

# The dual role of autophagy under hypoxia-involvement of interaction between autophagy and apoptosis

Mengmeng Li · Jin Tan · Yuyang Miao ·  
Ping Lei · Qiang Zhang

Published online: 27 February 2015  
© Springer Science+Business Media New York 2015

**Abstract** Hypoxia is one of severe cellular stress and it is well known to be associated with a worse outcome since a lack of oxygen accelerates the induction of apoptosis. Autophagy, an important and evolutionarily conserved mechanism for maintaining cellular homeostasis, is closely related to the apoptosis caused by hypoxia. Generally autophagy blocks the induction of apoptosis and inhibits the activation of apoptosis-associated caspase which could reduce cellular injury. However, in special cases, autophagy or autophagy-relevant proteins may help to induce apoptosis, which could aggravate cell damage under hypoxia condition. In addition, the activation of apoptosis-related proteins—caspase can also degrade autophagy-related proteins, such as Atg3, Atg4, Beclin1 protein, inhibiting autophagy. Although the relationship between autophagy and apoptosis has been known for rather complex for more than a decade, the underlying regulatory mechanisms have not been clearly understood. This short review discusses and summarizes the dual role of autophagy and the interaction and molecular regulatory mechanisms between autophagy and apoptosis under hypoxia.

**Keywords** Hypoxia · Autophagy · Apoptosis · Caspase · Cell damage

## Introduction

Hypoxia, as the main pathological mechanism of these diseases, such as sleep apnea-hypopnea syndrome, ischemic stroke, definitely can induce cellular injury including hippocampal neuronal injury, myocardial cell injury and renal cell injury. Ultimately, Hypoxia could result in serious complications, for instance, cognitive dysfunction [1], cardiac dysfunction [2], as well as renal dysfunction [3].

Recent studies have found that pathological mechanisms of multi-system injury induced by hypoxia may include excitotoxicity, acidosis, oxidative stress, inflammatory, necrosis and apoptosis [4]. These pathways interact with each other and finally result in severe cellular injury. As a major mechanism of regulated death, apoptosis has received more attention recently.

Autophagy is an intracellular process in which cytoplasmic materials are transported by double-membraned autophagosomes to lysosomes for degradation [5]. Autophagy was detected primarily by using electron microscopy to visualize autophagosomes in the 1990s. At preliminary stage, most studies about autophagy fasten on aging or starvation conditions. But recent studies have shown that autophagy also has a close and complex link with hypoxia [6]. Moreover, with the deepening research of autophagy, researchers found that autophagy plays a dual role in hypoxia-induced cell damage [7].

Recent studies have shown that autophagy and apoptosis often occur in the same cell, mostly in a sequence in which autophagy precedes apoptosis. There are many common upstream pathways between autophagy and apoptosis [8]. Furthermore, autophagy and apoptosis can regulate each other. The dual role of autophagy may be associated with the regulation of apoptosis. Autophagy could block the

M. Li · J. Tan · P. Lei · Q. Zhang (✉)  
Department of Geriatrics, Tianjin Medical University General Hospital, Tianjin Geriatrics Institute, Anshan Road No. 154, Tianjin 300052, China  
e-mail: zhangqiangyulv@163.com

Y. Miao  
Tianjin Medical University, Tianjin, China

induction of apoptosis and attenuate cellular injury. However, in special cases, autophagy or autophagy-relevant proteins may help to induce apoptosis, which could aggravate cellular injury. However, it is not entirely clear that the relationship between autophagy and apoptosis. In here, we discuss and summarize the dual role of autophagy and the interactions and molecular mechanisms between autophagy and apoptosis low-oxygen environments (Figs. 1, 2).

### The dual role of autophagy induced by hypoxia

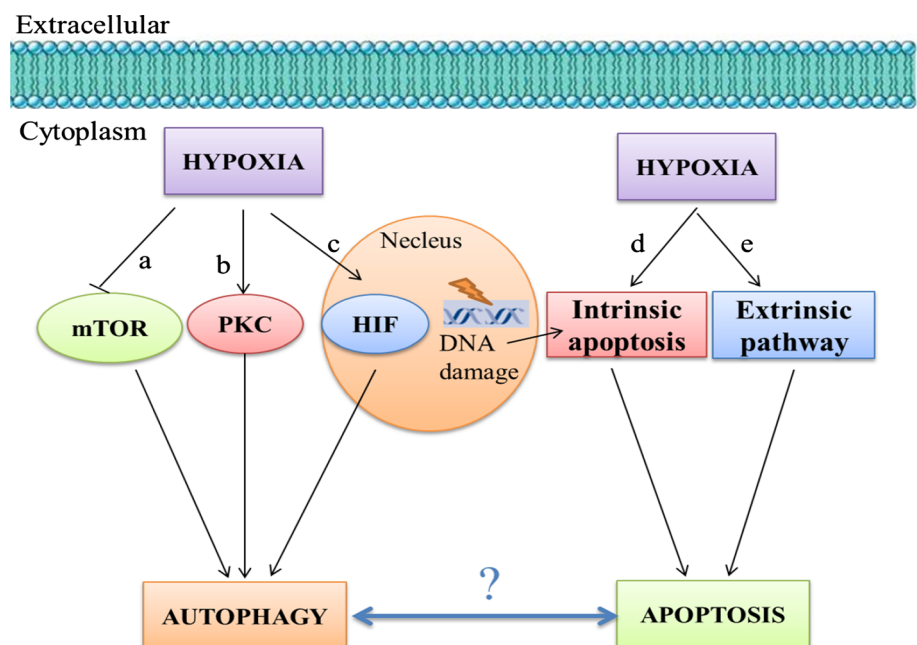
Recently, many studies have found that the role of autophagy in cardio-cerebral-vascular system injury induced by cerebral ischemia seemed to be duality, which not only could be cytoprotective, but also could lead to cell death and increase cardiovascular system and nervous system injury under certain conditions. This paradoxical role of autophagy always depends on the time of ischemia stimulation, the extent of ischemic reperfusion, and induction of autophagy [9].

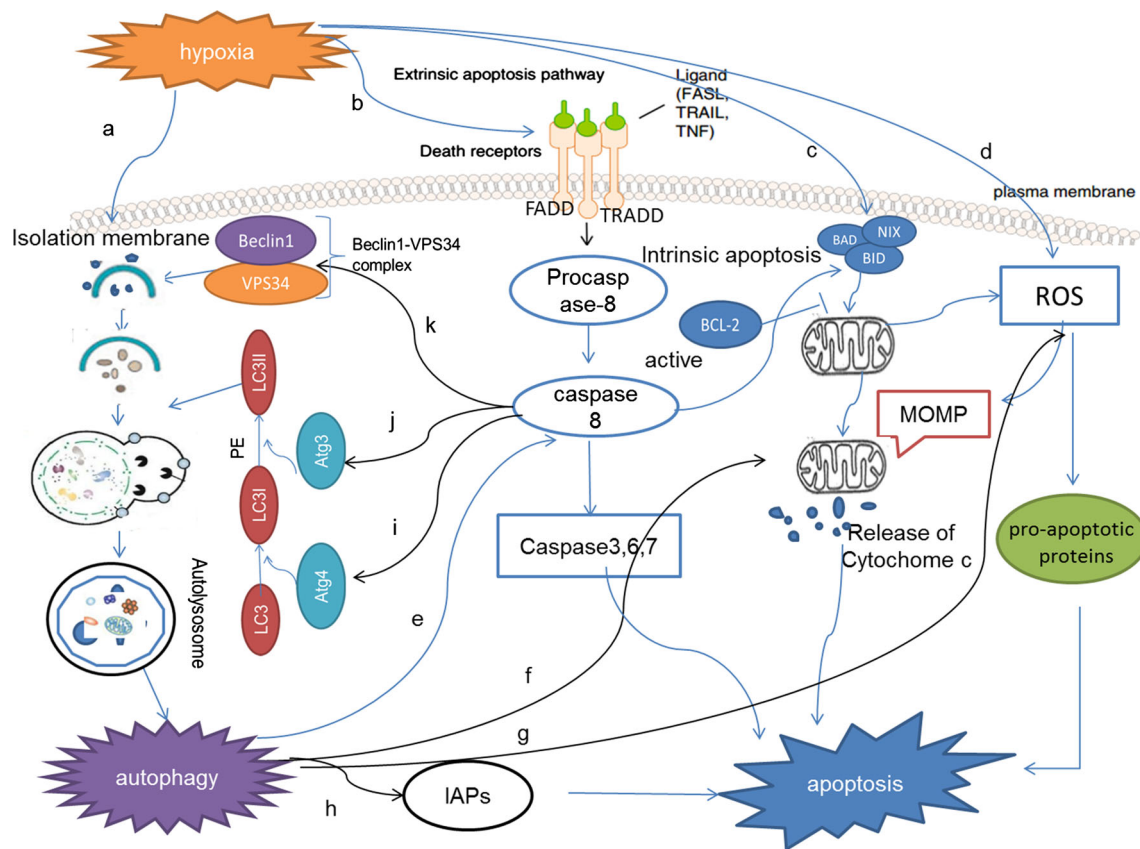
Studies have confirmed that sublethal ischemia can promote mild to moderate activation of autophagy. The moderate activation of autophagy played a protect role in ischemia and hypoxia conditions. Xia et al. [10] established the adult rat permanent middle cerebral artery occlusion (MCAO) model detected that sublethal ischemic preconditioning (IPC) performed before MCAO after ischemia can activate autophagy, as evidenced by Beclin1 expression and LC3-I/II conversion. Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) confirmed

that IPC protected against cerebral ischemic/reperfusion (I/R) injury. Critically, 3-methyladenine, an inhibitor of autophagy, abolished the neuroprotection of IPC and, by contrast, rapamycin, an autophagy inducer, potentiated it. Meanwhile, Kuma et al. [11] generated a transgenic mouse model in which autophagosomes are labelled with GFP-LC3 in tissues, found that autophagy was immediately upregulated in various tissues after mice birth by fluorescence microscope. Yet, mice deficient for Atg5, appeared almost normal at birth but die within 1 day of delivery. Atg5-deficient neonates exhibit reduced amino acid concentrations in plasma and tissues. These studies indicate that autophagy can play a cytoprotective role in hypoxic environment. This protective effect of autophagy may be accomplished by supplying cell metabolism with essential raw materials.

However, the activation of autophagy induced by hypoxia can not only play a protective role but also can aggravate cell injury. Hariharan et al. [12] established the mouse model of cardiac myocytes ischemia–reperfusion injury found that myocardial I/R increased both autophagosomes and autolysosomes, thereby increasing autophagic flux. Treatment with *N*-2-mercaptopropionyl glycine (MPG) which inhibits autophagy, attenuated I/R-induced increased in oxidative stress, accompanied by a decrease in the size of myocardial infarction (MI)/area at risk (AAR), suggesting that autophagy mediate myocardial injury during I/R. Meanwhile, Gao et al. [13] established the mouse model of cerebral ischemia-postconditioning (IPOC) detected that the increased accumulation of autophagy plays a deleterious role in 30 min of cerebral

**Fig. 1** Mechanisms of autophagy and apoptosis induced by hypoxia. *a* Hypoxia resulting in the AMP-activated protein kinase (AMPK) to be phosphorylated. AMPK phosphorylates and activates TSC1/TSC2, leading to inactivation of mTORC1 and induction of autophagy. *b* Hypoxia activates PKC $\delta$ , which activate the downstream of JNK1 and also disrupt Bcl-2-Beclin1 complex to promote autophagy. *c* Hypoxia activate autophagy by the HIF-1 signal pathway. *d, e* Two main pathways that control the activation of apoptosis have been characterized—the intrinsic pathway and the extrinsic (death receptor) pathway in hypoxic cells





**Fig. 2** Complex interplay between apoptosis and autophagy under hypoxia condition. *a–d* Hypoxia induced the activation of autophagy and apoptosis. *a* The activation of autophagy induced by hypoxia. The autophagy pathway induced by hypoxia starts with the formation of an isolation membrane. The ubiquitin-like protein conjugation system [light chain 3 (LC3)] that involve one protease (ATG4, which cleaves LC3 at its carboxyl terminus) and ATG3 (LC3 system), which together catalyse the covalent conjugation of phosphatidylethanolamine (PE) to LC3. *b* The extrinsic pathway is initiated by the ligation of death receptors with their cognate ligands, such as FASL, TRAIL or TNF. As a consequence, an adaptor molecule, FADD, couples death receptors and subsequently activates caspase-8. Activated Caspase-8 activates downstream effector caspases including caspase-3, -6 or -7, which degraded a broad range of cellular proteins, thereby executing the final steps of apoptosis. *c* The intrinsic apoptosis is marked by one central event, mitochondrial outer membrane permeabilization (MOMP), which leads to the release of cytochrome c from the mitochondrial intermembrane space. Cytosolic cytochrome c then triggers the assembly of a caspase-activating complex between caspase9 and apoptotic protease-activating factor1, the final activation of downstream caspase promote

apoptosis. *d* Hypoxia induce the production of ROS mainly from mitochondrion that can intently promote mitochondrial outer membrane permeabilization process. Simultaneously, the accumulation of ROS induced by hypoxia or ischemia could activate pro-apoptotic proteins and then induce apoptosis. *e–h* Regulation of apoptosis by autophagy. *e* Autophagosomes may acts as platforms for caspase8 activation and promote the activation of apoptosis. However, autophagy also can selective clean out the active caspase which delay the onset of extrinsic apoptosis after death receptor stimulation. *f* The selective autophagy of damaged mitochondria (mitophagy) delays intrinsic apoptosis by limiting the release of Cytochrome c. *g* The selective autophagy of ROS damaged mitochondria delays intrinsic apoptosis by inhibiting MOMP. *h* The selective autophagy of anti-apoptotic proteins, such as Bruce, contributes to the induction of apoptosis. *i, j, k* Regulation of autophagy by apoptosis. The apoptosis is usually accompanied by a high degree of caspase activation. Caspase can digest several essential autophagy proteins, such as Atg3, Atg4 and Beclin1, resulting in the inactivation of the autophagic programme, perhaps with the goal to abort its cytoprotective function and to accelerate cellular demise

ischemia followed by 12, 24, and 48 h of reperfusion. LC3II and Beclin1 expression were markedly enhanced after focal cerebral ischemia, accompanied by a down-regulation of P62, suggesting the upregulation of autophagy. Moreover, after a brief intermittent reperfusion during ischemia, IPOC treatment inhibits the induced of autophagy and cause a reduced infarct size, mitigated brain edema simultaneously. These data demonstrate that the

excessive activation of autophagy may be a contributing factor of hypoxia–ischemia-induced cellular injury by fusion with lysosomes in myocardial I/R and transient cerebral ischemia condition.

In short, the autophagy is a double-edged sword in hypoxic injury. A certain degree of autophagic activity may be beneficial to the deletion of denatured proteins and aging organelles and to provide the necessary raw materials

for cell metabolism. However, excessive activation of autophagy might actually lead to neuronal self-digestion and even apoptosis, which may further aggravate the ischemic and hypoxic cellular injury. There are many common pathways between autophagy and apoptosis, therefore the dual role of autophagy may be accomplished by regulating apoptosis.

### The mechanisms of autophagy and apoptosis induced by hypoxia

It is pertinent to briefly outline the mechanisms of hypoxia induced autophagy and effects of hypoxia on gene transcription and signal transduction pathway that confer the differential apoptotic responses in hypoxic cells, as this will help to streamline the overview of the interactions between autophagy and apoptosis.

In recent years, both laboratory and clinical evidence confirmed oxygen deprivation is one of the most established stimulus for induction of autophagy [14]. The signal pathways of autophagy induced by hypoxia are very complex. Hypoxia-inducible factor (HIF) is a key transcription factors, which regulates the transcription of more than 60 genes in response to hypoxia. Hypoxia can activate the HIF-1 that triggers downstream of BNIP3. The BNIP3 then can disrupt the Bcl-2-Beclin1 complex, thus releasing free Beclin1 to form of the vacuolar protein sorting 34 (VPS34)–Beclin 1 complex [15]. This complex is usually inactivated by anti-apoptotic proteins from the BCL-2 family and by other signalling compounds, but when active drives the nucleation of the isolation membrane.

The protein kinase C (PKC $\delta$ ), a serine/threonine kinases family, is activated by diverse stimuli and regulates a variety of cellular processes, such as cell death, proliferation and survival. In the stage of hypoxic response, the PKC $\delta$ , activated by hypoxia stress can activate the downstream of JNK1 and also can disrupt Bcl-2-Beclin1 complex to promote autophagy [16].

Under oxygen deprivation conditions, the serine/threonine kinase target of rapamycin (mTOR), a principal inhibition regulator of autophagy, dissociates from the ULK1 complex and phosphorylates ATG13 and FIP200 to further initiate autophagy process [17]. Moreover, hypoxia resulting in energy depletion, cause the AMP-activated protein kinase (AMPK) to be phosphorylated and activated by LKB1, CaMKK $\beta$  and TAK1, respectively. AMPK phosphorylates and activates TSC1/TSC2, leading to inactivation of mTORC1 and induction of autophagy [18, 19]. In short, we have described the cellular response to hypoxia/anoxia, which consists of several independent and important pathways including HIF-1 $\alpha$ , PKC&-JNK and mTOR signalings.

Hypoxia as a cellular stress can also induce cell apoptosis, and in recent years, large numbers of studies report that the cell damage induced by hypoxia is closely related to apoptosis [20]. Two main pathways that control the activation of apoptosis have been characterized—the intrinsic pathway and the extrinsic (death receptor) pathway in hypoxic cells.

Extrinsic pathway occurs in response to Ligand-mediated multimerization of death receptors like FAS, also referred to as CD95 or Apo1, and TNFR 1, all of which contain the death domain (DD) in their intracellular region to recruit downstream apoptotic proteins. This results in the recruitment of several proteins, including FAS-associated death domain (FADD), TNFR1-associated death domain (TRADD) [21]. FADD contains a DED in its N-terminal region that interacts with the DED in the prodomain of procaspase-8 and recruits caspase-8 to Fas. Fas, FADD, and procaspases-8 form death-inducing signaling complex (DISC), in which the initiator caspase-8 is activated. Activated caspase-8 releases into the cytoplasm and initiates further downstream caspase cascade, such as caspase-3, 6, 7 [22, 23], an effector caspase; Moreover, caspase also cleaves proapoptotic Bid into tBid [24], which translocates to mitochondria to execute apoptosis. In addition, the activity of caspases is negatively regulated by inhibitor of apoptosis proteins (IAPs).

Intrinsic apoptosis is marked by one central event, mitochondrial outer membrane permeabilization (MOMP), which leads to the release of cytochrome c from the mitochondrial intermembrane space. Cytosolic cytochrome c then triggers the assembly of a caspase-activating complex between caspase9 and apoptotic protease-activating factor1, the final activation of downstream caspase promote apoptosis [25].

At the level of genes, BH3-only proteins generally stimulate MOMP by inducing the oligomerization of BCL-2-associated X protein (BAX) in the outer mitochondrial membrane, thereby forming supramolecular channels that mediate the liberation of soluble proteins from the mitochondrial intermembrane space [26]. Besides, DNA damage induced by hypoxia can also stimulate the transactivation of genes encoding pro-apoptotic proteins [such as the BH3-only proteins p53 upregulated modulator of apoptosis (PUMA) and NOXA] by p53 [27]. Among the mitochondrial proteins that are released as a result of MOMP, apoptosis-inducing factor (AIF) and endonuclease G (EndoG) can promote caspase-independent cell death, which can also result from stimuli that cause lysosomal membrane permeabilization (LMP) to release cathepsin proteases into the cytosol. Cathepsins can also trigger MOMP, thereby stimulating the intrinsic apoptotic pathway.

As mentioned above, hypoxia can not only induce autophagy activation but also lead to cell apoptosis. Furthermore, such as BH3-only proteins, p53 and AMPK are not only present in autophagy signal pathways but also play an essential role in apoptosis pathways [28–30]. Hence, the relationship between autophagy and apoptosis is rather closed under hypoxia condition. Autophagy and apoptosis can regulate each other, which played an important role in hypoxia-induced multi-system injury.

### Regulation of apoptosis by autophagy under hypoxia

#### Suppression of apoptosis by autophagy under hypoxia

As mentioned above, there are two main pathways that control the activation of apoptosis in hypoxia cells. Many studies have shown that the suppression of apoptosis by autophagy under hypoxia is mainly associated with the committed steps and key proteins of two main pathways of apoptosis.

Qiang Li et al. [31] established the mouse model of the transient MCAO and observed that the expression of p62 increased in the mitochondrial fractions, in contrast to the decrease in the cytosolic fractions after rapamycin treatment in the peri-ischemic cortex at 24 h after tMCAO, which represents the activation of mitophagy. Moreover, Rapamycin significantly reduced infarct volumes compared with the control group. The 3MA blocked the protective effect of rapamycin on infarct volume and neurological deficit. These results further demonstrate that the activation of mitophagy by rapamycin could attenuate ischemic brain injury in the mouse model of the transient MCAO. In addition, in the cortex from mice subjected to temporary MCAO, Zhang et al. [32] found damage mitochondria engulfed by vacuolar structures, inhibition of autophagy either with 3-methyladenine or Atg7 knockdown enhanced the I-R-induced release of cytochrome c and the downstream activation of apoptosis. In support, administration of the mitophagy inhibitor mdivi-1 in the reperfusion phase aggravated the ischemia-induced neuronal injury both in vivo and in vitro. The study indicates that, the activation of autophagy induced by hypoxia cleared damage mitochondrion, which called mitophagy, preventing the release of cytochrome c and avoiding cytochrome c to activate caspase9-apoptotic protease activating factor-1 (APAF1) complexes, thus inhibiting the activation of caspase 3, 6, 7 and apoptosis downstream pathways. Furthermore, the ischemia-induced neuronal injury was reduced, proving that hypoxia-induced autophagy play a protective role in the cell by clearing damage mitochondrial and inhibiting apoptosis downstream pathways.

Autophagy inhibits apoptosis not only by mitochondria. Zhang R et al. [33] in EAhy926 cell line found that hypoxia treatment induced the upregulation of Beclin1 means the activation of autophagy. They summarized the flow cytometric analysis of Annexin V-FITC/PI activation in EAhy926 found that hypoxia treatment of cells with 3-MA showed an increase in Annexin V-FITC/to 15 %, compared with 8 % for the control. Moreover, the caspase-3 activity quantification test showed that 6 and 24 h of hypoxia could increase caspase-3 activity by 235.6 and 288.8 %, respectively, compared with the control group. Moreover, 24 h of hypoxia combined with 3-MA could increase the to 587.6 %. These results suggested that hypoxia can activate autophagy, moreover, the inhibition of autophagy by 3-MA can potentiate the caspase-3 activity and increase PI-positive cells. In addition, Yulia Grishchuk et al. [34] investigated the role of autophagy in the apoptosis of primary cortical neurons treated with the widely used and potent pro-apoptotic agent, staurosporine (STS). They found that inhibition of autophagy by 3-methyladenine, or by lentivirally-delivered shRNAs against Atg5 and Atg7, strongly reduced the activation of caspase-3, and gave partial protection against neuronal death. Together, these data suggested that the inhibition of hypoxia-induced autophagy could increase the activation of caspase 3 in cells, On the contrary the activation of autophagy keeps the activity of caspase-3 at bay and that inhibited apoptosis, which play a protective role for organisms.

Autophagy can inhibit two classical apoptosis pathways by clearing damage mitochondria and inhibiting caspase activation. Recent studies have demonstrated that autophagy also can inhibit apoptosis by clearing ROS damaged proteins and mitochondria which depend or independent mitophagy under hypoxia condition.

When there is occurrence of environmental factors or stressors such as starvation and hypoxia, there exists an imbalance in the homeostasis reflecting in the inadequate antioxidant capability of cells and the excessive production of ROS. ROS can intently promote MOMP process [35]. The increased ROS also enhances Bax/Bcl-2, Bax/Bcl-xL ratio, caspase 3 (CPP32) expression, and poly-(ADP-ribose)-polymerase (PARP) fragments subsequently resulting in apoptotic cell death. Hamacher-Brady A et al. [36] found that the main role of autophagy in cardiac myocytes subjected to IR is clearance of ROS damaged proteins and mitochondria as part of the cell's effort to minimize cell damage and promote survival. Chien CT's study in the kidney after IR also supported this view [37]. Taken together, autophagy could clear the ROS damaged proteins and mitochondria induced by hypoxia and then attenuate apoptosis.

In conclusion, the activation of autophagy can inhibits apoptosis by clearing damage mitochondrial, inhibiting

caspase 3 activation, as well as clearing ROS damaged proteins, which plays a protective role under the hypoxia conditions.

### Autophagy activates apoptosis under hypoxia

Autophagy could serve a dual function in the hypoxia-induced cell damage, in most cases the activated autophagy play a protective role in the cell, but excessive activation of autophagy may also mediate cell death named the autophagic cell death (ACD). Nevertheless, some studies suggest that the activation of autophagy may also promote the activation of apoptosis; some proteins that play an important role in the autophagy may also promote the apoptosis signal transduction.

Most autophagy-positive neurons also showed increased caspase-3 activation and were TUNEL-positive. Puyal et al. [38] used 12 days old rat cerebral ischemia model found the fact that inhibiting autophagy by 3-MA blocks the post-ischemic increases of caspase activation and prevents caspase-dependent apoptosis. Moreover, Young et al. [39] found that inhibition of the early steps of autophagy by knockout of Atg3 or Atg5 reduced the activation of caspase 8 and that of the effector caspase 3 in cells treated with SKI-I or bortezomib, whereas inhibition of the late steps of autophagy (by bafilomycin A1) increased caspase-dependent cell death. These data indicate that inhibition of the early steps of autophagosome formation will reduce the activation of caspase. Nevertheless, inhibition of late steps of autophagy show an opposite effect, certifying autophagosome formation, rather than the complete process of autophagy, enhancing the activation of caspase, which can activate apoptosis.

Previous studies had shown that apoptotic and anti-apoptotic signaling pathways are activated after cerebral HI, and a shift in the balance between apoptotic and anti-apoptotic cellular factors determines cells fate. A growing number of cellular proteins that have been shown to inhibit caspase activation including IAPs [40]. IAPs are a group of regulatory proteins that are structurally related. Their conserved homologues have been identified in various organisms. In human, eight IAP members have been recognized based on baculoviral IAP repeat (BIR) domains, including HIAP-1, HIAP-2, XIAP, ML-IAP, Survivin and ILP-2/Ts-IAP, NAIP, Bruce/apollon [41]. All members of the family contain N-terminal baculovirus IAP repeat (BIR) domains, and one conservative C-terminal RING domain. The BIR domains are zinc finger-like structures that can chelate zinc ions. These zinc fingers can bind to the surface of caspases so as to the amino acid sequences or linkers between BIR domains can prevent the catalyzing grooves of caspases. As a result, IAP can protect a cell

from apoptosis by inhibiting the activity of caspases. Bruce is an IAPs which have a molecular weight of 528 kD, and equipped BIR domain. Nezis et al. [42] found that autophagy is inhibited in atg1, atg13, and vps34 germline mutants of late *Drosophila melanogaster* oogenesis. Western blot analyses showed that autophagy germline mutant egg chambers contain higher levels of dBruce protein than wildtype egg chambers. Simultaneously, TUNEL showed that the death cell were reduced. These observations indicate that the inhibition of autophagy lead to Bruce accumulated in cytoplasm, thereby inhibiting apoptosis; in contrary, the activation of autophagy can consume Bruce, and then play a catalytic role for apoptosis.

In summary, autophagy can inhibit apoptosis by removing damage mitochondria, inhibiting caspase activation and clearing ROS damaged proteins in hypoxia cells. However, it also can promote apoptosis by activating caspase and clearing the Bruce, which plays a damage role under hypoxia conditions.

### Regulation of autophagy by apoptosis under hypoxia

Studies have reported that the cell signal pathways, responses to hypoxia, and regulation of apoptosis and autophagy are significantly different between normal and tumor cells. Simultaneously we found that there are few studies which report the regulation of autophagy by apoptosis in hypoxic normal cells. While in tumor or cancer cells, studies have demonstrated that the apoptosis has some impacts on the autophagy process. For instance, the activation of apoptosis-related proteins—caspase can degrade autophagy-related proteins, such as Atg3, Atg4, Beclin1 protein, which inhibiting autophagy in tumor or cancer cells.

Atg3 is a component of autophagy-related ubiquitination-like systems that are involved in autophagosome formation [43]. Oral et al. [44] found that Atg3 degradation following TNF and TRAIL treatment in the Jurkat human acute lymphoblastic leukemia T cells and U937 human histiocytic lymphoma cells with monocytic characteristics, whereas, administered the caspase-8 inhibitor (zIETD) or a pan-caspase inhibitor (zVAD), both inhibitors blocked Atg3 degradation and cell death following TRAIL/CHX treatment. These results suggest that Atg3 cleavage or degradation occurred needs caspase-8 activation. The degradation of Atg3 inhibited the autophagy-related ubiquitination-like systems, and then inhibited autophagy.

There is overwhelming evidence to suggest that besides Atg3, Atg4 do contributes significantly to the autophagy-related ubiquitination-like systems. The Atg4 family of endopeptidases can regulate autophagosome biogenesis by priming newly synthesized Atg8 to enable covalent

attachment of phosphatidylethanolamine, and by delipidating Atg8 at the lysosomal fusion step [45, 46], therefore the Atg4 play crucial roles in the autophagy process. Betin et al. [47] detected that induced apoptosis by STS treatment of A431 and HeLa cells and immunoblotted cell lysates for Atg4D and for caspase-cleaved PARP. Atg4D could no longer be detected 24 h following addition of staurosporine. Importantly, loss of Atg4D was prevented by the caspase inhibitor zVAD.fmk. They also found Caspase-3 cleaved Atg4D very efficiently in vitro, generating a ~47 kDa product, but very minimal Atg4D cleavage was detected using caspase-8. These data consistently demonstrate that Atg4 cleavage or degradation following caspase 3 and caspase 8 activation, which undermined the use of Atg8, and certainly inhibited autophagy process.

Among the autophagy related gene, Beclin1 cannot be ignored, Beclin1 possesses a Bcl-2 homology 3 domain (BH3-only), which is necessary for its binding to the BH3 receptor domain of Bcl-2 or Bcl-XL. Mutations of either the BH3-only domain within Beclin1 or the BH3 receptor domain within Bcl-2 or Bcl-XL disrupt the Beclin1/Bcl-2 complex, resulting in the stimulation of autophagy. Therefore Beclin1 plays a key role in the initiation of autophagy [48]. Li et al. [49] found that Beclin1 cleavage was observed in three additional colon cancer cell lines following CPT treatment. Furthermore, CPT-induced Beclin1 cleavage was suppressed in BAX knockout HCT116 cells, and in cells treated with CPT along with the pan-caspase inhibitor zVAD-fmk. Therefore, Beclin1 is likely to be cleaved by a caspase, through caspase8-mediated cleavage of Beclin1, apoptosis inhibits autophagy.

In conclusion, the activation of caspase during apoptosis process, mainly refers to caspase 8 and caspase 3 activation, can cleave autophagy-related proteins, such as Atg3, Atg4D and Beclin1, suppressing autophagy process and resulting in cell damage.

One unique example of the regulation of autophagy by apoptosis is the cleavage of autophagy-related gene by caspase 10. Caspase10 is an apical caspase that can contribute to the induction of apoptosis in response to TNF binding to death receptors. Caspase10 is closely related to many diseases. Caspase10 degradation of BCL2-associated transcription factor 1 (BCLAF1), thereby preventing BCLAF1 interaction with Bcl-2 and competitive disrupt BCL-2-Beclin1 complex, thereby inhibiting autophagy. In multiple myeloma cells, Lamy et al. [50] detected that inhibition of caspase 10 by Q-AEVD-OPH caused an uncontrolled increase in autophagic flux that culminates in cell death without the features of apoptosis. We can speculate that caspase10 could inhibit the excessive activation of autophagy and avoid cell ACD, thus playing a protective role for the cells.

In conclusion, the caspase activated in apoptosis process can cleave the autophagy-related proteins, which suppress autophagy process. However, the researches about the regulations of autophagy by apoptosis were mainly conducted in tumor cells. The regulations of autophagy by apoptosis in normal cells are not clear and need further research.

### Concluding remarks

This review has summarized the interactions and molecular mechanisms between autophagy and apoptosis under the hypoxia conditions. Apoptosis is an important pathological and physiological mechanism of the multiple system injury induced by hypoxia. Autophagy is an important and evolutionarily conserved mechanism for maintaining cellular homeostasis, which plays a dual role in hypoxia-induced cell damage. The dual role of autophagy may be accomplished by regulating the apoptosis. On the one hand, Autophagy could suppress apoptosis by removing damage mitochondria and clearing of apoptosis proteins and ROS damaged proteins, which played a cytoprotective role. Whereas, in special cases, autophagy or autophagy-relevant proteins may speculate the induction of apoptosis by promoting caspase activation and consuming apoptosis endogenous inhibitors, ultimately aggravating cell injury. It is important to note that the relationship between autophagy and apoptosis is mutual. Lots of studies about tumor or cancer cells have demonstrated that the activation of caspase8, 3 during apoptosis can cleavage of autophagy related gene Atg3, Atg4 and Beclin1 to inhibit autophagy, which could aggravate the cellular injury; Nevertheless, the activation of caspase10 can inhibit the excessive autophagy, reducing the cell damage. The interactions and mechanisms between autophagy and apoptosis are rather complex, still needing further study.

**Acknowledgments** This work was supported by National Natural Science Foundation of China (Grant No. 81370183), Tianjin Natural Science Foundation (Grant No. 14JCYBJC27800) and National Clinical Key Subject Construction Project of NHFPC Fund.

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

### References

1. Onen F, Onen H (2010) Obstructive sleep apnea and cognitive impairment in the elderly. *Psychol Neuropsychiatr Vieil* 8:163–169
2. Wang X, Ma S, Qi G (2012) Effect of hypoxia-inducible factor 1-alpha on hypoxia/reoxygenation-induced apoptosis in primary neonatal rat cardiomyocytes. *Biochem Biophys Res Commun* 417:1227–1234

3. Yang F, Liu GS, Lu XY, Kang JL (2009) Expression of caspase-3 in rat kidney with renal tubular damage induced by lipopolysaccharide and hypoxia. *Nan Fang Yi Ke Da Xue Xue Bao* 29:2091–2093
4. Nakka VP, Gusain A, Mehta SL, Raghurib R (2008) Molecular mechanisms of apoptosis in cerebral ischemia: multiple neuro-protective opportunities. *Mol Neurobiol* 37(1):7–38
5. Bras M, Queenan B, Susin SA (2005) Programmed cell death via mitochondria: different modes of dying. *Biochem (Mosc)* 70:231–239
6. Bellot G, Garcia-Medina R, Gounon P, Chiche J, Roux D, Pouyssegur J, Mazure NM (2009) Hypoxia-induced autophagy is mediated through hypoxia-inducible factor induction of BNIP3 and BNIP3L via their BH3 domains. *Mol Cell Biol* 29:2570–2581
7. Mazure MN, Pouyssegur J (2010) Hypoxia-induced autophagy: cell death or cell survival? *Curr Opin Cell Biol* 22:177–180
8. Mariño G, Niso-Santano M, Baehrecke EH, Kroemer G (2014) Self-consumption: the interplay of autophagy and apoptosis. *Nat Rev Mol Cell Biol* 15:81–94
9. Xu M, Zhang HL (2011) Death and survival of neuronal and astrocytic cells in ischemic brain injury: a role of autophagy. *Acta Pharmacol Sin* 32:1089–1099
10. Xia DY, Li W, Qian HR, Yao S, Liu JG, Qi XK (2013) Ischemia preconditioning is neuroprotective in a rat cerebral ischemic injury model through autophagy activation and apoptosis inhibition. *Braz J Med Biol Res* 46:580–588
11. Kuma A, Hatano M, Matsui M, Yamamoto A, Nakaya H, Yoshimori T, Ohsumi Y, Tokuhisa T, Mizushima N (2004) The role of autophagy during the early neonatal starvation period. *Nature* 432:1032–1036
12. Hariharan N, Zhai P, Sadoshima J (2011) Oxidative stress stimulates autophagic flux during ischemia/reperfusion. *Antioxid Redox Signal* 14:2179–2190
13. Gao L, Jiang T, Guo J, Liu Y, Cui G, Gu L, Su L, Zhang Y (2012) Inhibition of autophagy contributes to ischemic postconditioning-induced neuroprotection against focal cerebral ischemia in rats. *PLoS One* 7:e46092
14. Schaaf MB, Cojocari D, Keulers TG, Jutten B, Starmans MH, de Jong MC, Begg AC, Savelkoul KG, Bussink J, Vooijs M, Wouters BG, Rouschop KM (2013) The autophagy associated gene, ULK1, promotes tolerance to chronic and acute hypoxia. *Radiother Oncol* 108:529–534
15. Zhang H, Bosch-Marce M, Shimoda LA, Tan YS, Baek JH, Wesley JB, Gonzalez FJ, Semenza GL (2008) Mitochondrial autophagy is an HIF-1-dependent adaptive metabolic response to hypoxia. *J Biol Chem* 283(16):10892–10903
16. Chen JL, Lin HH, Kim KJ, Lin A, Ou JH, Ann DK (2009) PKC delta signaling: a dual role in regulating hypoxic stress-induced autophagy and apoptosis. *Autophagy* 5(2):244–246
17. Kim J, Guan KL (2011) Regulation of the autophagy initiating kinase ULK1 by nutrients: roles of mTORC1 and AMPK. *Cell Cycle* 10(9):1337–1338
18. Cam H, Easton JB, High A, Houghton PJ (2010) mTORC1 signaling under hypoxic conditions is controlled by ATM-dependent phosphorylation of HIF-1 $\alpha$ . *Mol Cell* 40(4):509–520
19. Papandreou I, Lim AL, Laderoute K, Denko NC (2008) Hypoxia signals autophagy in tumor cells via AMPK activity, independent of HIF-1, BNIP3, and BNIP3L. *Cell Death Differ* 15(10):1572–1581
20. Mattson MP (2000) Apoptosis in neurodegenerative disorders. *Nat Rev Mol Cell Biol* 1(2):120–129
21. Algeciras-Schimmich A, Shen L, Barnhart BC, Murmann AE, Burkhardt JK, Peter ME (2002) Molecular ordering of the initial signaling events of CD95. *Mol Cell Biol* 22:207–220
22. Adams JM (2003) Ways of dying: multiple pathways to apoptosis. *Genes Dev* 17:2481–2495
23. Ashkenazi A (2002) Targeting death and decoy receptors of the tumour-necrosis factor superfamily. *Nat Rev Cancer* 2:420–430
24. Li H, Zhu H, Xu CJ, Yuan J (1998) Cleavage of BID by caspase8 mediates the mitochondrial damage in the Fas pathway of apoptosis. *Cell* 94:491–501
25. Kroemer G, Galluzzi L, Brenner C (2007) Mitochondrial membrane permeabilization in cell death. *Physiol Rev* 87:99–163
26. Tokar T, Ulicny J (2013) The mathematical model of the Bcl-2 family mediated MOMP regulation can perform a non-trivial pattern recognition. *PLoS One* 8(12):e81861
27. Pheesse TJ, Myant KB, Cole AM, Ridgway RA, Pearson H, Muncan V, van den Brink GR, Vousden KH, Sears R, Vassilev LT, Clarke AR, Sansom OJ (2014) Endogenous c-Myc is essential for p53-induced apoptosis in response to DNA damage in vivo. *Cell Death Differ* 21(6):956–966
28. Gallagher PJ, Blue EK (2014) Post-translational regulation of the cellular levels of DAPK. *Apoptosis* 19(2):306–315
29. Zhang L, Wang H, Xu J, Zhu J, Ding K (2014) Inhibition of cathepsin S induces autophagy and apoptosis in human glioblastoma cell lines through ROS-mediated PI3K/AKT/mTOR/p70S6K and JNK signaling pathways. *Toxicol Lett* 228(3):248–259
30. Chen JL, Lin HH, Kim KJ, Lin A, Ou JH, Ann DK (2009) PKC delta signaling: a dual role in regulating hypoxic stress-induced autophagy and apoptosis. *Autophagy* 5(2):244–246
31. Li Q, Zhang T, Wang J, Zhang Z, Zhai Y, Yang GY, Sun X (2014) Rapamycin attenuates mitochondrial dysfunction via activation of mitophagy in experimental ischemic stroke. *Biochem Biophys Res Commun* 444(2):182–188
32. Zhang X, Yan H, Yuan Y, Gao J, Shen Z, Cheng Y, Shen Y, Wang RR, Wang X, Hu WW, Wang G, Chen Z (2013) Cerebral ischemia-reperfusion-induced autophagy protects against neuronal injury by mitochondrial clearance. *Autophagy* 9:1321–1333
33. Zhang R, Zhu F, Ren J, Huang L, Liu P, Wu G (2011) Beclin1/PI3K-mediated autophagy prevents hypoxia-induced apoptosis in EAhy926 cell line. *Cancer Biother Radiopharm* 26(3):335–343
34. Grishchuk Y, Ginet V, Truttmann AC, Clarke PG, Puyal J (2011) Beclin 1-independent autophagy contributes to apoptosis in cortical neurons. *Autophagy* 7(10):1115–1131
35. Grohm J, Plesnila N, Culmsee C (2010) Bid mediates fission, membrane permeabilization and peri-nuclear accumulation of mitochondria as a prerequisite for oxidative neuronal cell death. *Brain Behav Immun* 24(5):831–838
36. Hamacher-Brady A, Brady NR, Gottlieb RA (2006) Enhancing macroautophagy protects against ischemia/reperfusion injury in cardiac myocytes. *J Biol Chem* 281(40):29776–29787
37. Chien CT, Shyue SK, Lai MK (2007) Bcl-xL augmentation potentially reduces ischemia/reperfusion induced proximal and distal tubular apoptosis and autophagy. *Transplantation* 84(9):1183–1190
38. Puyal J, Vaslin A, Mottier V, Clarke PG (2009) Postischemic treatment of neonatal cerebral ischemia should target autophagy. *Ann Neurol* 66(3):378–389
39. Young MM, Takahashi Y, Khan O, Park S, Hori T, Yun J, Sharma AK, Amin S, Hu CD, Zhang J, Kester M, Wang HG (2012) Autophagosomal membrane serves as platform for intracellular death-inducing signaling complex (iDISC)-mediated caspase-8 activation and apoptosis. *J Biol Chem* 287:12455–12468
40. Russell JC, Whiting H, Szufflita N, Hossain MA (2008) Nuclear translocation of X-linked inhibitor of apoptosis (XIAP) determines cell fate after hypoxia ischemia in neonatal brain. *J Neurochem* 106(3):1357–1370
41. Nachmias B, Ashhab Y, Ben-Yehuda D (2004) The inhibitor of apoptosis protein family (IAPs): an emerging therapeutic target in cancer. *Semin Cancer Biol* 14:231–243



42. Nezis IP, Shrivage BV, Sagona AP, Lamark T, Bjørkøy G, Johansen T, Rusten TE, Brech A, Baehrecke EH, Stenmark H (2010) Autophagic degradation of dBruce controls DNA fragmentation in nurse cells during late *Drosophila melanogaster* oogenesis. *J Cell Biol* 190:523–531
43. Mizushima N, Yoshimori T, Ohsumi Y (2011) The role of atg proteins in autophagosome formation. *Annu Rev Cell Dev Biol* 27:107–132
44. Oral O, Oz-Arslan D, Itah Z, Naghavi A, Deveci R, Karacali S, Gozuacik D (2012) Cleavage of Atg3 protein by caspase-8 regulates autophagy during receptor-activated cell death. *Apoptosis* 17:810–820
45. Kirisako T, Baba M, Ishihara N, Miyazawa K, Ohsumi M, Yoshimori T, Noda T, Ohsumi Y (1999) Formation process of autophagosome is traced with Apg8/Aut7p in yeast. *J Cell Biol* 147:435–446
46. Kim J, Huang WP, Klionsky DJ (2001) Membrane recruitment of Aut7p in the autophagy and cytoplasm to vacuole targeting pathways requires Aut1p, Aut2p, and the autophagy conjugation complex. *J Cell Biol* 152:51–64
47. Betin VM, Lane JD (2009) Caspase cleavage of Atg4D stimulates GABARAP-L1 processing and triggers mitochondrial targeting and apoptosis. *J Cell Sci* 122:2554–2566
48. Luo S, Rubinsztein DC (2010) Apoptosis blocks Beclin 1-dependent autophagosome synthesis: an effect rescued by Bcl-xL. *Cell Death Differ* 17:268–277
49. Li H, Wang P, Sun Q, Ding WX, Yin XM, Sobol RW, Stolz DB, Yu J, Zhang L (2011) Following cytochrome c release, autophagy is inhibited during chemotherapy-induced apoptosis by caspase-8-mediated cleavage of Beclin-1. *Cancer Res* 71:3625–3634
50. Lamy L, Ngo VN, Emre NC, Shaffer AL 3rd, Yang Y, Tian E, Nair V, Kruhlak MJ, Zingone A, Landgren O, Staudt LM (2013) Control of autophagic cell death by caspase-10 in multiple myeloma. *Cancer Cell* 23:435–449