Chapter 11
The Cross Talk Between Apoptosis and Autophagy

Megan M. Young and Hong-Gang Wang

Abstract The cross talk between autophagy and apoptosis is complex. Autophagy serves as a cytoprotective mechanism in response to stress to generate nutrients during starvation, remove damaged proteins and organelles during metabolic stress, and eliminate intracellular pathogens. Alternatively, autophagy can promote cell death by serving as an independent cell death mechanism or by enabling the induction of apoptosis. While the induction of "autophagic cell death" remains controversial, the molecular cross talk between autophagic and apoptotic pathways is evident and functions to dynamically maintain cellular homeostasis and respond to stress. This chapter summarizes the molecular regulators of the cross talk between apoptosis and autophagy, including the Bcl-2 protein family; mediators of the extrinsic apoptotic pathway; the Beclin 1-interacting molecules Ambra 1 and Bif-1; several autophagy proteins; the transcription factors p53, E2F1, and NF-κB; PI3K/Akt/mTOR and JNK signal transduction pathways; and microRNAs. Collectively, these molecules function at multiple levels, from direct protein–protein interactions to transcriptional regulation, to control the interplay between apoptosis and autophagy and maintain cellular homeostasis. Molecular mediators of the cross talk between apoptosis and autophagy are continuously being identified; and ultimately, a greater understanding of the cross talk between apoptosis and autophagy will be critical for enhancing the efficacy of anticancer therapies.

Keywords Autophagy • Apoptosis • Cross talk • Bcl-2 • Beclin 1 • Ambra 1 • Bif-1 • iDISC • p53 • NF-κB • microRNA • mTOR • JNK • Cancer

M.M. Young • H.-G. Wang (✉)
Department of Pharmacology and Penn State Hershey Cancer Institute, The Pennsylvania State University College of Medicine, 500 University Drive, Hershey, PA 17033, USA
e-mail: huw11@psu.edu

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1 Functional Relationship Between Autophagy and Apoptosis

The functional cross talk between autophagy and apoptosis is complex (Fig. 11.1). Autophagy is an evolutionarily conserved stress response that antagonizes apoptosis to promote cell survival under various cellular settings, such as starvation, metabolic stress, or intracellular infection. Indeed, *Atg* gene knockout is observed to enhance apoptotic cell death in *Caenorhabditis elegans* and in mice (Takacs-Vellai et al. 2005; Hara et al. 2006; Komatsu et al. 2006; Pua et al. 2007). Moreover, dysfunctional autophagy is associated with a number of human diseases, including cancer, neurodegeneration, microbial infection, and aging (Mizushima et al. 2008). In contrast, under other cellular conditions, autophagy can promote cell death by serving as an alternate cell death mechanism or by enabling the induction of apoptosis. For example, mouse embryonic fibroblasts (MEFs) from *Bax/Bak* double-knockout mice fail to undergo apoptosis in response to the DNA-damaging agent etoposide and instead are observed to induce massive autophagy followed by *Atg* gene-dependent cell death (Shimizu et al. 2004). Additionally, autophagy has been

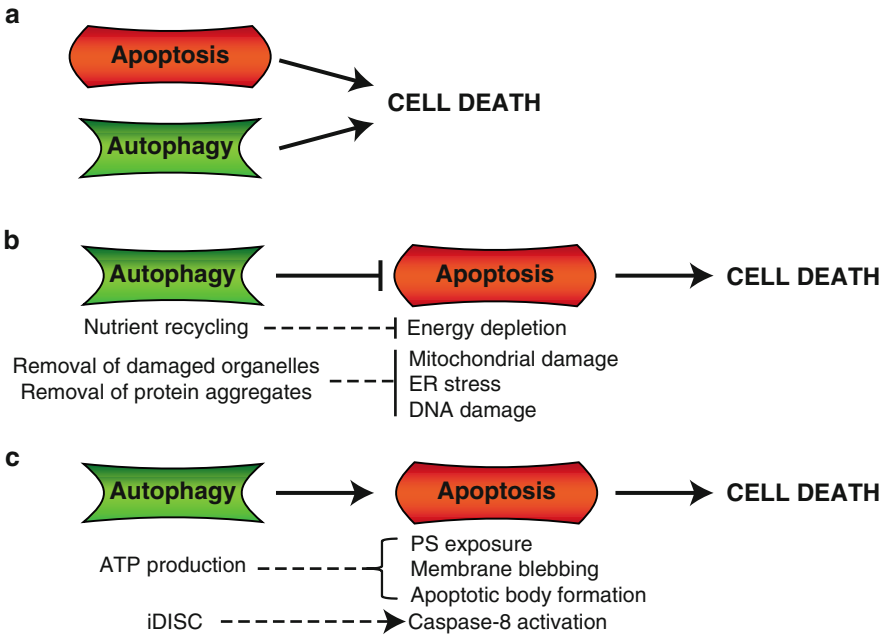

Fig. 11.1 Schematic representation of the functional relationship between autophagy and apoptosis. (**a**) Apoptosis and autophagy function as independent, parallel partners for the induction of cell death. (**b**) Autophagy antagonizes apoptosis through the recycling of nutrients, and the removal of damaged organelles and protein aggregates. (**c**) Autophagy enables apoptosis by providing ATP for phosphatidylserine (PS) exposure, membrane blebbing, and apoptotic body formation. Alternatively, autophagosomal membranes serve as platforms for the formation of an intracellular death-inducing signaling complex (iDISC), caspase-8 activation, and the initiation of apoptosis

reported to be essential for the induction of apoptosis during Drosophila development (Berry and Baehrecke 2007; Mohseni et al. 2009) as well as in mammalian cells during T-cell proliferation (Bell et al. 2008), human adenovirus infection (Jiang et al. 2011), and exposure to interferon-gamma (Pyo et al. 2005) as well as several pharmacological inhibitors (Laussmann et al. 2011; Young et al. 2012). However, the possibility that Atg proteins may have autophagy-independent roles must also be considered and closely investigated when attempting to dissect the roles of autophagy in cell death.

While the induction of "autophagic cell death" remains an area of intense debate (Kroemer and Levine 2008), it is evident that autophagy and apoptosis are not mutually exclusive processes. Rather, the autophagic and apoptotic machinery assemble an intricate signaling network capable of acting in synergy as well as antagonism to maintain cellular homeostasis (Maiuri et al. 2007c; Giansanti et al. 2011). Here, we discuss several of the molecular mediators in the interplay between apoptosis and autophagy. Ultimately, we hope to demonstrate how an understanding of the cross talk between apoptosis and autophagy can have a significant clinical impact for enhancing the efficacy of anticancer therapies.

2 Apoptosis

Apoptosis is a widely studied cell death program that is characterized by several hallmark morphological features: cell shrinkage, nuclear fragmentation, membrane blebbing, and generation of apoptotic bodies. Notably, the induction of apoptosis is mediated by a family of cysteine proteases, known as caspases. Caspases are synthesized as inactive precursors and undergo proteolytic maturation and activation in response to extracellular (extrinsic) or intracellular (intrinsic) signals (Fulda and Debatin 2006; Li and Yuan 2008).

2.1 Extrinsic Pathway

The extrinsic pathway is initiated upon the binding of extracellular death ligands [tumor necrosis factor (TNF), Fas ligand, and TNF-related apoptosis-inducing ligand (TRAIL)] to cell surface death receptors belonging to the TNF receptor superfamily (Fulda and Debatin 2006). Ligation of the receptors stimulates receptor clustering for the assembly of the death-inducing signaling complex (DISC). Notably, Fas, TRAIL receptor 1, and TRAIL receptor 2 induce a DISC composed of the adaptor protein Fas-associated death domain (FADD) and procaspase-8, while the DISC of TNF receptor 1 signaling contains the additional signaling molecules, TNFR-associated death domain (TRADD) and TNFR-associated factor 2 (TRAF2). DISC formation promotes procaspase-8 oligomerization for auto-activation through self-cleavage and the initiation of the caspase cascade.

2.2 *Intrinsic Pathway*

The intrinsic pathway is initiated in response to intracellular signals, such as DNA damage or cytotoxic stress (Fulda and Debatin 2006). These signals trigger mitochondrial outer membrane permeabilization (MOMP) for the release of apoptogenic factors to the cytosol. Upon release from the mitochondria, cytochrome *c* associates with procaspase-9 and apoptotic protease-activating factor 1 (Apaf-1) in a multiprotein complex known as the apoptosome. Furthermore, the release of second mitochondria-derived activator of caspase (Smac/DIABLO) and OMI/HTRA2 promote caspase activation through the neutralization of inhibitor of apoptosis proteins (IAPs). Ultimately, the extrinsic and intrinsic pathways converge upon the activation of effector caspases (caspase-3, -6, -7), which cleave cytosolic and nuclear substrates to execute the apoptotic program.

3 Molecular Cross Talk Between Autophagy and Apoptosis

3.1 *Intrinsic Pathway and Autophagy*

The Bcl-2 family of proteins provides a molecular link between the intrinsic pathway of apoptosis and autophagy (Fig. 11.2). Bcl-2 proteins contain at least one of the four conserved Bcl-2 homology (BH) domains (BH1–4) and undergo

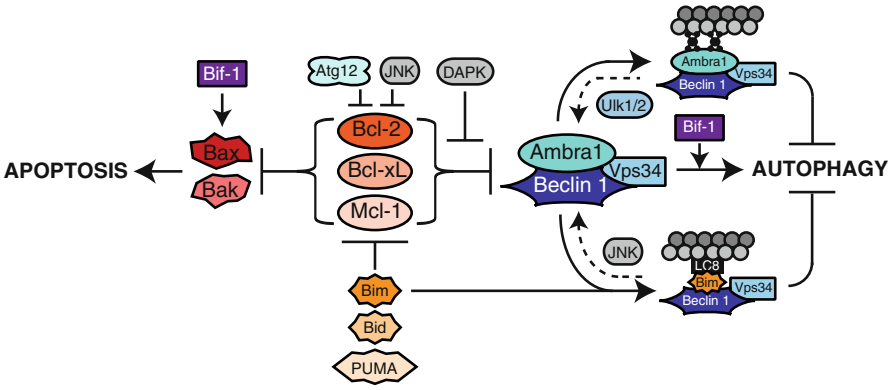

Fig. 11.2 Beclin 1-interacting proteins regulate the cross talk between apoptosis and autophagy. Antiapoptotic Bcl-2 family proteins (Bcl-2, Bcl-xL, Mcl-1) suppress apoptosis and autophagy through the inhibition of proapoptotic effector proteins (Bax, Bak) and Beclin 1, respectively. Activation of JNK and DAPK promote autophagy by liberating Beclin 1. Additionally, Ambra 1 and Bim suppress autophagy by sequestering Beclin 1 at the cytoskeleton. Activation of ULK1/2 or JNK releases Beclin 1 for the induction of autophagy. Bif-1 positively regulates Bax/Bak activation and autophagosome formation. Finally, Atg12 interacts with antiapoptotic Bcl-2 proteins to sensitize cells to cell death

dimerization through BH3 domain-dependent interactions (Chipuk et al. 2010). Furthermore, the protein family is categorized according to their BH domain organization: (1) proapoptotic effector proteins containing BH1–BH3 domains, (2) antiapoptotic proteins containing BH1–BH4 domains, and (3) proapoptotic BH3-only proteins. Activation of the proapoptotic effector proteins, Bax and Bak, triggers a conformational change that promotes homo-oligomerization within the outer mitochondrial membrane for the induction of MOMP. Antiapoptotic Bcl-2 proteins (Bcl-2, Bcl-xL, and Mcl-1) bind and inhibit the effector proteins to preserve mitochondrial integrity. In contrast, proapoptotic BH3-only proteins promote MOMP through the inhibition of antiapoptotic proteins (e.g., Bad and Noxa) and/or the direct activation of Bax and Bak (e.g., Bid, Bim, and Puma).

Beclin 1, the mammalian ortholog of Atg6, is a positive regulator of class III type phosphoinositide 3-kinase (PI3KC3/Vps34). The core PI3KC3 complex (Beclin 1–Vps34–Vps15) mediates the nucleation of autophagosomal membranes during the initiation of autophagy. Notably, Beclin 1 is a BH3-only protein that is directly antagonized by the binding of Bcl-2/Bcl-xL (Oberstein et al. 2007). Importantly, it is believed that only an endoplasmic reticulum pool of Bcl-2/Bcl-xL is responsible for the inhibition of autophagy, thus suggesting that the antiautophagic and antiapoptotic effects of Bcl-2/Bcl-xL are spatially regulated within the cell (Pattingre et al. 2005; Maiuri et al. 2007b). The Beclin 1:Bcl-2/Bcl-xL complex is disrupted in response to signals that induce autophagy. For example, the calcium/calmodulin-regulated serine/threonine kinase death-associated protein kinase (DAPK) phosphorylates Thr 119 within the BH3 domain of Beclin 1 to release Beclin 1 and stimulate autophagy (Wei et al. 2008; Zalckvar et al. 2009). Interestingly, DAPK is a tumor suppressor that promotes apoptosis by several pathways, including the activation of p19ARF, a negative regulator of the p53-destabilizing oncogene MDM2 (Raveh et al. 2001). In addition, the stress-activated kinase c-Jun N-terminal kinase 1 (JNK1) phosphorylates several residues within the nonstructural loop of Bcl-2 to displace Beclin 1 (Wei et al. 2008; Zalckvar et al. 2009). Moreover, degradation of the antiapoptotic protein Mcl-1 has been suggested to be an early event for the activation of autophagy (Germain et al. 2011).

Proapoptotic BH3-only proteins (Bad, BNIP3) and BH3-mimetics also stimulate the release of Beclin 1 for autophagy induction by competitively binding Bcl-2 or Bcl-xL (Maiuri et al. 2007a; Lian et al. 2011). BNIP3-mediated autophagy is reported to occur in response to hypoxia as well as ceramide treatment (Daido et al. 2004; Hamacher-Brady et al. 2007). Moreover, the BNIP3 homolog, BNIP3L/NIX, is a well-established regulator of mitophagy, or the selective degradation of mitochondria by autophagy, during erythroid cell development (Schweers et al. 2007). Interestingly, a recent report has demonstrated that inactive Bim inhibits autophagy by mislocalizing Beclin 1 to microtubules (Luo et al. 2012). Upon nutrient starvation, Bim is phosphorylated and activated for apoptosis by JNK, consequently disrupting its association with dynein light chain 1 (DYNLL1/LC8) and releasing Beclin 1 for the induction of autophagy (Luo et al. 2012). Therefore, Bim is capable of functioning as both an apoptosis inducer and autophagy inhibitor under different cellular conditions. Collectively, the Bcl-2 family of proteins dynamically regulates the cross talk between apoptosis and autophagy.

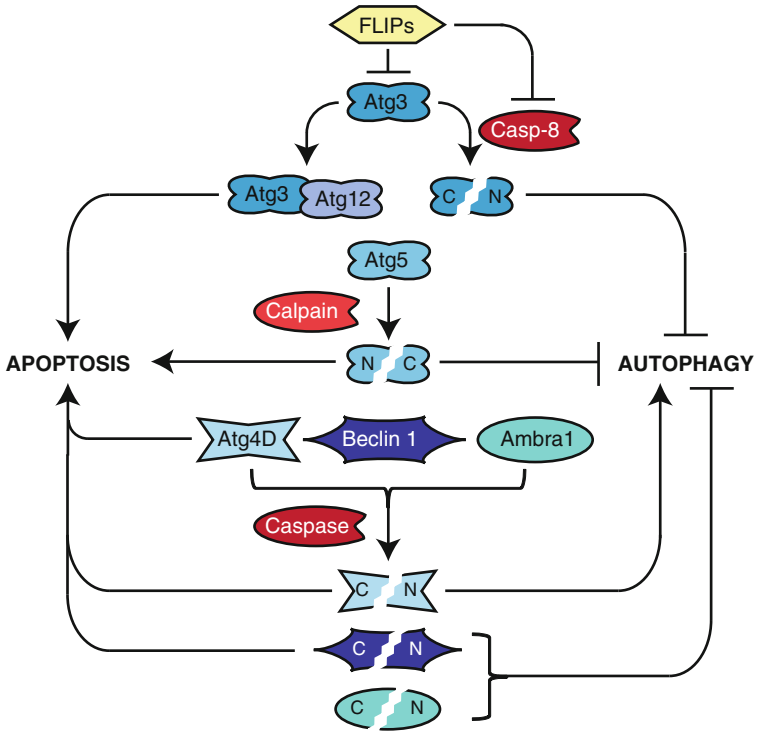

Fig. 11.3 Autophagic proteins exert proapoptotic functions. The calpain-mediated cleavage of Atg5 and caspase-mediated cleavage of Atg3, Beclin 1, and Ambra 1 generate protein fragments that are unable to induce autophagy. Notably, cleavage fragments of Atg5 and Beclin 1 localize to the mitochondria to enhance apoptosis. In addition, the cleavage fragment of Atg4D exhibits enhanced autophagic activity, while Atg4D exhibits cytotoxic effects independent of caspase cleavage through its recruitment to mitochondria and putative C-terminal BH3 domain. Atg3 also serves as a substrate for Atg12 conjugation to generate an Atg12–Atg3 conjugate that sensitizes cells to apoptosis. In addition to inhibiting caspase-8 activation, FLIPs bind Atg3 to prevent LC3 modification and suppress autophagy

3.2 Extrinsic Pathway and Autophagy

Several mediators of the extrinsic pathway of apoptosis also regulate the interplay between autophagy and apoptosis (Fig. 11.3). Atg3 serves as the E2-like enzyme in the ubiquitin-like conjugation of LC3/Atg8 and phosphatidylethanolamine (PE) during autophagosome biogenesis. Activation of caspase-8 during death receptor-mediated apoptosis triggers the cleavage of Atg3 to suppress autophagy and promote cell death (Oral et al. 2012). Moreover, the inhibition or loss of caspase-8 promotes the induction of excessive autophagy leading to cell death (Yu et al. 2004; Bell et al. 2008). Furthermore in addition to suppressing death receptor-mediated apoptosis, FLICE-like inhibitor proteins (FLIPs) directly bind Atg3 to prevent the

association and processing of LC3/Atg8 and thus inhibit autophagy (Lee et al. 2009). In contrast, cells deficient in TRAIL-mediated apoptosis as a result of the overexpression of FLIP or loss of *Bax* have been demonstrated to upregulate Beclin 1 in response to TRAIL ligation for the induction of cytoprotective autophagy (Han et al. 2008; Herrero-Martin et al. 2009). Lastly, active caspase-8 has been identified as an autophagic substrate (Hou et al. 2010). Altogether, molecular cross talk among the autophagic and extrinsic machinery allows each pathway to negatively regulate and restrain one another.

3.3 Beclin 1-Interacting Proteins

3.3.1 Ambra 1

Activating molecule in Beclin 1-regulated autophagy (Ambra 1) is a Beclin 1-binding protein that regulates the balance between apoptosis and autophagy (Fig. 11.2). Ambra 1 promotes autophagosome formation by binding Beclin 1 and stabilizing the PI3KC3 complex (Fimia et al. 2007). Notably, Ambra 1 interacts with Beclin 1 at a region adjacent to the BH3 domain and is therefore able to compete with Bcl-2 for binding (Strappazzon et al. 2011). In the absence of autophagic stimuli, Ambra 1 localizes Beclin 1 and Vps34 to the dynein complex (Di Bartolomeo et al. 2010). Upon autophagy induction, Ambra 1 is phosphorylated by UNC-51-like kinase 1 (Ulk1) to release the complex and allow for its translocation to the ER for autophagy induction (Di Bartolomeo et al. 2010). Investigation of whether Bim and Ambra 1 regulate two distinct pools of Beclin 1 at the cytoskeleton is an interesting future direction that would aid in understanding the cross talk between autophagy and apoptosis. Additionally, Ambra 1 directly interacts with Bcl-2 at the mitochondria; and as this interaction is disrupted during nutrient starvation and apoptosis, the association of Ambra 1 and Bcl-2 may regulate Beclin 1-dependent autophagy as well as apoptosis (Strappazzon et al. 2011). Furthermore, Ambra 1 undergoes caspase- and calpain-mediated cleavage and degradation during apoptosis to suppress autophagy and increase the susceptibility to apoptotic stimuli (Pagliarini et al. 2012). Collectively, Ambra 1 appears to be a critical regulator of the cross talk between autophagy and apoptosis.

3.3.2 Bif-1

Endophilin B1/Bax-interacting factor 1 (Bif-1) interacts with Beclin 1 through ultraviolet radiation resistance-associated gene (UVRAG) to positively regulate PI3KC3 activity and the induction of autophagy (Takahashi et al. 2005). Interestingly, Bif-1 was initially identified as a Bax-binding protein that promotes the activation of Bax/Bak to induce apoptosis (Cuddeback et al. 2001; Takahashi et al. 2005). Thus, Bif-1 serves as an additional molecule that bridges autophagic and apoptotic pathways.

3.4 Proapoptotic Functions of Autophagy Proteins

3.4.1 Atg12

Several autophagy proteins can also function as positive regulators of apoptosis (Fig. 11.3). Atg12 is an ubiquitin-like modifier that is covalently conjugated to Atg5 to promote LC3/Atg8 modification and the expansion of autophagosomal membranes during autophagy induction. However, Atg3, the E2 enzyme necessary for LC3/Atg8 modification, has been identified as an additional substrate of Atg12 conjugation (Radoshevich et al. 2010). Interestingly, the Atg12–Atg3 conjugate regulates mitochondrial homeostasis and cell death, as disruption of the conjugate has no effect on autophagy but rather enhances mitochondrial mass and the resistance to cell death by mitochondrial pathways (Radoshevich et al. 2010). Additionally, Atg12 has been shown to directly interact with antiapoptotic Bcl-2 proteins to promote MOMP in a manner independent of its autophagic function (Rubinstein et al. 2011).

3.4.2 Proteolytic Cleavage of Autophagic Proteins

Furthermore, the activation of apoptotic proteases stimulates the cleavage of autophagy proteins to generate protein fragments that enhance cell death (Fig. 11.3). The calpain-mediated cleavage of Atg5 produces an amino-terminal protein fragment that translocates to the mitochondria and associates with Bcl-xL to promote apoptosis (Yousefi et al. 2006). Interestingly, Atg5 cleavage was observed to be independent of cell type or apoptotic stimuli, suggesting that it is a universal phenomenon in apoptotic cells (Yousefi et al. 2006). In a similar manner, the caspase-3-, 7-, and 8-mediated cleavage of Beclin 1 generates protein fragments that are incapable of inducing autophagy (Cho et al. 2009; Luo and Rubinsztein 2010; Wirawan et al. 2010). Moreover, the C-terminal cleavage product translocates to the mitochondria to sensitize cells to apoptosis; however, as the C-terminal fragment lacks the BH3-like domain of Beclin 1, the mechanism by which this fragment enhances apoptosis is unclear (Cho et al. 2009; Luo and Rubinsztein 2010; Wirawan et al. 2010). In addition, as described above, Ambra 1 is targeted by apoptotic proteases to dismantle autophagy and promote apoptosis (Pagliarini et al. 2012). Notably, the caspase-3-mediated cleavage of Atg4D enhances its ability to prime and delipidate the Atg8 paralogue gamma-aminobutyric acid receptor-associated protein-like 1 (GABARAP-L1), suggesting that caspases can stimulate autophagy (Betin and Lane 2009). Interestingly, Atg4D also has a cytotoxic function independent of caspase cleavage, as the protease associates with mitochondria prior to apoptosis in a manner likely mediated by a putative C-terminal BH3 domain (Betin and Lane 2009). Finally, several additional autophagy proteins, such as Atg3, Atg6, Atg7, and Atg9, have also been reported as caspase substrates in vitro (Norman et al. 2010); however, the physiological relevance of such cleavages remains to be demonstrated. In summary, several autophagy proteins exert proapoptotic functions in response to cleavage by apoptotic proteases.

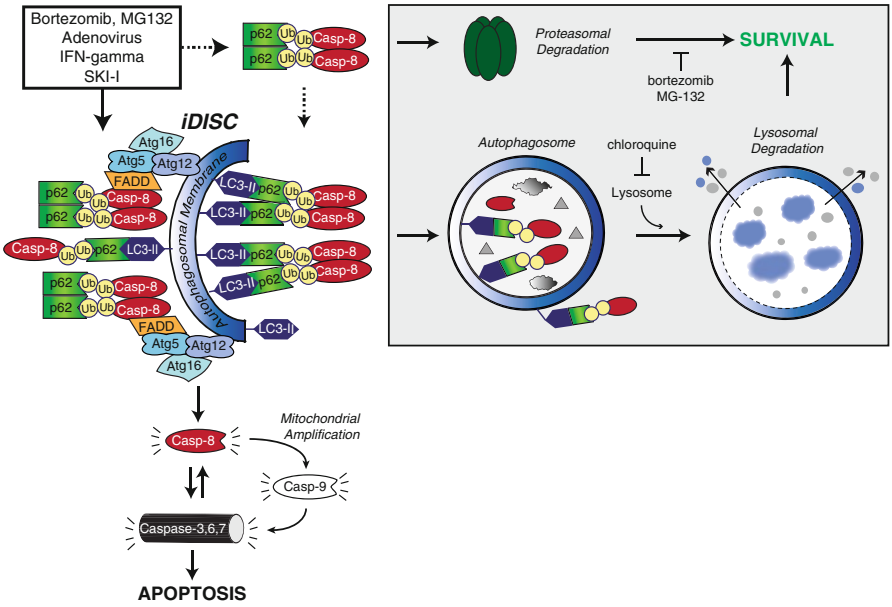

Fig. 11.4 The intracellular death-inducing signaling complex (iDISC) regulates cross talk between autophagy and apoptosis. iDISC-mediated apoptosis has been reported to occur in response to the proteasome inhibitors bortezomib and MG-132, interferon (IFN)-gamma, pan-sphingosine kinase inhibitor SKI-I, and during adenovirus-induced cell lysis. Atg5 recruits FADD to expanding autophagosomal membranes, and in a manner analogous to DISC formation, FADD recruits caspase-8 to promote caspase-8 oligomerization and self-activation. In addition, interaction of p62 and LC3-II recruits polyubiquitinated caspase-8 to autophagosomal membranes to promote caspase-8 activation. Autophagosome maturation and proteasomal degradation (indicated in the *grey box*) serve as cytoprotective mechanisms to limit iDISC-mediated caspase-8 activation. The use of proteasomal inhibitors (e.g., bortezomib) or lysosomal inhibitors (e.g., chloroquine) may suppress these "survival" pathways to enhance iDISC formation, caspase-8 activation, and apoptosis

3.5 Intracellular Death-Inducing Signaling Complex

In addition to promoting mitochondrial apoptosis upon proteolytic cleavage, emerging evidence demonstrates that autophagic machinery enables caspase-8-mediated apoptosis (Fig. 11.4). Assembly of an intracellular death-inducing signaling complex (iDISC) on the autophagosomal membrane promotes caspase-8 activation in response to several stimuli, including proteasome inhibitors, cytokine interferon-gamma, and pan-sphingosine kinase inhibitor, SKI-I (Pyo et al. 2005; Bell et al. 2008; Jiang et al. 2011; Laussmann et al. 2011; Pan et al. 2011; Young et al. 2012). The iDISC consists of two independent arms that facilitate caspase-8 activation: (1) Atg5–FADD–caspase-8 and (2) LC3–p62 (sequestosome-1)–caspase-8. Notably, iDISC-mediated cell death is negatively regulated by autophagic flux and the

proteasomal degradation of p62; and thus the use of lysosomal inhibitors, which impair autophagosome–lysosome fusion, or proteasome inhibitors to prevent the degradation of p62 can stabilize the iDISC to enhance the cross talk between autophagy and apoptosis (Young et al. 2012).

Atg5 is an essential autophagy protein that is covalently conjugated to Atg12 and localizes to expanding autophagosomal membranes. Interestingly, Atg5 directly interacts with FADD to recruit the adapter protein to the autophagosomal membrane (Pyo et al. 2005; Bell et al. 2008; Young et al. 2012). In a manner analogous to DISC formation, FADD associates with procaspase-8 to facilitate caspase-8 oligomerization and self-cleavage for the initiation of the caspase cascade (Bell et al. 2008; Jiang et al. 2011; Laussmann et al. 2011; Young et al. 2012). iDISC-mediated caspase-8 activation also occurs through a p62- and LC3-dependent mechanism. p62 contains an LC3-interacting region (LIR) and ubiquitin-binding domain that serves to recruit polyubiquitinated cargo to the autophagosomal membrane for degradation (Bjorkoy et al. 2005; Pankiv et al. 2007). In addition to serving as an autophagy adaptor protein, p62 can promote the aggregation of polyubiquitinated caspase-8 for caspase-8 self-cleavage and the induction of apoptosis (Jin et al. 2009). In response to iDISC stimuli, p62 associates with autophagosomal membrane-bound LC3-II to recruit self-associated caspase-8 and facilitate caspase-8 activation for the initiation of apoptosis (Jin et al. 2009; Pan et al. 2011; Young et al. 2012). Collectively, the autophagosomal membrane can serve as a platform for the assembly of iDISCs and caspase-8 activation.

3.6 Transcriptional Regulators

3.6.1 p53

Cross talk between apoptosis and autophagy also occurs at the transcriptional level (Fig. 11.5a). The tumor-suppressor p53 plays a dynamic role in the cross talk between apoptosis and autophagy. p53 is a well-established activator of apoptosis that transcriptionally upregulates proapoptotic genes, such as *Bax*, *NOXA*, and *PUMA*, and suppresses the transcription of antiapoptotic genes, such as *Bcl-2* (Fridman and Lowe 2003). Moreover, cytoplasmic p53 enhances Bax-induced MOMP (Wolff et al. 2008). In addition, p53 is a dual regulator of autophagy that exerts its effects through transcription-independent and -dependent mechanisms. First, activation of p53 inhibits mTOR in an AMP kinase (AMPK) and tuberous sclerosis (TSC)1/TSC2-dependent manner to induce autophagy (Feng et al. 2005). Furthermore, nuclear p53 transcriptionally activates the lysosomal membrane protein damage-regulated autophagy modulator (DRAM) to enhance autophagy in response to DNA damage (Crighton et al. 2006; Gao et al. 2011). Interestingly, DRAM expression is required for p53-induced autophagy as well as the subsequent induction of apoptosis in response to DNA-damaging agents (Crighton et al. 2006; Gao et al. 2011), thus highlighting a secondary mediator of cross talk. Likewise,

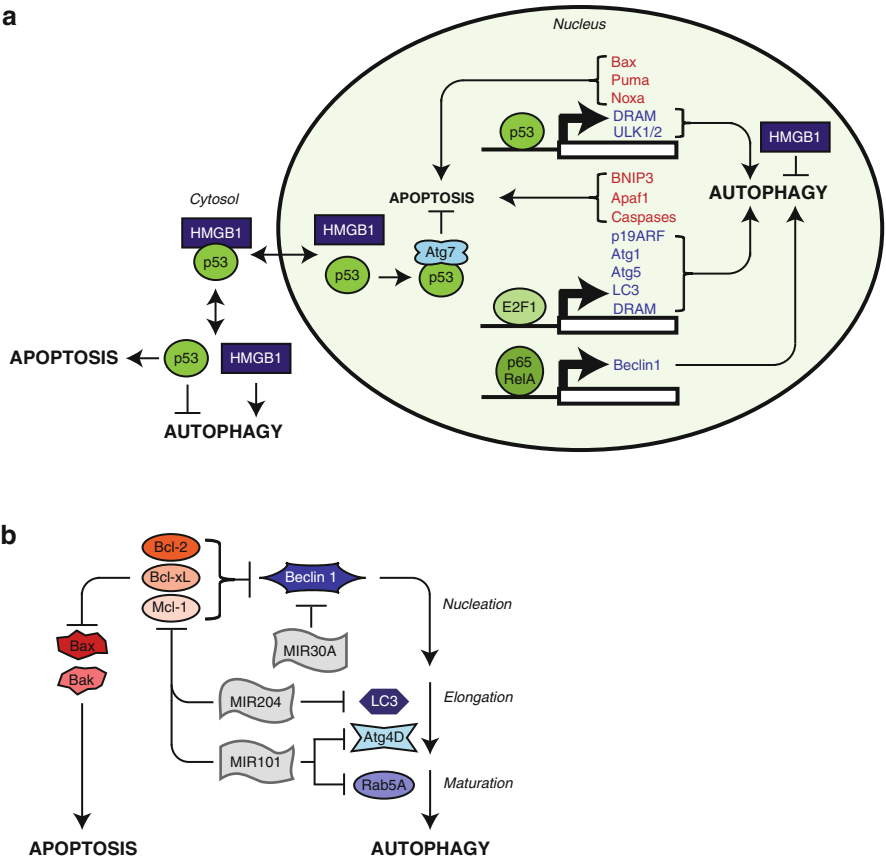

Fig. 11.5 Transcriptional (**a**) and microRNA (**b**)-mediated regulation of autophagy and apoptosis. (**a**) p53 regulates autophagy through transcriptional dependent and independent mechanisms. p53 transactivates proapoptotic genes *Bax*, *Puma*, and *Noxa* and proautophagic genes *DRAM* and *ULK1/2*. In addition, p53 forms a complex with HMGB1 to regulate the intracellular localization of the respective proteins. Cytosolic HMGB1 activates autophagy by binding Beclin 1, while nuclear HMGB1 suppresses autophagy. In contrast, cytosolic p53 inhibits autophagy. In addition, the autophagy protein Atg7 interacts with p53 to suppress apoptosis. The transcription factor E2F1 promotes autophagy and apoptosis through the transcriptional upregulation of multiple target genes, while the canonical NF-κB member p65/RelA upregulates Beclin 1 expression to induce autophagy. (**b**) MIR30A represses Beclin 1 expression to limit autophagy. Furthermore, MIR101 regulates the cross talk between autophagy and apoptosis by suppressing the mRNA and protein levels of autophagy protein Atg4D and LC3-II and antiapoptotic molecule Mcl-1. MIR204 suppresses autophagy through Rab5A and enhances apoptosis by targeting antiapoptotic Bcl-2

ULK1 and ULK2 are transcriptional targets of p53 that are upregulated following DNA damage and necessary for p53-mediated autophagy and subsequent cell death (Crighton et al. 2006; Gao et al. 2011).

Loss of p53 function activates autophagy, thus suggesting that p53 also functions as a negative regulator of autophagy (Tasdemir et al. 2008). The antiautophagic activity is attributed to cytoplasmic p53; and in contrast to the autophagy discussed above, the induction of autophagy in response to p53 depletion promotes cell survival (Tasdemir et al. 2008). Interestingly, high-mobility group box 1 (HMGB1) and p53 form a complex to regulate the cytoplasmic localization of the respective proteins (Livesey et al. 2012). Specifically, knockout of p53 was seen to enhance the expression of cytosolic HMGB1 for the induction of autophagy, while knockout of HMGB1 enhanced cytosolic p53 to suppress autophagy (Livesey et al. 2012). Cytosolic HMGB1 induces autophagy by directly binding Beclin 1 to displace Bcl-2/Bcl-xL (Kang et al. 2010; Tang et al. 2010). Intriguingly, the autophagy protein Atg7 has recently been shown to bind p53 during nutrient deprivation, independent of its E1-like enzymatic function, to regulate p53-dependent cell cycle arrest and cell death (Lee et al. 2012). Therefore, in addition to regulating autophagy, p53 is reciprocally regulated by the autophagy protein, Atg7. In summary, p53 promotes apoptosis while also serving as a dual regulator of autophagy.

3.6.2 E2F1 and ARF

E2F transcription factor 1 (E2F1) is implicated in apoptosis and autophagy. E2F1 upregulates expression of the proapoptotic genes, such as *BNIP3*, *Apaf1*, and *caspases*, as well as the autophagy genes *LC3*, *Atg1*, *Atg5*, and *DRAM* (Polager et al. 2008). In addition, E2F1 targets the tumor-suppressor *ARF* (p19ARF in mouse and p14ARF in human), which antagonizes Mdm2 to stabilize p53. The activation of p53, in turn, mediates the transactivation of proapoptotic genes to promote cell death (Iaquinta and Lees 2007). Additionally, the p19ARF mRNA can also produce a smaller isoform, smARF, which localizes to the mitochondria to induce mitochondrial depolarization and autophagic cell death (Reef et al. 2007). Furthermore, p14ARF has been reported to bind Bcl-2/Bcl-xL on the outer mitochondrial membrane to reduce the association of Bcl-2/Bcl-xL and Beclin 1; however, exactly how the interaction at the mitochondria affects ER-localized Bcl-2/Beclin 1 complex remains unknown (Pimkina et al. 2009). In total, E2F1 and ARF mediate the interplay of apoptosis and autophagy through several mechanisms.

3.6.3 NF-κB

NF-κB signaling also regulates the cross talk between autophagy and apoptosis. Activation of NF-κB suppresses apoptosis through the transcriptional upregulation of antiapoptotic gene expression. Furthermore, NF-κB activation has been shown to both positively and negatively regulate autophagy. In T-cells, the canonical NF-κB protein p65/RelA transcriptionally upregulates Beclin 1

expression to enhance autophagy for T-cell cellular homeostasis (Copetti et al. 2009). In contrast, prolonged NF-κB activation in macrophages negatively regulates autophagy through the suppression of Atg5 and Beclin 1 expression (Schlottmann et al. 2008). Furthermore, NF-κB activation has been associated with the suppression of autophagy in response to TNF-α and starvation (Djavaheri-Mergny et al. 2006; Fabre et al. 2007). Additional investigation is necessary in order to understand the dual roles of NF-κB in autophagy in relationship to its antiapoptotic effects.

3.7 microRNA

microRNAs (miRNAs) are ~22-nucleotide-long noncoding RNAs that reside in protein-coding, intronic, or intergenic, regions throughout the genome (Xu et al. 2012). miRNAs are transcribed as hundreds or thousands of nucleotide long primary miRNA products, which are cleaved by the DROSHA nuclease complex within the nucleus to generate 70-nucleotide hairpins designated precursor-miRNAs. Precursor-miRNAs are further processed in the cytosol by the RNase III DICER1 to mature miRNAs. miRNAs are emerging as regulators of autophagy as well as the cross talk between autophagy and apoptosis (Xu et al. 2012) (Fig. 11.5b). *MIR30A* was the first identified miRNA regulator of autophagy that targets *BECN1* in human breast, lung, and glioma cancer cell lines (Zhu et al. 2009). Importantly, *MIR30A* expression is inhibited in response to nutrient deprivation or rapamycin, and overexpression of *MIR30A* results in the *BECN1*-dependent suppression of autophagy (Zhu et al. 2009).

Furthermore, *MIR101* targets the autophagy genes *RAB5A*, *ATG4D*, and *STMN1*, and the antiapoptotic gene *MCL-1* to suppress autophagy and apoptosis, respectively (Su et al. 2009; Frankel et al. 2011). Moreover, *MIR204* is reported to negatively regulate autophagy during hypoxia–reoxygenation by targeting LC3-II expression and has also been shown to repress BCL-2 expression for enhanced chemotherapeutic drug-induced apoptosis, thus suggesting that *MIR204* may also mediate the cross talk between apoptosis and autophagy (Chen et al. 2009; Jian et al. 2011). Finally, *MIR17* modulates *p62/SQSTM1* gene expression, which has dual roles in autophagy and apoptosis (Meenhuis et al. 2011). In summary, miRNAs are emerging as dual regulators of apoptotic and autophagic machinery. Notably, transcripts involved in autophagy and apoptosis are proposed to indirectly modulate one another by competing for common miRNA-binding sites (Xu et al. 2012). For example, when autophagy-related proteins are repressed by miRNAs, additional miRNA molecules will be released into the free miRNA pool and in turn will be able to target apoptosis-related gene products. In contrast, abundant expression of autophagy-related genes will bind more miRNA molecules to suppress the free miRNA pool available for binding to apoptosis-related genes. Therefore, miRNAs are capable of dynamically regulating the cross talk between apoptosis and autophagy.

3.8 Kinase Signaling

3.8.1 PI3K/Akt/mTOR

The apoptotic and autophagic pathways are also linked through shared kinase regulation. The PI3K/Akt/mTOR pathway is a well-known regulator of apoptosis and autophagy. Akt activation promotes cell survival through many mechanisms, including activation of the NF-κB pathway (Ozes et al. 1999). In addition, Akt phosphorylates Bad to trigger its release from Bcl-xL and association with the chaperone protein, 14-3-3, for enhanced cell survival (Zha et al. 1996; Datta et al. 1997). Moreover, Akt phosphorylation of Bax at Ser184 suppresses its activation and translocation to mitochondria (Yamaguchi and Wang 2001; Tsuruta et al. 2002; Gardai et al. 2004). Similarly, Akt phosphorylates XIAP to enhance its stability and anti-apoptotic function (Dan et al. 2004). In autophagy, the activation of Akt upstream of mTORC1 negatively regulates the Ulk1 autophagic complex to suppress the autophagosome biogenesis. Conversely, activation of phosphatase and tensin homolog (PTEN) inhibits Akt to indirectly suppress mTORC1 and promote the induction of autophagy and apoptosis. Collectively, the mTOR signaling axis is a key regulator of the interplay between autophagy and apoptosis.

3.8.2 JNK

The stress-activated JNK pathway represents an additional kinase signaling pathway that mediates cross talk. JNK promotes apoptosis through several mechanisms, including the phosphorylation and AP1-mediated transcriptional regulation of pro-apoptotic Bcl-2 family proteins, Bax and Bad (Weston and Davis 2007). In addition, JNK phosphorylates the chaperone protein, 14-3-3, to antagonize Akt-mediated survival signals and trigger the release of proapoptotic proteins, such as Bad, for enhanced apoptosis (Sunayama et al. 2005). As discussed above, JNK activates Beclin 1-dependent autophagy through the phosphorylation of Bcl-2 (Wei et al. 2008; Park et al. 2009; Pattingre et al. 2009). Additionally, the JNK-mediated activation of transcription factor c-Jun positively regulates Beclin 1 expression to enhance autophagy (Wei et al. 2008; Park et al. 2009; Pattingre et al. 2009). In summary, the stress-activated JNK signaling pathway dually regulates apoptosis and autophagy.

4 Summary and Future Perspectives

The interplay between apoptosis and autophagy is complex as autophagy can (1) suppress apoptosis, (2) function as an alternative cell death mechanism, or (3) facilitate the induction of apoptosis. We propose that outcome of autophagy is dependent

upon the balance of autophagosome initiation and maturation (Fig. 11.4). For example, if autophagosomal biogenesis and degradation are in balance with one another, autophagy functions as a cell survival mechanism to allow for the recycling of nutrients, removal of damaged organelles, and degradation of aggregated proteins to prevent cell death. However, if autophagic degradation occurs at an excessive rate, the extensive catabolism of cytosolic components will trigger caspase-independent cell death. In contrast, the accumulation of autophagosomal membranes or immature autophagosomes as a result of a defect in autophagosome maturation will initiate iDISC-mediated caspase-8 activation and the induction of apoptosis. Collectively, any disruption in autophagic flux has the potential to alter cellular homeostasis.

The paradoxical functions of autophagy present a challenge when attempting to determine the appropriate modulation of autophagy for cancer therapy. Autophagy is induced in response to many chemotherapeutic agents as a cytoprotective mechanism, thereby limiting the efficacy of many apoptosis-inducing drugs. In this scenario, inhibition of autophagy would be beneficial for enhanced tumor cell killing. However, as basal autophagy is critical for maintaining cellular homeostasis in nontransformed cells, the ideal autophagy modulator would selectively inhibit autophagy in tumor cells while sparing normal cells. Chloroquine is a FDA-approved lysosomal inhibitor that is in clinical trials as an autophagy-suppressing agent to enhance chemotherapeutic efficacy. Although chloroquine is relatively well tolerated, the drug has been reported to have autophagy-independent toxicity attributable primarily to the disruptions of endosomal trafficking. Therefore, there is a significant need for autophagy-specific inhibitors in both experimental and clinical settings. Vps34 and Atg4 are two potential "druggable" targets for the development of such an agent. Moreover, the recent identification of miRNA regulators of autophagy may allow for the identification of additional mechanisms by which to modulate autophagy. In contrast to serving as a cytoprotective mechanism, autophagy may also function as an alternative caspase-independent mechanism of cell death in apoptosis-deficient tumor cells; and thus, the development of autophagy-activating agents is also of interest.

Elucidation of the molecular mediators of cross talk between apoptosis and autophagy allows for the identification of convergence points that can be targeted to switch one pathway to another. For example, the accumulation of autophagosomal membranes in response to the inhibition of autophagic flux (e.g., chloroquine) switches cytoprotective autophagy toward apoptosis through the stabilization of iDISCs that facilitate caspase-8 activation and the initiation of apoptosis independent of the mitochondrial pathway (Fig. 11.4). Notably, the Atg12–Atg5 conjugate dissociates from the autophagosomal membrane upon autophagosomal closure to limit caspase-8 activation. Furthermore, the sealing of autophagosomal membranes sequesters iDISC-associated caspase-8 from its cytosolic substrates. As a result, the development of inhibitors of autophagosome sealing would be of great interest to enhance iDISC-mediated cell death and switch autophagy to apoptosis. Moreover, identification of specific inhibitors of autophagosome–lysosome fusion that function independently of the endosomal trafficking system would be of great benefit. In addition to enhancing iDISC-mediated apoptosis, inhibition of autophagosome

maturation will promote apoptosis by preventing the autophagic degradation of proapoptotic molecules, damaged organelles (e.g., mitochondria), and aggregated proteins to enhance cellular stress and promote cell death.

Elucidation of the complex interplay between apoptosis and autophagy has begun to identify convergence points that can be exploited to enhance cell death. However, many questions still exist. For example, which proteins are critical in setting the threshold and regulating the induction of cytoprotective versus cytotoxic autophagy? Furthermore, the potential for non-autophagic functions of Atg proteins needs to be closely examined in order to predict potential side effects of autophagy-modulating drugs. Finally as autophagy functions as a tumor suppressor and a cell survival mechanism during tumorigenesis (see Chaps. 5 & 6), the identification of novel biomarkers will be critical for identifying appropriate context- and stage-dependent modulation of autophagy. Collectively, exploitation of the intricate network of cross talk between apoptosis and autophagy provides a novel strategy to improve cancer therapy.

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