochondrial stress. Mitochondria can undergo the so-called permeability transition (PT), a sudden increase in the permeability of the inner membrane that causes the dissipation of $\Delta\Psi_m$ and, subsequently, MOMP. Mitochondria that undergo PT are usually removed by specific autophagy (mitophagy)⁷³, provided that PT only affects a fraction of mitochondria; for example, when the stimulus that induces PT (such as Ca²⁺ overload) is provided at a low subapoptotic intensity. When PT affects a large fraction of mitochondria, it causes cell death. This was initially documented in hepatocytes in which individual mitochondria that lose $\Delta\Psi_m$ colocalize rapidly with lysosomal markers. In hepatocytes, the inhibition of autophagy sensitizes cells to apoptosis, presumably due to the failure to keep permeabilized mitochondria in check⁷³. These findings

Oncogene
A gene of which overexpression or gain-of-function mutation contributes to oncogenesis.

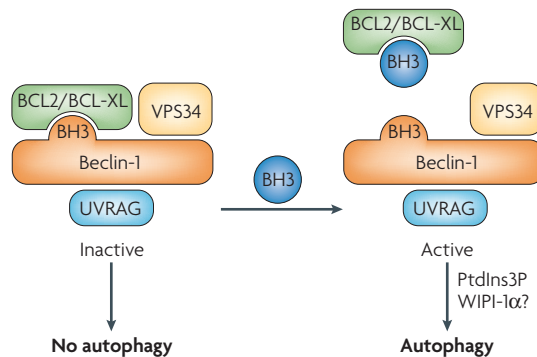


Figure 3 | BH3 proteins and mimetics act on the beclin-1-BCL2 interaction. Proteins that contain BCL2 homology-3 (BH3) domains or small molecules that mimic BH3 domains can bind to the BH3-receptor domain of BCL2 or BCL-XL and, hence, competitively disrupt the interaction between BCL2 or BCL-XL and beclin-1. This probably leads to the activation of the lipid kinase activity of the class III phosphatidylinositol 3-kinase VPS34 (which depends on the interaction with UVRAG (UV irradiation resistance-associated tumour suppressor gene), thereby provoking the production of phosphatidylinositol-3-phosphate (PtdIns3P). In turn, this leads to vesicle nucleation in a manner that probably involves WIPI-1 α (WD-repeat protein interacting with phosphoinositides).

are reminiscent of those obtained for BNIP3, a BH3-only protein induced by hypoxia that causes $\Delta\Psi_m$ dissipation⁷⁴ and mitophagy⁶¹, as well as for ABT737, which induces specific mitophagy⁵⁸. In these cases, mitophagy serves as a stop-gap mechanism to limit cellular damage.

In other systems, mitophagy in itself can have fatal consequences. In cultured neuroblastoma cells and primary neurons, induction of MOMP with the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine causes mitophagy and subsequent non-apoptotic death that is inhibited by knockdown of *ATG5*, *ATG7* and *LC3*, yet is insensitive to the knockout of *BECN1* and the inhibition of VPS34 (REF. 75).

ER stress. Stress that affects the ER is widely known to induce apoptosis through the intrinsic pathway⁷⁶. ER stress is also a particularly efficient stimulus of autophagy that specifically affects the ER (this is known as reticulophagy)⁷⁷. ER stress is usually caused by the accumulation of incorrectly folded proteins in the ER lumen, giving rise to the unfolded protein response (UPR)⁷⁸. The three main transducers of the UPR in multicellular organisms are inositol-requiring protein-1 (IRE1), protein kinase RNA (PKR)-like ER kinase (PERK) and activating transcription factor-6 (ATF6), all of which sense the presence of unfolded proteins in the ER lumen and transduce signals to the nucleus or cytosol. ER stress can be induced by overexpressing proteins that contain polyglutamine tracts or by drugs such as tunicamycin (an inhibitor of *N*-glycosylation), thapsigargin (an inhibitor of the ER Ca²⁺ ATPase) or brefeldin A (which disrupts the ER-to-Golgi trafficking). Overexpression of BCL2 or BCL-XL fails to prevent autophagy that is induced by thapsigargin or tunicamycin⁷⁹, although both proteins can inhibit the

ER stress response by a direct interaction with IRE1 (REF. 80) and can inhibit apoptosis induced by ER stress⁸¹. In one study, it was found that MEFs that lack IRE1 α (but not those that lack PERK or ATF6) were unable to mount an autophagic response to ER stress⁸². ER stress induced by polyQ72 aggregates caused autophagy in a fashion that was inhibited by dominant-negative PERK, as well as replacement of the PERK substrate eIF2 α by a non-phosphorylatable mutant (eIF2 α S51A)⁸³.

Altogether, the most recent data indicate that ER stress is a major stimulator of an autophagic response that participates in the degradation of unfolded proteins and in the removal of superfluous ER membranes. Consistent with this cytoprotective mechanism, it has been found that in many cellular settings, inhibition of autophagy that is induced by ER stress (for example, with 3-methyladenine, *BECN1* or *LC3* knockdown and *ATG5* knockout) increased apoptosis^{82,84}. Yet, in some cases, especially in normal untransformed cells, inhibition of autophagy instead protected the cells from cell death. This suggests that the ER-stress-elicited autophagy may be either cytoprotective or lethal, depending on the cellular context^{82,84}.

Polarization between autophagy and apoptosis

As discussed above, autophagy and apoptosis share many common inducers. Yet in many instances, cross-inhibitory interactions between apoptosis and autophagy may cause polarization between the two processes. The type of the initiating stimulus (stressors in FIGS 1, 4b, 5) might determine which process will dominate. For example, during nutrient deprivation the default pathway would be autophagy, which creates a metabolic state with high (or increased)²⁸ ATP that is anti-apoptotic. Successful removal of the damaged organelles followed by repair and adaptation would allow for survival, while failure to restore homeostasis would result in delayed apoptosis. By contrast, the default pathway that is triggered by other signals such as DNA damage or death-receptor activation would be immediate apoptosis, which occurs in a rapid, self-amplifying process, precluding simultaneous autophagic responses. Moreover, several precise molecular events may account for the polarization between apoptosis and autophagy.

ATG5 in autophagy and apoptosis. Overexpression of *ATG5* can induce autophagy and also enhance the susceptibility of tumour cells to activate the intrinsic cell-death pathway, for instance by ceramide or DNA-damaging agents⁵¹. Knockout or knockdown of *ATG5* abolishes autophagy and reduces the incidence of apoptotic events in human cancer cells treated with staurosporin or with the anthracycline doxorubicin⁵¹. Indeed, *ATG5* may have a dual role in autophagy and apoptosis. This dual function is regulated by the proteolysis of *ATG5* (FIG. 4a). Upon lethal stress, the 33-kDa full-length *ATG5* protein is cleaved by calpains to remove the C terminus, which generates a 24-kDa fragment. This fragment loses its autophagy-inducing activity and instead acquires a pro-apoptotic one. The *ATG5* fragment (but not full-length *ATG5*) translocates to mitochondria where it favours

Unfolded protein response (UPR). A signalling pathway that is activated in response to stress in the endoplasmic reticulum (ER). The UPR can cope effectively with stress by reducing the amount of misfolded protein overload in the ER.

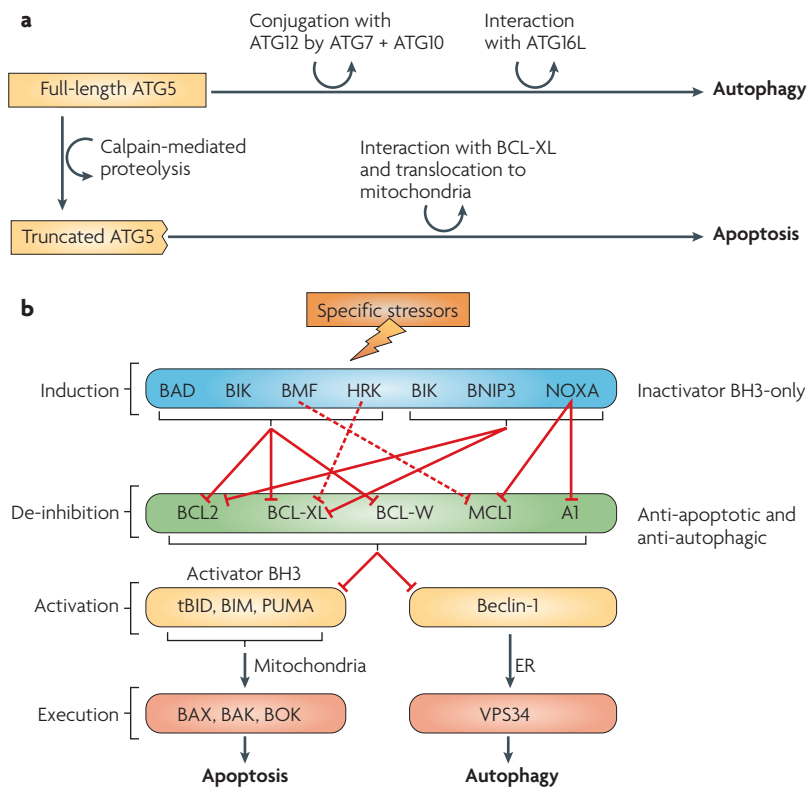


Figure 4 | Molecular switches between apoptosis and autophagy. **a** | Dual function of autophagy protein-5 (ATG5) in autophagy. Full-length ATG5 can participate in the initial stages of autophagy with the help of a ubiquitin-like conjugation system. ATG12 (activated by ATG7 and then transferred to ATG10) is conjugated with ATG5 at a lysine- ϵ -amino group, allowing it to bind to ATG16. The resulting ATG12–ATG5–ATG16 complex imposes curvature on the crescent phagophore and recruits activated LC3 to the elongating membrane (LC3 is one of the mammalian homologues of Atg8, which is conjugated to phosphatidylethanolamine by the action of ATG4, ATG7 and ATG3) (BOX 1). Proteolysis by a calpain results in a truncated ATG5, which can translocate to mitochondria and induce MOMP. **b** | BCL2 homology-3 (BH3)-only proteins as inducers of apoptosis and autophagy. Depending on their specificity (for pro- or anti-apoptotic and autophagy-inhibitory proteins that contain BH3 receptors), as well as their preferential subcellular localization (mitochondria or endoplasmic reticulum), BH3-only proteins can preferentially activate apoptosis or autophagy. A specific class of inactivator BH3-only protein interacts with anti-apoptotic and anti-autophagic multidomain proteins of the BCL2 family (red blocking arrows symbolize preferential affinities), thereby releasing activator BH3-only proteins that allosterically activate BAX and BAK (for the induction of apoptosis). Alternatively, they release beclin-1 from its inhibitory interaction with multidomain proteins of the BCL2 family, allowing it to activate the lipid kinase activity of VPS34 and, hence, to activate autophagy. Several steps in this working model still need further experimental validation.

MOMP, as shown in a cell-free system. MOMP induction by the 24-kDa fragment may be linked to its capacity to interact with (and, possibly, to inhibit) BCL-XL (REF. 51).

In another scenario, ATG5 may interact directly with FADD (Fas-associated via death domain) through its C terminus, thereby stimulating caspase-dependent death⁸⁵. The interaction with FADD would be required for apoptotic cell-death induction but not for vacuolization⁸⁵. Thus, ATG5 could trigger apoptosis through several mechanisms and be part of the molecular mechanisms that govern the inhibitory crosstalk between apoptosis and autophagy (FIG. 4a).

Interaction between BCL2 and beclin-1. The interaction between beclin-1 and its binding partners regulates the initial steps of autophagy^{4,8}. The association of beclin-1 with mammalian VPS34 and another protein, UVRAG (UV irradiation resistance-associated tumour suppressor gene), is essential for the induction of autophagy, and UVRAG stimulates the interaction between VPS34 and beclin-1 as well as the lipid kinase activity of VPS34 (REF. 86). Importantly, both beclin-1 and UVRAG⁸⁶ may act as tumour suppressors, although it is not known whether this activity is truly linked to the modulation of autophagy or to unrelated functions. Ambra-1 is another autophagy-stimulatory beclin-1-interacting protein that stimulates the capacity of beclin-1 to activate VPS34 (REF. 87).

The autophagy-inducing activity of beclin-1 is inhibited by multidomain proteins of the BCL2 family, including BCL2 itself, BCL-XL and MCL1 (REFS 58,88). BCL2 and its homologues are likely to inhibit autophagy by a direct physical interaction with beclin-1, because beclin-1 mutations that abolish its interaction with BCL2 or BCL-XL confer a gain-of-function phenotype with respect to beclin-1-mediated autophagy and abrogate its inhibition by BCL2 or BCL-XL. Similarly, mutations in BCL-XL that prevent its interaction with beclin-1 also eliminate its capacity to antagonize autophagy induction by beclin-1 (REFS 58,88).

The autophagy-inhibitory effects of BCL2 or BCL-XL depend on their subcellular localization. Only ER-localized (but not mitochondrial) BCL2 or BCL-XL inhibit autophagy induced by starvation, beclin-1 (REF. 88), BH3 mimetics⁵⁸, inhibition of inositol trisphosphate (InsP₃) receptors⁷⁹ or autophagy-stimulatory Ca²⁺ fluxes⁴⁹. Moreover, only beclin-1–BCL2/BCL-XL complexes that are present in the ER (but not those present on heavy membrane fractions enriched in mitochondria) are disrupted by ABT737 or by starvation⁵⁸. These findings suggest that the beclin-1–BCL2/BCL-XL complexes that normally inhibit autophagy are specifically located in the ER, which points to an organelle-specific regulation of autophagy. Furthermore, these data suggest a spatial organization of autophagy and apoptosis control in which BH3-only proteins exert two separate functions, depending on their subcellular localization. On the one hand, they induce apoptosis by (directly or indirectly) activating MOMP. On the other hand, they can activate autophagy by liberating beclin-1 from its inhibition by BCL2 or BCL-XL at the level of the ER.

In apparent contrast to this hypothesis, however, it appears that BNIP3 and SPIN1, both of which are BH3-only proteins that preferentially (but not exclusively) localize to mitochondria, are potent inducers of autophagy and — in some settings — non-apoptotic cell death^{74,89}. So, the question as to how BH3 proteins preferentially induce autophagy or apoptosis remains unresolved. Nonetheless, one might predict that BH3-only proteins that directly target the pro-apoptotic members of the BCL2 family should induce apoptosis only (by triggering MOMP), whereas those that target anti-apoptotic and anti-autophagic BCL2 proteins may induce both apoptosis and autophagy (FIG. 4b). Thus, the decision between autophagy and apoptosis might be determined

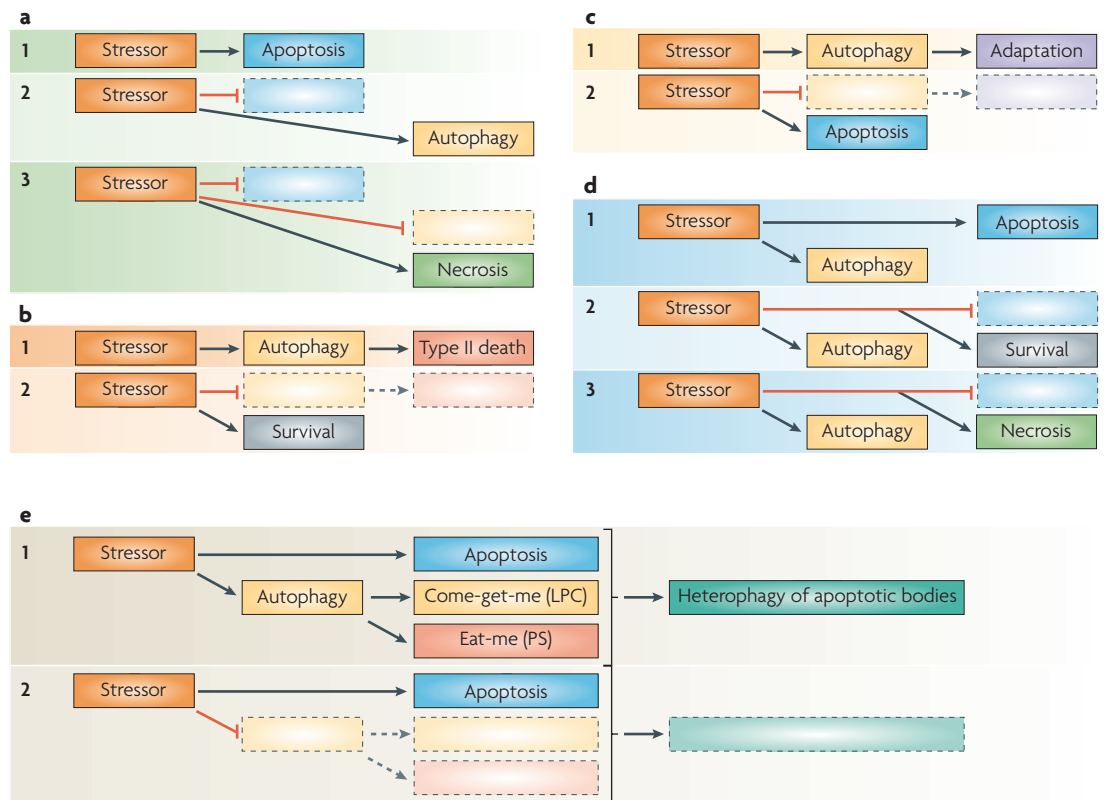


Figure 5 | Scenarios for the interaction between apoptosis and autophagy. **a** | Schematically, a particular set of insults induces apoptosis (part 1), which, if inhibited, can switch to autophagy. At least in some cellular settings, autophagy serves as a defence mechanism that prevents or retards necrosis (parts 2,3). **b** | Some conditions can trigger a lethal autophagic response that is responsible for cell death, for example, in naïve cells (parts 1,2) or in cells in which the apoptotic pathways have been interrupted. **c** | Another set of stimuli (or perhaps simply a lower dose of insults) provokes a protective autophagic response (part 1), which is required for adaptation of the cell and the avoidance of apoptosis (part 2). **d** | Frequently, lethal conditions trigger an autophagic response that, independently of the autophagic response, is followed by apoptosis (part 1). In this case, inhibition of apoptosis causes either cell survival (part 2) or necrosis (part 3). In this scenario, the order of events (autophagy, then apoptosis) is chronological, not hierarchical, meaning that inhibition of autophagy does not prevent apoptosis. **e** | Autophagy can be indispensable for sustaining the high ATP levels that are required for cells to emit signals to phagocytic cells that engulf the apoptotic bodies (part 1). Inhibition of autophagy does not affect apoptotic cell death, yet it abolishes the heterophagic removal of apoptotic material (part 2). LPC, lysophosphatidylcholine; PS, phosphatidylserine.

by the organellar localization of BCL2 or BCL-XL, which is regulated by post-translational modifications such as phosphorylation.

Conclusions and perspectives

Undoubtedly, there are multiple connections between the apoptotic and autophagic processes, and the two phenomena jointly seal the fate of the cell. Here, we have discussed multiple connections between autophagy and cell death that may occur in a hierarchical (FIG. 5a–c) or independent fashion. Frequently, autophagy occurs before, but independently from, apoptosis. This ordered sequence of autophagy and apoptosis (FIG. 5d) may serve either of two purposes, in teleological terms. First, autophagy may constitute a mechanism through which the cell destined to die initiates its catabolism, thus accelerating the disappearance of the cell. Second, autophagy may also help to maintain optimal, high ATP levels that may facilitate the apoptotic process.

Recent data have been obtained regarding embryonic cavitation, which is the earliest programmed cell-death process in mammalian development. These data indicate that autophagy is required for the maintenance of high ATP levels, which, in turn, may serve to ensure the efficient clearance of apoptotic cells. Thus, cells contained in the inner mass of the embryoid bodies undergo normal apoptosis even when they lack *Becn1* or *Atg5*. However, such cells fail to secrete the ‘come-get-me’ signal lysophosphatidylcholine and do not display signs of phosphatidylserine exposure (the ‘eat-me’ signal), which suggests that they are not engulfed by neighbouring cells and remain within the lumen of the embryoid body⁹⁰ (FIG. 5e). Strikingly, this phenomenon is observed for *Becn1*^{-/-} embryos *in vitro* and *in vivo* (with consequent early embryonic lethality), but for *Atg5*^{-/-} embryos only *in vitro* but not *in vivo* (with near-to-normal embryonic development), which casts some doubt on the general applicability of these findings. However, *Atg5*^{-/-} embryos

Heterophagy

Phagocytosis of a cell by another cell. Heterophagy has an important role in the efficient removal of apoptotic corpses. Efficient heterophagy is indispensable for avoiding inflammatory responses that are triggered by apoptotic material.

do manifest a deficient clearance of apoptotic cells; for example, in the lung and retina⁹⁰. Thus, as a possibility, autophagy-dependent optimal clearance of apoptotic cells could prevent a detrimental inflammatory response both during normal development and after exposure to pathological stimuli.

The distinction between the aforementioned pathways is not trivial because it dictates how pharmacological strategies that induce cell death, protect cells or switch between cell-death modalities will be designed, case by case. For this, the following problems await urgent solution.

First, although it is relatively easy to target the core proteins of the apoptotic machinery (such as caspases), so far no pharmacological inhibitors (that would act with an acceptable specificity) are available for the experimental or therapeutic inhibition of autophagy. Thus, specific inhibitors of 'druggable' Atg proteins (such as VPS34 and the ATG4 protease) are urgently needed, both for the experimental manipulation of autophagy and as lead compounds for future drugs.

Second, it is clear that the propensity of cells to undergo apoptosis is determined by a cornucopia of factors (such as caspases and their endogenous inhibitors,

as well as a multiplicity of MOMP-inducing and MOMP-inhibitory factors). But are there proteins (or non-proteinaceous molecules) that determine the threshold of autophagic responses beyond BCL2-like proteins? Such threshold regulators might constitute important pharmacological targets, in part because compounds that influence threshold effects might have fewer side-effects than inhibitors of the autophagic core machinery.

Third, it will be essential to gather more information on the possible non-autophagic roles of Atg proteins and autophagy modulators because this could predict the possible side-effects of pharmacological agents that target Atg proteins and autophagy. Thus, the ideal pharmacological target for modulation of the autophagic pathway would not be involved in any metabolic or signal-transducing pathways other than autophagy.

Finally, the current knowledge on the molecular intersections between the autophagic and apoptotic pathways is incomplete and fragmented. Systems-biology approaches should yield precious information on the cross-regulation, hierarchy and interdependence between both catabolic phenomena and, hence, open the way to new concepts and novel strategies of intervention.

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Competing interests statement

The authors declare no competing financial interests.

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OMIM: <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM>
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ATG5 | ATG7 | BAK | BAX | BECN1 | p19ARF | Vps34

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