

## Review

# Physiological and pathological significance of the molecular cross-talk between autophagy and apoptosis

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**Summary.** Autophagy and apoptosis are two important molecular mechanisms that maintain cellular homeostasis under stress conditions. Autophagy represents an intracellular mechanism responsible for turnover of organelles and long-lived proteins through a lysosome-dependent degradation pathway. Cell death signals or sustained stress might trigger programmed cell death pathways, and among them, apoptosis is the most extensively studied one. Recent studies indicate the presence of a complex interplay between autophagy and apoptosis. Physiological relevance of autophagy-apoptosis crosstalk was mainly shown *in vitro*. However, *in vivo* consequences possibly exist both during health and disease. In this review, we will summarize the current knowledge about molecular mechanisms connecting autophagy and apoptosis, and about the significance of this crosstalk for human health.

**Key words:** Autophagy, Apoptosis, Signaling, Cross-talk, Diseases

## Introduction

Autophagy is an evolutionary conserved, cellular degradation mechanism functioning under basal conditions and it is activated during cellular stress conditions, including nutrient limitation, energy

deficiency, oxidative stress, and protein or organelle accumulation. It is initiated by formation of double or multi-membrane vesicles in the cytoplasm. These vesicles, termed autophagosomes, engulf portions of the cytoplasm and organelles. After the fusion of the outer membrane with lysosomes, the inner membrane and the cargo are degraded by hydrolytic enzymes in lysosomes and then its constituents are recycled (Gozuacik and Kimchi, 2007; Kuma and Mizushima, 2010). Autophagy is genetically regulated by the protein products of a group of autophagy-related (ATG) genes that have concerted action which results in autophagosome biogenesis and maturation (Yang and Klionsky, 2010). Two essential ubiquitin-like conjugation systems control autophagosome biogenesis. The first system results in the covalent attachment of ATG12-ATG5 proteins. In the second system, LC3 protein is conjugated to a lipid molecule, generally to a phosphatidylethanolamine (PE). Both conjugation systems require the function of E1- (ATG7) and E2-like enzymes (ATG3 and ATG10). ATG12-ATG5 stable complex eventually forms a larger complex with ATG16, resulting in the formation of an E3-like enzyme complex that catalyzes LC3-PE conjugation (Kuma et al., 2002) (Fig. 1a).

Under stress conditions, autophagic activity contributes to cell survival which maintains cellular homeostasis. This homeostatic role of autophagy is particularly critical in post-mitotic differentiated cells, such as neurons and cardiomyocytes. However, excessive autophagy or activation of autophagy in the context of specific diseases might result in cell death which carries the hallmarks of classical apoptosis, necrosis (Mizushima et al., 2008; Mizushima and Levine, 2010). Moreover, a caspase-independent cell

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death type that requires autophagy genes was also reported by us and others (Gozuacik and Kimchi, 2007). Although molecular mechanisms of autophagic cell death as a separate entity require further investigation, signaling pathways for apoptosis have been investigated in the last 4-5 decades. Apoptotic cell death is morphologically defined by cellular and nuclear shrinkage, chromatin condensation, nuclear fragmentation, formation of apoptotic bodies and eventually phagocytosis by neighboring cells or phagocytes. Morphological changes and eventual death of cells undergoing apoptosis is a result of the activation of specific cysteine aspartase enzymes, called caspases (Hengartner, 2000). At least two major pathways were defined to activate caspases. The first pathway is called "the extrinsic or receptor-mediated apoptotic pathway", which is triggered by binding of death receptor ligands, including tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), fas ligand or tumor necrosis factor-related apoptosis inducing ligand (TRAIL) to their respective death receptors (Lavrik et al., 2005). Activated death receptors lead to the recruitment of adaptor proteins, such as FADD or

TRADD, and subsequent recruitment of upstream caspases, mainly caspase-8 or caspase-10. Engagement of upstream caspases allows activation of downstream executionary caspases, caspase-3, caspase-6 and caspase-7. Caspases are responsible for the destruction of survival-related proteins, cell division proteins, signaling proteins and proteins responsible for cellular integrity. Caspase-activated DNA degradation into small nucleosomal fragments (laddering) is one of the hallmarks of apoptotic cell death. The second pathway, called "the intrinsic apoptotic pathway", relies on mitochondrial depolarization and permeability increase, resulting in the release of critical factors, including cytochrome-c. In the cytosol, cytochrome-c initiates formation of an APAF-1/caspase-9 complex and activation of downstream executionary caspases, leading to cell death (Kroemer et al., 2007) (Fig. 1b).

Both autophagy and apoptosis are well-controlled biological processes, and recent studies indicate a complex crosstalk between components of these two pathways. Here, we will summarize the recent literature about the molecular crosstalk and discuss their

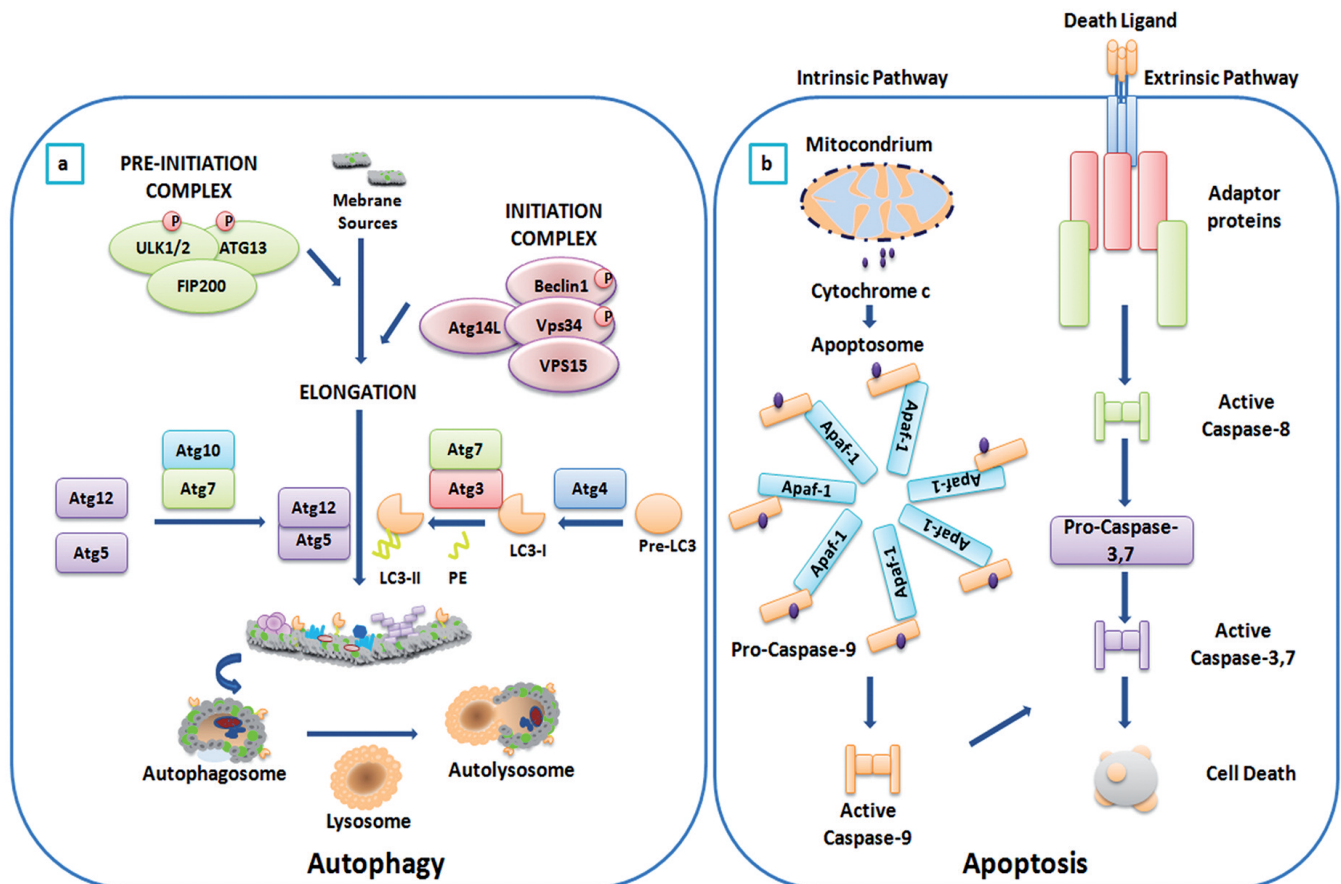


Fig. 1. Molecular Mechanisms of Autophagy (a) and Apoptosis (b).

physiological and pathological consequences.

### **Molecular connections between autophagy and apoptosis**

Over the last decade, several molecular studies have been published about the interface of autophagy and apoptosis. This complex interplay can be classified into three different types, which are cell type, stimulus and context-dependent. 1) autophagy and apoptosis can act in a coordinated and cooperative manner to induce cell death; 2) autophagy blocks apoptotic cell death by promoting cell survival and counterbalancing apoptosis; 3) autophagy facilitates execution of apoptotic cell death. Several genes and proteins are implicated in both pathways: These include mTor signaling components, ATG5 and ATG12 proteins, death adaptor proteins such as FADD, BECN1 (Beclin 1), BCL2 family proteins, p53, p19ARF, DAPk and microRNAs.

#### *mTor signaling pathway*

Mammalian target of rapamycin (mTor) and its regulators are molecular sensors of cellular energy, growth factor and nutrient levels. They play crucial roles in the control of cell growth, protein synthesis and autophagy. Suppression of mTor kinase releases its inhibitory effects on Unc-51-like kinase (Ulk-1) complex, which is necessary for the induction of autophagy (Yang and Klionsky, 2010). The tuberous sclerosis complex 1/2 (TSC1/TSC2) proteins negatively regulate mTor activity through inactivation of the small GTP-binding protein, Rheb (Guertin and Sabatini, 2007). Regulatory factors such as AKT, ERK or RSK are activated by growth factors of the class I PI3K pathway and this activation inhibits Rheb through TSC1/TSC2. Consequently, mTor is activated and lead to autophagy inhibition. On the contrary, AMP-activated kinase (AMPK) induces autophagy through stimulation of TSC1/TSC2 activity via phosphorylation (Inoki et al., 2003).

mTor has been reported to affect apoptosis through several downstream targets such as p53, BAD and BCL2 proteins (Castedo et al., 2002). AKT or RSK-mediated phosphorylation of BAD leads to its dissociation from BCL2 and inhibits BAX/BAK-mediated apoptosis. Additionally, AKT phosphorylates the Forkhead box O (FOXO) family of transcription factors and a key tumor suppressor p53, which results in reduced expression of several pro-apoptotic genes. Conversely, phosphorylation of FOXO by AKT can induce expression of several autophagy genes, e.g. LC3, GABARAPL1, BNIP3 and BNIP3L (Mammucari et al., 2007). AKT also negatively regulates several kinases upstream of Jun- N-terminal protein kinase 1 (JNK1, also known as MAPK8), thereby inhibiting JNK1 activation and its pro-apoptotic functions. Recently, proline-rich AKT substrate (PRAS40) and PRR50-like protein have been

identified as two new mTor interactors, which control the balance between cell growth and cell death by regulating apoptosis (Thedieck et al., 2007). Moreover, anti-apoptotic BCL-2 homolog MCL-1 was shown to control both autophagy and apoptosis. mTor inhibition by nutrient deprivation was suggested to cause MCL-1 degradation that finally led to downstream interplay between BAX and BECN1 activation (Germain et al., 2011).

#### *ATG5 and ATG12*

ATG5 is one of the key proteins of the basic autophagic machinery. It is involved in ubiquitin-like conjugation systems which are essential for autophagosome biogenesis. In addition to its essential role in autophagy, ATG5 can also impact apoptotic cell death. Some apoptotic stimuli were shown to result in the N-terminal cleavage of ATG5 by calpain. The cleavage product was shown to translocate to mitochondria by an unknown mechanism, and promoted cytochrome-c release through interaction with the pro-survival BCL2 family member, BCLXL (Yousefi et al., 2006). ATG5 was also shown to interact with FADD and thereby induce autophagic cell death (Pyo et al., 2005) (Fig. 2a,b).

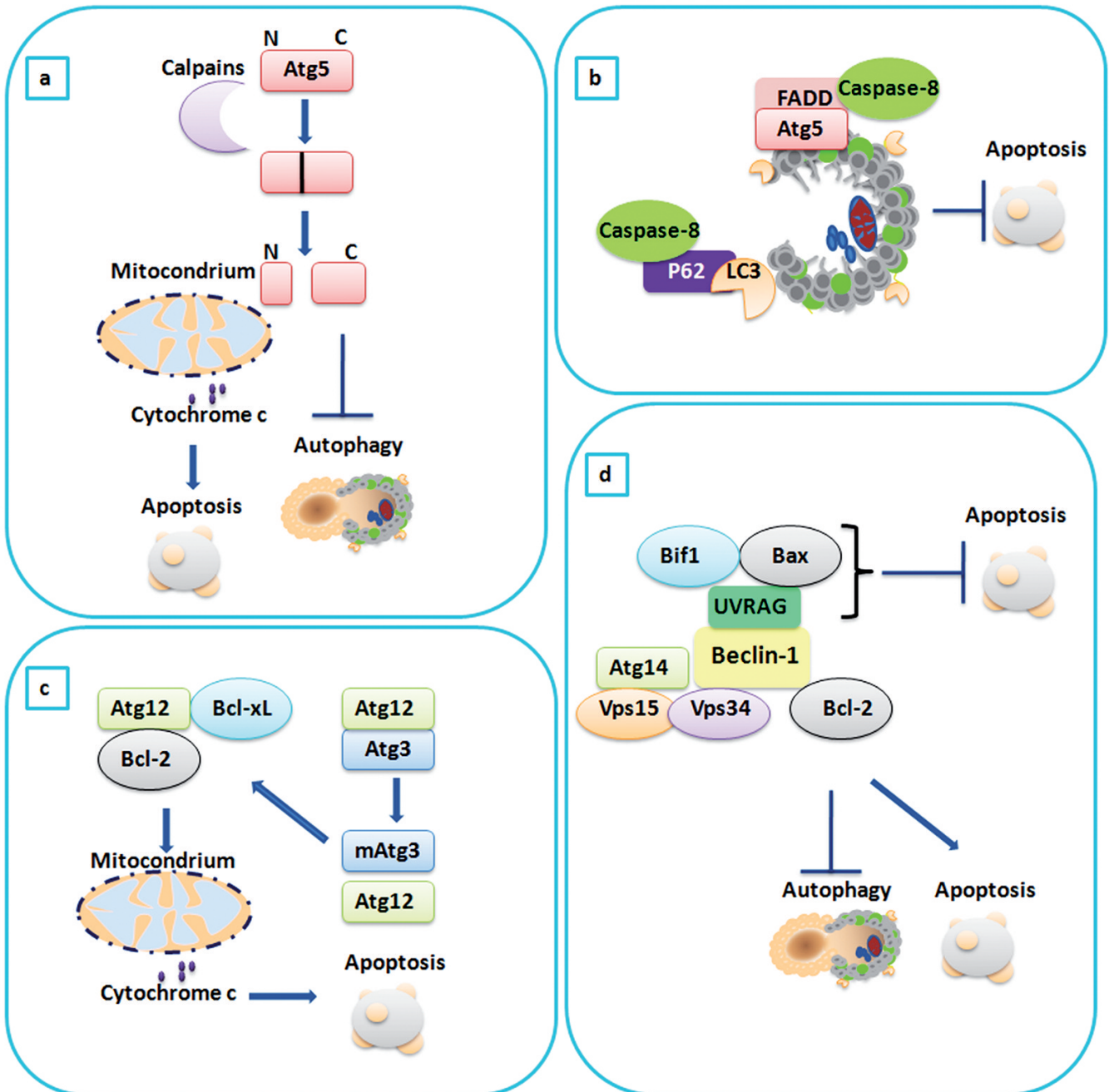
ATG12 is another protein involved in autophagic vesicle formation. It is covalently conjugated to ATG5 in the first ubiquitin-like conjugation system. ATG12 was shown to be required for caspase activation in response to a range of apoptotic stress inducers (Rubinstein et al., 2011). Interaction assays revealed that non-conjugated ATG12 is able to bind and inhibit two anti-apoptotic members of the BCL2 family, namely BCL2 and MCL1. They further revealed that a BH3-like domain on ATG12 is required for both interaction and pro-apoptotic function of ATG12. Knockdown of several other essential autophagy genes did not show a significant effect on death induced by apoptotic signals, supporting a specific role for ATG12 in apoptosis, independent of its role in autophagosome biogenesis (Rubinstein et al., 2011). Recently, it has been found that a stable conjugate of ATG12 and ATG3 was involved in the regulation of mitochondrial homeostasis and apoptotic cell death via a mitochondrial pathway, but by the death-receptor mediated pathway (Radoshevich and Debnath, 2011) (Fig. 2c). Increased mitochondrial mass was detected in cells expressing a non-conjugated mutant form of ATG3. In addition, disrupting ATG12-ATG3 conjugation rendered cells resistant to mitochondrial cell death. Interestingly, ATG12-ATG3 conjugation here seems not to affect regulation of autophagy (Radoshevich et al., 2010).

Although calpain-mediated cleavage of ATG5 was suggested to play a role in the switch between autophagy to apoptosis in selected systems, the implications of the pro-apoptotic function of ATG12 on autophagy-apoptosis crosstalk need to be clarified.

*Class III PI3-kinase VPS34 complex*

Autophagosomal membrane nucleation requires the function of a Class-III phosphoinositol-3 kinase complex containing VPS34 and BECN1. This complex serves as a central regulator of autophagic machinery, and it is necessary for labeling membranes with phosphoinositol-3 phosphate (PI3P) molecules, allowing recruitment of

critical autophagic proteins to these sites of autophagosome complex formation. BECN1 was identified as a new member of the BH3-only family of proteins (Maiuri et al., 2007; Oberstein et al., 2007). It is now well-documented that functional and structural interaction between BECN1 and the anti-apoptotic BCL2 proteins is important for autophagy-apoptosis coordination (Pattingre et al., 2005). Binding of BCL2 to



**Fig. 2.** Atg5/Atg12- (a-c) and class III PI3-kinase VPS34 complex-(d) dependent regulation of autophagy by apoptotic proteins.



the BH3 domain of BECN1 was found to inhibit autophagy (Maiuri et al., 2007). Moreover, anti-apoptotic activity of BCL2 was shown to be retained when it was bound to BECN1 (Ciechomska et al., 2009a,b). In fact, BECN1/BCL2 binding did not modify apoptosis even in autophagy-deficient ATG5 <sup>-/-</sup> mouse embryonic fibroblasts (MEF) (Ciechomska et al., 2009a,b). Thus, the functional outcome of BECN1/BCL2 interaction is the inhibition of autophagy with little or no effect on apoptosis. Indeed, an endoplasmic reticulum associated fraction rather than mitochondria-associated and cell death related BCL2 proteins were reported to be critical for autophagy control through BECN1 interaction (Pattingre et al., 2005). This observation is consistent with the idea that ER-associated Class-III PI3K activity is necessary for nucleation of autophagosomes. Yet, BECN1 can also co-localize with BCLX<sub>L</sub> in mitochondria, suggesting that BECN1/BCLX<sub>L</sub> complexes might carry on divergent roles compared to BECN1/BCL2 (Noble et al., 2008).

UVRAG protein was suggested to play a role in endosome and autophagosome maturation through its contribution to Class-III phosphoinositol-3 kinase complexes (Liang et al., 2006). UVRAG was also shown to inhibit apoptosis by preventing translocation of pro-apoptotic BAX proteins to mitochondria (Yin et al., 2011). Data showed that UVRAG could bind to BAX independently of BECN1 (Yin et al., 2011).

Bif-1, a member of the endophilin B family, was shown to participate in the Class-III PI3-kinase VPS34 complex during the nucleation of autophagosomal membranes. It was shown that Bif-1 regulated apoptosis through binding and activation of pro-apoptotic BAX/BAK proteins (Takahashi et al., 2005). Recently, same group demonstrated insufficient suppression of apoptosis by the loss of BAX activating Bif-1 function under metabolic stress. Concomitantly, they observed enhanced expression of the anti-apoptotic proteins MCL1 and BCL2L1, as well as an increase in mitochondrial mass and accumulation of genomic DNA damage in the same cells. Furthermore, they found a decrease in mitochondrial matrix proteins in PARK2-expressing MEFs, indicating impairment of mitophagy (clearance of mitochondria by autophagy) by the loss of Bif-1 function (Takahashi et al., 2013) (Fig. 2d).

### *BCL2 family proteins*

In addition to their role in apoptosis regulation, BCL2 proteins were associated with autophagy control. Under basal conditions, BCL2 proteins were found to be constitutively bound to BECN1, and allowing only basal levels of autophagy to proceed. However, BCL2 proteins dissociated from BECN1 under stress conditions, allowing an increase of autophagy levels in the cells (Pattingre et al., 2005). Competitive displacement of BECN1 BH3-like domain by other BH3-only BCL2 family proteins, such as BAD (Maiuri et al., 2007), BNIP3 (Mellor and Harris, 2007; Zhu et al., 2007a,b),

BIM (Luo et al., 2012), NOXA (Elgendy et al., 2011) or BH3 mimetic drugs (Maiuri et al., 2007) was reported (Fig. 3a).

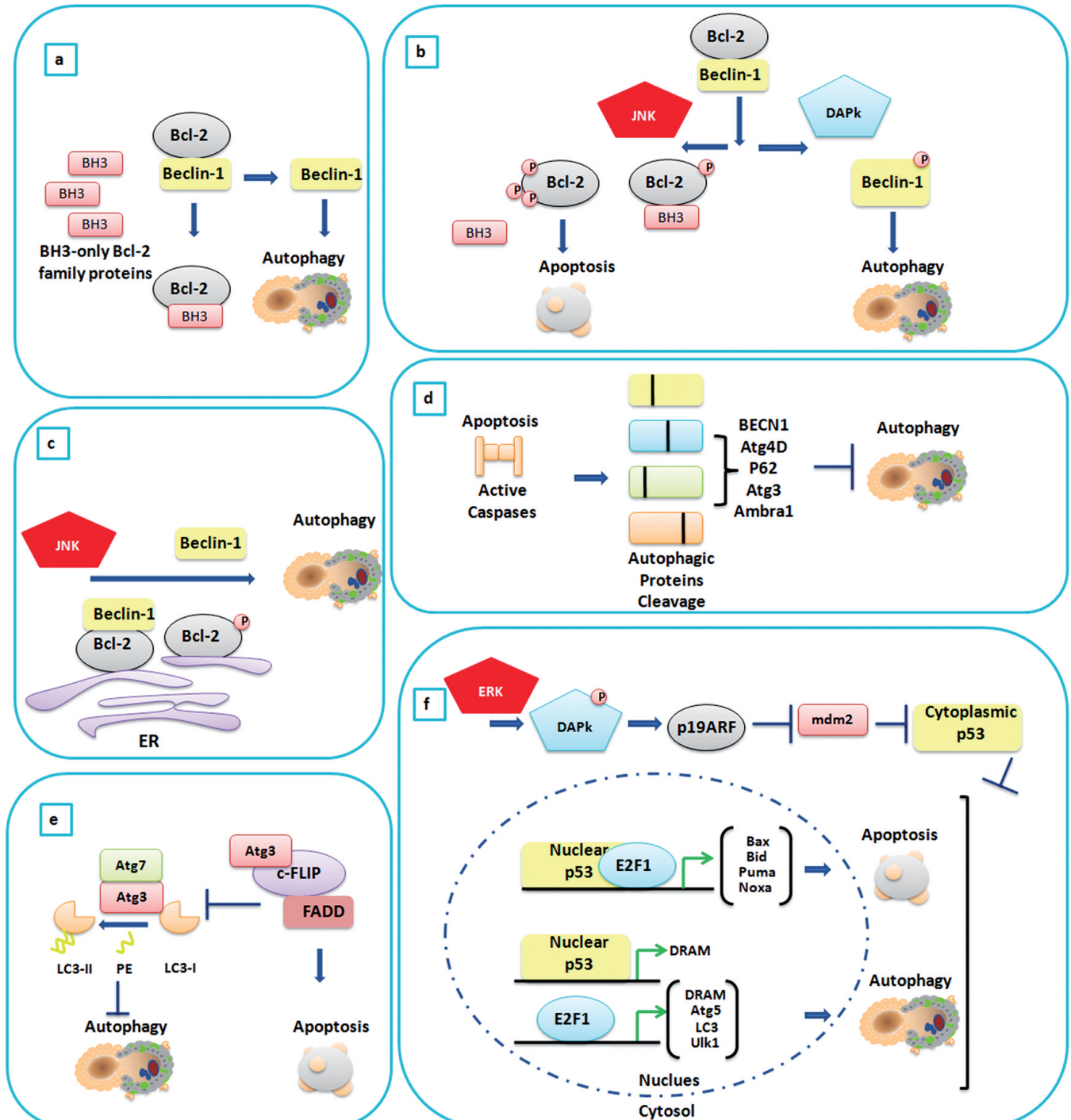
For example, Maiuri et al. showed that starvation led to an increase in the interaction between BCLX<sub>L</sub> and BAD, which correlated with decreased binding of BCLX<sub>L</sub> to BECN1 and elevated levels of autophagy (Maiuri et al., 2007). BNIP3 triggers apoptosis by sequestering anti-apoptotic BCL2 family proteins, and promoting BAX/BAD dependent mitochondrial release of pro-apoptotic mediators. Recently, BNIP3 was also shown to stimulate mitophagy by disrupting the interaction between BECN1 and BCL2 (Mellor and Harris, 2007; Zhu et al., 2007a,b). On the other hand, Luo et al. showed that BIM over-expression was insufficient to disrupt BECN1/BCL2 interaction. Instead, they showed a role of BIM as an inhibitor of autophagy during BECN1-microtubule binding (Luo et al., 2012). NOXA, another BH3-only pro-apoptotic protein, was found to displace BCL2 family protein MCL-1 from BECN1, promoting autophagic cell death (Elgendy et al., 2011).

Phosphorylation of BECN1 or BCL2 constitutes another mechanism in controlling the disruption of BECN1/BCL2 association. BECN1 is phosphorylated by death-associated protein kinase (DAPK) in its BH3 domain, which induced autophagy by promoting its dissociation from BCLX<sub>L</sub> (Zalcvar et al., 2009). Likewise, phosphorylation of BCL2 by JNK1 and ERK at multiple sites have been shown to reduce binding of BCL2 to BECN1, leading to activation of autophagy in response to starvation (Wei et al., 2008) or ceramide (Pattingre et al., 2009) treatment. Note that both JNK1 and DAPK were implicated in the apoptosis control as well (Raveh et al., 2001; Jang et al., 2002; Bialik and Kimchi, 2006). In this phosphorylation context, one of the most interesting mechanisms is differential binding affinities of proteins, which provide a sequential activation of autophagy and apoptosis through a single regulator. For example, at early time points of amino acid starvation, JNK1-mediated phosphorylation of BCL2 leads to its rapid dissociation from BECN1 and subsequent induction of pro-survival autophagy (Wei et al., 2008a,b). Since pro-apoptotic proteins, such as BAX, can bind to BCL2 with higher affinity than BECN1, this low level of BCL2 phosphorylation is insufficient to displace them from BCL2. On the other hand, if starvation continues much longer, hyper-phosphorylated BCL2 is accumulated and results in the dissociation of BCL2 from BAX, which is sequentially activated apoptosis (Fig. 3b).

BCL2 proteins were reported to localize in multiple subcellular compartments, including endoplasmic reticulum (ER) and mitochondrial membranes (Akao et al., 1994). Differential localization of BCL2 enables dynamic and independent modulation of organelle-specific stress and death pathways. As mentioned earlier, ER-localized BCL2, but not mitochondria-localized BCL2 was reported to regulate autophagy through

BECN1 binding (Pattingre et al., 2005). ER protein NAF-1, a nutrient-deprivation-related autophagy factor, was reported to be inhibited by an ER-localized BH3-only protein, namely BIK (Chang et al., 2010).

Additionally, JNK1 specifically phosphorylated ER-localized BCL2 during starvation to induce autophagy (Wei et al., 2008a,b). In contrast to ER-localized BCL2, interaction of BH3-only proteins BAX or BAK with



**Fig. 3.** Regulation of Autophagic Machinery by Apoptosis. BCL2 family proteins (a-c), caspases (d), FLICE-like Inhibitory Protein (FLIP) (e), p53, ARF protein and E2F1 transcription factor, and DAPk (f) regulation.

mitochondria-localized BCL2 was reported to control activation of apoptosis (Adams and Cory, 2007; Martin, 2011) (Fig. 3c).

In summary, BECN1/BCL2 protein interactions seem to be a critical junction for the control and coordination of apoptosis and autophagy in cells.

### Caspase regulation

Caspases are an essential family of cysteine proteases that are required for apoptotic programmed cell death. They are crucial for developmental, physiological and stress-related cell death mechanisms. Recent findings revealed a role for caspases in the regulation of the molecular interplay between autophagy and apoptosis.

Caspase-8 is activated by a death inducing signaling complex (DISC) assembling on endosomal membranes (Laussmann et al., 2011; Young et al., 2012). Interestingly, recruitment of caspase-8 to autophagosomes was described as well. For example, ubiquitylated caspase-8 was found to bind autophagic cargo receptor p62 (also known as SQSTM1) through ubiquitin-binding domain (UBD) of p62, leading to its engulfment by autophagosomes (Jin et al., 2009; Young et al., 2012). Additionally, caspase-8 was recruited to the autophagosome machinery through an interaction between the adaptor protein FADD and ATG5 (Pyo et al., 2005; Jin et al., 2009; Young et al., 2012). Autophagosome-related activation of caspase-8 was reported upon treatment with proteasome inhibitors, although activation did not require ligand binding, indicating that intrinsic stress signals were sufficient for activation in this context (Laussmann et al., 2011). Moreover, caspase-8 activation by autophagy was also implicated in a non-apoptotic pathway in T cells. In these cells, caspase-8 regulated autophagy in caspase-8 or FADD-deficient cells by increasing autophagy (Bell et al., 2008). Interestingly, it was recently reported that while depletion of autophagy genes attenuated caspase-8 activation, pharmacological inhibition of autophagosome-lysosome fusion was found to enhance caspase-8 activation, suggesting that autophagosome-mediated activation of caspase-8 required autophagosome formation, but not autophagolysosomal activity (Young et al., 2012).

Caspases were reported to directly cleave autophagy proteins, and inactivated or modified their function. To date, several autophagy proteins, including BECN1, ATG4D, p62, ATG3 and AMBRA1 were identified as targets of caspases (Betin and Lane, 2009; Cho et al., 2009; Luo and Rubinshtein, 2010; Norman et al., 2010; Oral et al., 2012; Pagliarini et al., 2012) (Fig. 3d). For example, caspase-6 cleaved BECN1, and cleaved BECN1 fragments failed to bind the VPS34 PI3 kinase complex (Cho et al., 2009; Wirawan et al., 2010; Young et al., 2012). Instead, they translocated to mitochondria where they acquired a pro-apoptotic function. Moreover, a non-cleavable form of BECN1 was shown to restore autophagy (Oberstein et al., 2007). Caspase-3 processing

of ATG4D resulted in an enzyme with increased LC3 protein priming and delipidation activities. However, truncated ATG4D induced apoptosis, following recruitment of ATG4D to mitochondria (Betin and Lane, 2009). p62 protein was reported to be cleaved by caspase-6 and caspase-8, and interestingly cleavage affected formation of lipidated-LC3 and inhibited the autophagy process (Norman et al., 2010). Recently, Pagliarini et al. demonstrated that caspases were responsible for AMBRA1 cleavage, whereas calpains were involved in AMBRA1 complete degradation. They showed that AMBRA1 levels were critical for apoptosis induction, and expression of a mutant form of AMBRA1 that could not be cleaved by caspases conferred partial protection from apoptotic cell death and cells displayed high levels of autophagy (Pagliarini et al., 2012).

We recently showed that caspase-8 mediated cleavage of ATG3 protein upon TNF- $\alpha$  and TRAIL stimulation modulated apoptosis and autophagy responses of leukemia and lymphoma cells (Oral et al., 2012). We found that caspase-8 overexpression led to ATG3 degradation, and this event depended on caspase-8 enzymatic activity. Indeed, mutation of the caspase-8 cleavage site on ATG3 abolished its cleavage, demonstrating that ATG3 was a direct target of caspase-8. Our data demonstrated that caspase-mediated cleavage led to inactivation of survival-promoting autophagy during death receptor-activated apoptosis.

### FLICE-like Inhibitory Protein (FLIP)

Cellular FLIP (c-FLIP) has been identified as a pro-caspase-8-like regulator of the extrinsic apoptotic pathway. It impeded with death ligand (TNF- $\alpha$ , Fas-L, and TRAIL etc)-induced apoptosis through binding FADD and/or caspase-8/10 in a ligand-dependent fashion, preventing DISC formation and subsequent activation of the caspase cascade. Both c-FLIP and viral orthologs were found to inhibit apoptosis triggered by death receptors (Irmeler et al., 1997). A recent study revealed a novel function of FLIP as a negative regulator of autophagy (Lee et al., 2009). Under normal conditions, c-FLIP inhibited autophagy by interfering with the function of ATG3 in the LC3 conjugation system. However, under stress conditions, c-FLIP allowed ATG3-LC3 interaction and thus induced autophagy. Interestingly, different regions within the FLIP protein were responsible for the anti-autophagic and anti-apoptotic activities of FLIP. Inhibition of apoptosis by c-FLIP was shown to take place at the plasma membrane, while inhibition of autophagy occurred at sites of autophagosome formation (Fig. 3e).

### p53

p53 is a key tumor suppressor protein that can be activated by various stress conditions, including DNA damage, hypoxia, DNA repair, cellular senescence, aberrant oncogene expression and apoptosis (Levine,



1997). Induction of apoptosis by p53 is often context-dependent, relying on the transcriptional activation of BCL2 family member BAX and BH3-only members PUMA, BID and NOXA, and/or the attenuation of BCL2 expression (Fridman and Lowe, 2003) (Fig. 3f). In addition to controlling transcription of pro-apoptotic members of the BCL2 family, p53 can directly or indirectly regulate transcriptional expression of caspases (Green and Kroemer, 2009; Vaseva et al., 2012).

Even though most studies focused on the apoptotic role of p53, recent reports highlighted its function in autophagy regulation, and its role in the link between autophagy and apoptosis. Intriguingly, p53 plays a dual role in autophagy regulation. The nuclear form of p53 regulated expression of damage-regulated autophagic modulator (DRAM), a gene encoding a lysosomal protein involved in autophagy activation (Feng et al., 2005; Crighton et al., 2006). DRAM-mediated induction of autophagy by nuclear p53 contributed to apoptotic cell death in response to genotoxic stress (Fig. 3f). More recently, Contreras et al. revealed that p53 is deacetylated by histone deacetylase (HDAC) upon interferon- $\gamma$  stimulation, leading to its nuclear accumulation and direct suppression of BCL2-modifying factor (Bmf) promoter. Thereby, BECN1 and BCL2 interaction was reduced and autophagy was facilitated (Contreras et al., 2013).

In contrast with the nuclear form, cytoplasmic p53 was associated with suppression of autophagy. In this context, loss of p53 function either through genetic manipulation or pharmacological inhibition was itself sufficient for triggering autophagy activation (Tasdemir et al., 2008a,b). Similarly, autophagy-inducing chemical stimuli led to proteasomal degradation of p53, enabling autophagy activation (Tasdemir et al., 2008a,b).

Thus, the autophagy regulatory capacity of p53 depended on p53 localization. While nuclear p53 favored inducing autophagy and led to cell death, cytoplasmic p53 repressed basal pro-survival autophagy. Collectively, p53 links autophagy and apoptosis in a complex, context-dependent manner in order to ensure cellular homeostasis (Tasdemir et al., 2008a,b).

#### *Alternative Reading Frame (ARF) protein and E2F1 transcription factor*

ARF is a tumor suppressor protein (termed p14ARF in human and p19ARF in mouse) functioning in both autophagy and apoptosis. p19ARF is an upstream activator of p53, leading its stabilization by antagonizing p53's negative regulator Mdm2 proteins (Hdm2 in human). p19ARF mRNA has a second small variant, known as smARF, which is generated by internal translation. smARF lacks the N-terminal domains mediating nuclear localization and Mdm2 binding. Instead of the nucleus, it localized to mitochondria and induced mitochondrial depolarization and autophagic cell death (Reef et al., 2006). An additional study showed that human p14ARF also localized to the outer

membrane of mitochondria where it interacted with BCL2X<sub>L</sub>. Although reduced association of BECN1 to BCL2 was reported in this context, it is still unclear how an interaction in mitochondria affected ER-localized BCL2/BECN1 complex (Pimkina et al., 2009).

The E2F family of transcription factors are involved in not only cellular proliferation, but also in DNA repair, differentiation and development (DeGregori and Johnson, 2006). Moreover, E2F was reported to have important roles in signaling pathways leading to cell survival or cell death. E2F1 was described to trigger apoptosis through upregulation of p53, enhancing p53-mediated transactivation of pro-apoptotic genes (Iaquinta and Lees, 2007). Activation of E2F1 transcription factor was shown to induce autophagy through direct upregulation of the expression of the autophagy genes LC3, ULK1 and DRAM, or through indirect upregulation of ATG5 (Polager et al., 2008; Mehrpour et al., 2010) (Fig. 3f). Although an effect of E2F1 on BECN1 expression remains to be demonstrated, E2F1 was shown to bind to the promoter of BECN1 gene as well (Weinmann et al., 2001).

#### *Death-associated protein kinase (DAPk)*

DAPk, a Ca<sup>2+</sup>/calmodulin-regulated Ser/Thr kinase, is a tumor suppressor protein and a metastasis inhibitor (Bialik and Kimchi, 2006). Both tumor and metastasis suppressive roles of DAPk are related to its ability to regulate apoptosis, while it also affects cytoskeleton and cell motility (Wang et al., 2002; Kuo et al., 2006). DAPk activity was induced under several stress conditions, including death receptor activation, forced oncogene expression, anoikis, ceramide and TGF $\beta$  (Bialik and Kimchi, 2006). The role of DAPk in the regulation of autophagy was first established in mammalian cell culture (Deiss et al., 1995; Inbal et al., 2002) and in *C. elegans* (Kang et al., 2007). Down-regulation of *dapk*-, the worm ortholog of DAPk, reduced starvation-induced autophagy in the pharyngeal muscle of *C. elegans* (Kang et al., 2007). Interestingly, experiments using DAPk knockout mice showed that both autophagic and apoptotic activities were attenuated *in vitro* in knockout fibroblasts, and *in vivo* in kidney toxicity tests (Gozuacik et al., 2008). Indeed, tunicamycin- or thapsigargin-induced ER stress activated DAPk activity, triggering a cell death response that was of a mixed autophagic and apoptotic character. Therefore, DAPk integrated signals from autophagic and apoptotic pathways to induce cell death during ER-stress. Thus, in this system, DAPk was shown to play a critical role in autophagy and apoptosis cross-talk.

As mentioned above, DAPk phosphorylated BECN1, thereby reducing its binding to BCLXL to induce autophagy (Zalckvar et al., 2009). Induction of membrane blebbing and stress fiber formation by DAPk correlated with myosin-II regulatory light chain (MLC) phosphorylation (Kuo et al., 2003; Bialik et al., 2004). DAPk has also been shown to phosphorylate protein



kinase D (PKD), an event that led to JNK activation and cell death during oxidative stress (Eisenberg-Lerner and Kimchi, 2007). p53 is upregulated by DAPk through a mechanism that requires p19ARF, potentially linking DAPk to both autophagic and apoptotic pathways (Raveh et al., 2001; Inbal et al., 2002). ERK can phosphorylate and activate DAPk, which in turn promotes ERK retention in the cytoplasm. This results in inhibition of ERK's nuclear functions, which is antagonistic to apoptotic cell death (Anjum et al., 2005; Chen et al., 2005) (Fig. 3f).

### miRNA regulation

MicroRNAs (miRNAs) are phylogenetically conserved short single-stranded non-protein coding regulatory RNAs, playing vital roles in regulation of around 30% of gene expressions. They are ~22 nucleotide long and regulate important biological processes, including differentiation, proliferation, cell death and cell cycle (Bartel, 2009). More recent studies introduce miRNAs as new players in the regulation of autophagy (Tekirdag et al., 2014). Since autophagy and apoptosis share several essential proteins, targeting of these proteins by miRNAs will directly affect signaling switch between two pathways. So far, several confirmed targets of autophagy-miRNAs are found to be important mediators in the crosstalk between autophagy and apoptosis. MIR30A was shown to reduce the cytoplasmic level of BECN1, and several other miRNAs such as MIR15A and MIR16 were demonstrated to reduce the expression level of the anti-apoptotic family member, BCL2 (Cimmino et al., 2005; Zhu et al., 2009; Zou et al., 2012). Thus, miRNAs are actively involved in the regulation of both autophagy and apoptosis based on modulation of the BECN1/BCL2 interaction. Another common mediator under the control of miRNAs (MIR17, 20, 93 and 106) is SQSTM1 (Meenhuus et al., 2011). Recent data suggest that SQSTM1 can modulate the polyubiquitination and aggregation of CASP8, which is essential for the extrinsic apoptotic pathway (Jin et al., 2009). Conversely, SQSTM1 can negatively regulate the degradation of LC3 protein (Gao et al., 2010). As a result, collective evidence implicates a potential mechanism of SQSTM1 underlying the interplay between apoptotic and autophagic pathways (Xu et al., 2012). Moreover, some miRNAs can simultaneously target multiple genes, which function either in autophagy or in apoptosis. MIR101 potently targets the genes encoding the autophagy-associated proteins RAB5A, ATG4D and STMN1, and also the anti-apoptotic protein MCL1 (Su et al., 2009; Frankel et al., 2011). On the other hand, MIR204 blocks autophagy by modulating the LC3-II protein during hypoxia-reoxygenation, whereas in cholangiocarcinoma cells the exogenous expression of MIR204 negatively regulates BCL2 and facilitates chemotherapeutic drug-triggered apoptosis (Chen et al., 2009).

### Physiological importance of cross-talk

The role of apoptotic cell death was broadly studied in several diseases and treatment of some diseases relies on apoptosis manipulation strategies. Autophagy abnormalities were also shown to play a role in the pathogenesis of human diseases. In this section, we will summarize the significance of autophagy-apoptosis crosstalk in common and well-studied diseases: Cancer, neurodegenerative disorders and cardiovascular diseases.

#### Cancer

It is now well established that activation of apoptotic cell death plays a suppressive role on cancerous transformation and tumorigenesis. However, the role of autophagy and autophagy-apoptosis interplay in a cancer context seems to be more complex. Recent findings indicate a stage-dependent function for autophagy in tumor development (Eisenberg-Lerner et al., 2009; Murrow and Debnath, 2013; Karakas and Gozuacik, 2014). At a pre-cancerous stage, various types of environmental stresses causing DNA and mitochondrial damage lead to ROS releasing and mutated gene accumulation in cells. Such mutations might disrupt vital control mechanisms such as cell cycle, DNA repair and cell death. At this stage, autophagy functions as a recycling mechanism to maintain cellular homeostasis. Elimination of damaged organelles and maintaining genomic stability by autophagy inhibits transformation of the cells and tumor development (Jin, 2006). For example, in this context, in *BECN1* heterozygous cells, a protective role of autophagy was shown to be more prominent when apoptosis was defective (Mathew et al., 2007). Consistently, defective autophagy and genomic instability in apoptosis-deficient cells facilitated tumor progression (Degenhardt et al., 2006; Mantovani et al., 2010). By contrast, in non-small-cell lung carcinoma (NSCLC) cells, suppression of autophagy resulted in the inhibition of cell proliferation and sensitized cells to cisplatin-induced apoptosis (Kaminsky et al., 2012). Another example of interplay between autophagy and apoptosis in transformed cells is observed when autophagy is not enough to maintain genomic instability. In this case, a cell death program may be initiated in order to block transformation. Cells might die by either apoptosis or by an autophagy-related cell death (or autophagic cell death) pathway, a programmed cell death type that was particularly studied in apoptosis-deficient cancer cells (Gozuacik and Kimchi, 2007; Eberhart et al., 2013).

In central regions of poorly vascularized solid tumors, hypoxia and metabolic stress might trigger apoptotic cell death. In such regions, autophagy activation might promote tumor survival. Indeed, in *in vivo* cancer models, autophagy was shown to favor tumor metabolism and cancer cell survival (Yang et al., 2011; Guo et al., 2013).

Autophagy was described as a key determinant of cancer drug resistance. For example, inhibition of autophagy through knockdown of BECN1 in cancer cells or chemical blockage of autophagy in *in vivo* cancer models enhanced apoptosis following chemotherapy (Ropolo et al., 2012).

#### Neurodegenerative disorders

In addition to extracellular inclusions leading to synaptic dysfunction, abnormal or misfolded protein accumulation in the cytoplasm and nucleus result in organelle damage in the pathogenesis of neurodegenerative disorders (Walker and LeVine, 2000). Autophagy is a major elimination pathway for misfolded and long-lived proteins, and impairment of autophagy-dependent degradation was associated with neurodegenerative diseases, including Alzheimer's Disease, Parkinson's Disease, Huntington's Disease and Amyotrophic Lateral Sclerosis (Hara et al., 2006; Amelio et al., 2011; Ghavami et al., 2014; Frake et al., 2015). Autophagy problems were shown to exacerbate the pathology and contribute to apoptotic activation and neurodegeneration (Agostini et al., 2011; Hellwig et al., 2011).

Alzheimer's Disease (AD) is characterized by an accumulation of misfolded proteins, inflammatory changes and oxidative damage, leading to region-specific loss of synaptic contacts and neuronal cell death (Querfurth and LaFerla, 2010). Recent studies underline the fact that mitochondrial dysfunction resulting from autophagy defects add up to abnormal metabolism of amyloid precursor protein (APP) in Alzheimer's disease pathology. In this disease, problems in APP metabolism lead to neurotoxic peptide amyloid- $\beta$  (A $\beta$ ) accumulation, tau phosphorylation and ROS generation, conducing to apoptosis induction and dementia (Lustbader et al., 2004). All these events trigger hyperactive autophagy, while autophagic degradation is inefficient under these conditions. Translocation of misfolded proteins into the mitochondrial membranes was also reported, contributing to the disruption of oxidative phosphorylation, damaged mitochondria accumulation and autophagy induction (Rhein et al., 2009; Smaili et al., 2011). An aging-related decline in autophagic degradative capacity of cells seems to be another factor predisposing elderly population to AD. An aging-related accumulation of A $\beta$  and alpha-synuclein ( $\alpha$ -syn) oligomers in the mitochondrial membrane could modify cell death sensitivity and trigger caspase-dependent cell death (Yang et al., 2008; Rubinsztein et al., 2011; Taylor and Dillin, 2011). Furthermore, a massive neuronal accumulation of autophagosomes in dystrophic and degenerating neuritis was shown to interfere with the axonal transport, an event that was also observed in AD models (Silva et al., 2011; Friedman et al., 2012). Consequently, although elimination of damaged mitochondria and/or misfolded proteins by autophagy seem to be important for the

protective mechanism in order to protect neurons from apoptotic cell death in AD, defective autophagic vesicle accumulation was shown to be toxic as well (Jiang et al., 2014).

Parkinson's Disease (PD) is a progressive neurological disorder without any protective or effective long-term treatment (Habibi et al., 2011). A well-known characteristic of PD is the aggregation of wild-type and mutant forms of  $\alpha$ -synuclein ( $\alpha$ -syn) forming accumulation of Lewy bodies in brain regions affected by the disease (Polymeropoulos et al., 1997; Mizuno et al., 2008). In transgenic mouse models, increased level and accumulation of mutant  $\alpha$ -syn was shown to cause paralysis and neuronal cell death (Giasson et al., 2002). Autophagic degradation, particularly chaperone-mediated autophagy (CMA) was described as an elimination pathway for wild-type  $\alpha$ -syn, while its mutant form clogged the pathway by blocking its receptors (Cuervo et al., 2004). An attenuated or incomplete autophagic degradation pathway resulted in the accumulation of more aggregated proteins, suggesting that autophagy defects are among the contributing factors in PD (Cuervo et al., 2004; Dehay et al., 2010). On the other hand, the induction of autophagy by different chemical compounds could improve molecular signs of PD (Dehay et al., 2010; Mendelsohn and Larrick, 2011; Wu et al., 2011; Filomeni et al., 2012; Lu et al., 2012). In addition to the accumulation of  $\alpha$ -syn in familial PD cases, mutations in the PARK2 gene encoding PARKIN protein were observed (Yamamoto et al., 2005). In *in vitro* studies, suppression of PARKIN led to the accumulation of unfolded proteins in endoplasmic reticulum (ER) and consequently induced apoptosis (Zou et al., 2012a,b). PARKIN, as well as other familial PD-related proteins, PINK1 (PARK6), DJ-1 (PARK7) and LRRK2 (PARK8) play key roles in the control of mitochondrial maintenance through mitochondrial autophagy (mitophagy) (Geisler et al., 2010; Lazarou et al., 2012). For example, PINK1 deficiency resulted in Ca<sup>2+</sup> accumulation in mitochondria, increased generation of ROS and subsequently triggered apoptotic cell death (Akundi et al., 2011; Heeman et al., 2011). PARK7 protected neuronal cells from apoptosis by activating the ERK pathway that triggered mitophagy induction (Gao et al., 2012). On the other hand, mutations in PARK7 gene lead to mitochondrial dysfunction, elevated ROS generation and decreased autophagic degradation, causing accumulation of damaged mitochondria in neurons (Krebiel et al., 2010; Schapira, 2012; Youle and van der Bliek, 2012). Although ER stress is a well-known inducer of apoptotic cell death (Nakagawa et al., 2000), recent studies show its beneficial effects on neuronal cell survival (Pallepati and Averill-Bates, 2011). Consistently, induction of autophagy by ER stress was shown to protect cells from apoptosis in animal and cellular models of PD (Fouillet et al., 2012; Matus et al., 2012). Accordingly, autophagy and mitophagy activation might be exploited as a novel strategy for PD treatment,

preventing neuronal loss and potentially improving disease symptoms.

Huntington's disease (HD) is an autosomal dominant neurological disorder, caused by a mutation in the huntingtin gene. It affects basal ganglia in the brain and causes dystonia, incoordination, cognitive impairment and behavioral difficulties (Walker, 2007). Huntingtin protein (Htt) has a role in controlling brain-derived neurotrophic factor production, vesicular transport, neuronal gene transcription and synaptic transmission (Cattaneo et al., 2005). Although the molecular mechanism of neurodegeneration in HD is not fully known, proteolytic cleavage of mutant Htt by caspases (Wellington et al., 2000; Kim et al., 2001) and release of high poly-glutamine (pQ)-containing N-terminal fragment was shown to form aggregates between neurons. These aggregates are suggested to be the main reason for inhibition of neurotransmission (Rubinsztein and Carmichael, 2003; Ratovitski et al., 2007). Furthermore, generated mutant Htt fragments may cause excitotoxicity, abnormal histone modifications, mitochondrial dysfunction, caspase activation and autophagy stimulation (Sadri-Vakili and Cha, 2006). Interestingly, in some studies using immortalized cell lines derived from HD patients, in addition to mitochondrial membrane hyperpolarization and apoptosis, increased autophagic degradation was observed as well (Mormone et al., 2006). Mutant Htt itself was repeatedly described as an autophagy target. Moreover, Ravikumar et al. showed that damaged mitochondria were degraded and eliminated by autophagy (mitophagy), preventing cytochrome-c release, and inhibiting apoptotic cell death and neurodegeneration in HD (Ravikumar et al., 2006). In line with this, another *in vitro* study showed mutant Htt clearance by autophagy when the level of lysosomal proteases were elevated in HEK (Liang et al., 2011). On the other hand, in murine models and HD patients, Martinez-Vicente et al. found normal autophagosome formation and degradation, although they suggested that cargo recognition could be severely affected, leading to undegraded cytoplasmic cargo accumulation, i.e. huntingtin aggregates, and consequently resulting in neuronal damage (Martinez-Vicente et al., 2010).

Amyotrophic Lateral Sclerosis (ALS) is diagnosed with degeneration of spinal motoneurons leading to muscle weakness and atrophy, spasticity, paralysis or dementia (Martin, 2011). Mutation in the superoxide dismutase 1 (SOD1) gene encoding antioxidant enzyme is the most common genetic cause of ALS (Carri and Cozzolino, 2011). Mutations in the TARDBP gene encoding TAR DNA binding protein 43 kDa (TDP-43) (Sreedharan et al., 2008) and mutations in the FUS gene encoding a RNA binding protein (Vance et al., 2009) were also identified among genes involved in ALS. Moreover, a mutant form of valosin-containing protein (VCP), an ATP-driven chaperon that is essential for autophagosome maturation, was found to be associated with ALS (Johnson et al., 2010). VCP protein was

suggested to control mitochondrial quality (Yamanaka et al., 2012) and as seen in several other neurological disorders, mitochondrial dysfunction was suggested to contribute to neuronal loss in ALS (Reyes et al., 2010; Shi et al., 2010). An *in vivo* study using transgenic SOD1 mice showed that dysfunction of mitochondria was observed in addition to accumulation of aggregates of misfolded proteins, mainly SOD1 and TDP-43 (Neumann et al., 2006; Magrane et al., 2009; Shi et al., 2010). Elimination of these aggregates and damaged mitochondria through autophagy induction resulted in the suppression of apoptosis and neuronal loss and slowed down the progression of ALS (Fornai et al., 2008; Matus et al., 2009; Crippa et al., 2010). Protective effect of autophagy was also observed in lymphomononuclear cells of ALS patients (Sala et al., 2012). Although the protective role of autophagy in ALS was described by the above-mentioned and many other studies, treatment of mutant SOD1 mice with an autophagy inducer, rapamycin, increased pro-apoptotic BAX levels and triggered motor neuron degeneration via apoptosis (Zhang et al., 2011).

Interplay between autophagy and apoptosis was suggested to be involved in neuronal development and central nervous system homeostasis (Hara et al., 2006). Komatsu et al. observed the accumulation of ubiquitin-containing inclusion bodies in Atg7-deficient mice and this ended up in a catastrophic level of neuronal loss (Komatsu et al., 2006). A similar neuronal phenotype was observed in other models of autophagy deficiency. The autophagic mechanism was shown to protect neurons from apoptotic cell death in other studies as well (He et al., 2012). In cerebral ischemia models, clearance of damaged mitochondria by autophagy prevented caspase-dependent apoptosis of neurons that was observed in the reperfusion phase (Zhang et al., 2013). In addition, autophagy was revealed to prevent translocation of pro-apoptotic BAX on mitochondria, resulting in a decrease in cytochrome-c release. Moreover, autophagy was shown to block apoptotic mechanisms after subarachnoid hemorrhage (Chen et al., 2014). Accumulation of autolysosomes was demonstrated in cultured cerebellar granule cells upon potassium deprivation, and accumulation of autolysosomes in this case was found to be involved in the leakage of hydrolyses into the cytosol, triggering apoptotic cell death (Heitz et al., 2009).

### Cardiovascular diseases

Cardiovascular diseases are among the leading causes of mortality in the world. Changes in life style-related factors as well as genetic tendency are considered as the main risk factors for cardiovascular diseases. Autophagy and apoptosis abnormalities were detected in a number of cardiac pathologies, including myocardial infarction (MI), cardiac hypertrophy, heart failure, cardiomyopathy and atherosclerosis. In some cases, both mechanisms were analyzed in the heart under the same



pathological conditions, underlining the fact that autophagy and apoptosis signaling pathways are critical for cellular fate determination.

Ischemia is a restriction of blood supply to tissues, causing a shortage of oxygen, glucose and nutrients that are crucial for cellular metabolism. Cardiac ischemia that occurs in heart muscle cells (myocardium) is generally caused by poor flow of blood through vessels supplying the heart. Insufficient oxygen delivery to cells leads to the accumulation of damaged mitochondria, the release of ROS and subsequently initiates apoptosis. During mild or chronic ischemia, activation of autophagy that was activated by the AMPK-mediated mTor inhibition was shown to inhibit apoptosis and attenuated cardiac tissue damage (Yan et al., 2005; Depre and Vatner, 2007; Matsui et al., 2007; Troncoso et al., 2012). Impaired autophagic flux was detected following ischemia/reperfusion (I/R) in cardiac HL-1 cells. Induction of autophagy via overexpression of BECN1 decreased pro-apoptotic BAX activation and protected cells from apoptotic cell death (Hamacher-Brady et al., 2006). Elevated autophagic activity was also shown in patient tissue samples with ischemic heart disease (Singh et al., 2014). Under these conditions, autophagy played a protective role by supplying cellular metabolic needs and eliminating damaged mitochondria (Valentim et al., 2006; Zhang et al., 2008). On the other hand, experimental results support that autophagy might contribute to both adaptive and maladaptive responses to I/R in cardiomyocytes. Pharmacologic suppression of autophagy in cultured neonatal cardiomyocytes enhanced cell viability (Valentim et al., 2006), and in line with this, other studies reported that autophagy protected cardiomyocytes from apoptotic cell death (Gurusamy et al., 2009; Huang et al., 2010). Recently, BECN1 expression levels were revealed as important determinants of adaptive or maladaptive role of autophagy in cardiomyocytes upon I/R (Ma et al., 2012). For example, reperfusion increased expression of BECN1 and reduced LAMP-2, which resulted in impairment in autophagosome-lysosome fusion, and triggered apoptotic cell death. These results were confirmed in knockdown models of BECN1, where a strong decrease in BECN1 levels blocked autophagy, but even low levels of BECN1 obtained by partial knockdown restored autophagy and protected cardiomyocytes from I/R-induced apoptotic cell death.

Cardiac hypertrophy is the thickening of myocardium resulting in a decrease in the chamber size of the left and right ventricles. Metabolic substrate utilization, alterations in  $Ca^{2+}$  handling, systolic/diastolic dysfunction or abnormal contractility are considered as factors involved in cardiac hypertrophy (Hill and Olson, 2008). Conditional deletion of ATG5 was found to be involved in cardiac hypertrophy and contractile dysfunction. Accumulation of damaged organelles, particularly mitochondria, was reported to induce apoptosis and, these events affected cell fate and resulted in cardiac dysfunction (Nakai et al., 2007). In an *in vivo*

study, haploinsufficiency of BECN1 was revealed to protect the heart from transverse aortic constriction (TAC)-induced hypertrophy (Zhu et al., 2007a,b). Interplay between autophagy and apoptosis in cardiac hypertrophy was shown to be influenced by a balance between oxidized and reduced forms of nicotinamide adenine dinucleotide (NAD). Keeping in a certain level of rate limiting enzyme in the mammalian NAD<sup>+</sup> pathway inhibited apoptosis and stimulated autophagic flux and thus, protected myocardium from death (Hsu et al., 2009; Imai, 2009). In a hypertensive rat model, caloric restriction attenuated mortality, cardiac hypertrophy and cardiomyocyte apoptosis, and this was linked to increased autophagy (Finckenberg et al., 2012).

Cardiomyopathies diseases are characterized by cardiac dysfunction due to abnormality of the heart muscle. It is among the leading factors of heart failure (HF). In addition to the most common form of cardiomyopathy (dilated), other types of cardiomyopathies (hypertrophic and restrictive) also cause the heart to pump a reduced volume of blood to the rest of the body. Protein aggregations, glycogen storage, diabetes, atrial fibrillation and aging are considered as main factors for the cause of cardiomyopathy pathology. Autophagy was shown to protect cardiomyocytes from the toxicity of abnormal protein aggregation in mouse models of cardiomyopathy (Hoshino et al., 2013). Indeed, defective autophagy either by LAMP-2 or ATG5 deficiency in mice was shown to contribute to disease progression (Nishino et al., 2000; Tanaka et al., 2000; Fukuda et al., 2006). Additionally, induction of ATG7-dependent autophagy improved the pathology and extended survival of cardiomyocytes (Bhuiyan et al., 2013).

Heart failure (HF) is a disease characterized by a failure in the blood pumping capacity of the heart. Common causes of HF include myocardial infarction history, high blood pressure, atrial fibrillation and valvular heart disease. Analysis of samples from patients with HF pointed to a correlation between autophagy and apoptosis (Kostin et al., 2003; Saijo et al., 2004). In a conditional cardiac knockout mouse model of mitochondrial fusion 2 (MFN-2) gene, impairment of mitophagy was associated with ROS production and contributed to HF pathology (Chen and Dorn, 2013).

Atherosclerosis, also known as arteriosclerotic vascular disease (ASVD), is defined by an increase in the artery wall thickness, secondary to accumulation of lipoproteins and inflammation of the wall. In this disease, plaque formation and plaque rupture are found to closely involve apoptotic cell death and autophagy. Cross-talk between these two mechanisms was shown to be critical for disease progression, particularly in vascular smooth muscle cells and endothelial cells (Weissberg and Bennett, 1999; Martinet and De Meyer, 2009; Razani et al., 2012). Transmission electron microscopy of vascular smooth muscle cells and atherosclerotic plaques revealed increased autophagic activity (Martinet and De Meyer, 2009). A protective



role of autophagy in this context was demonstrated in several studies (Martinet et al., 2004; Kiffin et al., 2006; Hill et al., 2008). However, treatment of the vascular smooth muscle cell line A7r5 with a calcium channel inhibitor induced apoptosis and autophagy (Park et al., 2011). In another study, treatment of vascular smooth muscle cells with TNF- $\alpha$  stimulated autophagic cell death (Jia et al., 2006). These last two studies revealed the involvement of autophagy in the promotion of cell death rather than cytoprotection. Additionally, excessive autophagic activity was shown to provoke plaque destabilization and thrombotic events (Debnath et al., 2005; Martinet and De Meyer, 2009). Liao et al. showed that silencing of the major autophagic player ATG5 triggered apoptosis and resulted in plaque instability because of the loss of contact cell layer (Liao et al., 2012). Strikingly, both autophagy and apoptosis were found in the endothelial layer of carotid artery, and it was involved in neointima formation during atherosclerosis (Ye et al., 2014).

## Conclusion and perspective

Under stress conditions, autophagy recycles cellular components and is generally defined as a survival mechanism. In addition to its homeostatic role, it plays complicated roles in programmed cell death and today it is well known that both the autophagy and apoptotic pathways share many regulatory molecules. An understanding of the entire molecular machinery of autophagy and apoptosis, as well as the coordination to influence cell fate decisions, will hopefully pave the way to the generation of novel therapies for diseases.

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