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# The autophagy–lysosomal system in subarachnoid haemorrhage

**Haijian Wu [a], Huanjiang Niu [a], Cheng Wu [a], Yong Li [b], Kun Wang [a], Jianmin Zhang [c], Yirong Wang [a, *], Shuxu Yang [a, *]**

*[a] Department of Neurosurgery, Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University, Hangzhou, China*
*[b] Department of Neurosurgery, School of Medicine, Ningbo University, Ningbo, China*
*[c] Department of Neurosurgery, Second Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, China*

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## Abstract

The autophagy–lysosomal pathway is a self-catabolic process by which dysfunctional or unnecessary intracellular components are degraded by lysosomal enzymes. Proper function of this pathway is critical for maintaining cell homeostasis and survival. Subarachnoid haemorrhage (SAH) is one of the most devastating forms of stroke. Multiple pathogenic mechanisms, such as inflammation, apoptosis, and oxidative stress, are all responsible for brain injury and poor outcome after SAH. Most recently, accumulating evidence has demonstrated that the autophagy–lysosomal pathway plays a crucial role in the pathophysiological process after SAH. Appropriate activity of autophagy–lysosomal pathway acts as a pro-survival mechanism in SAH, while excessive self-digestion results in cell death after SAH. Consequently, in this review article, we will give an overview of the pathophysiological roles of autophagy–lysosomal pathway in the pathogenesis of SAH. And approaching the molecular mechanisms underlying this pathway in SAH pathology is anticipated, which may ultimately allow development of effective therapeutic strategies for SAH patients through regulating the autophagy–lysosomal machinery.

**Keywords:** autophagy • lysosome • cell death • neuroprotection • subarachnoid haemorrhage

## Introduction

The autophagy–lysosomal system is a self-destructive process by which cytoplasmic substrates are delivered to lysosomes for degradation [1]. It serves as an important homeostatic mechanism that is responsible for clearance of damaged organelles and protein aggregates in eukaryotic cells [2]. Dysfunction of autophagy–lysosomal pathway has been linked to various disease states, such as cancers, infectious diseases and neurodegenerative diseases [3–6]. Thus, deciphering molecular mechanisms of this degradation pathway would contribute to harness this process for therapeutic purposes.

Subarachnoid haemorrhage (SAH) is a serious, life-threatening type of stroke, which denotes the presence of blood within the subarachnoid space [7]. Approximately 85% of cases of spontaneous SAH occur from the rupture of intracranial aneurysms, 10% fit into the pattern of peri-mesencephalic non-aneurysmal haemorrhages and the remaining 5% are caused by other medical conditions, such as inflammatory or non-inflammatory lesions of intracerebral vessels, sickle cell disease, coagulopathies, neoplasms or drugs [8]. It is noteworthy that although SAH accounts for only 5% of all strokes, its burden on individuals, their families and society is significant, because of high mortality and disability rates, and remarkable incidence among young adults [9]. On the other hand, despite considerable advances in diagnosis and treatment of SAH, clinical outcome remains disappointing and effective therapeutic strategies are yet to

*Correspondence to: Yirong WANG
E-mail: wang.yr@163.com

Shuxu YANG
E-mail: yangsxsy@163.com

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be established [9]. As a consequence, further improving the understanding of SAH pathophysiology is emphasized.

Currently, early brain injury (EBI) and delayed brain injury (DBI) are conceived as two most important mechanisms for SAH pathology [10]. Experimental evidence has demonstrated that the function of subcellular organelles is altered and is implicated in the pathogenesis of brain injury after SAH [11]. Molecular events, such as transcription factor translocation, endoplasmic reticulum stress and mitochondrial dysfunction, occur in the neurovascular unit after SAH [12–14]. More importantly, the 'self-eating' autophagy–lysosomal cascades are activated and play an important role in the pathophysiology of SAH [15]. Thus, this review aims to survey the role and underlying mechanism of autophagy–lysosomal system in the pathogenesis of EBI and DBI after SAH, which may ultimately contribute to develop novel therapeutic targets for SAH treatment *via* modulating this pathway.

## The mechanism and regulation of autophagy–lysosomal system

Autophagy is a sophisticated catabolic process in which cytosolic components and organelles are transported to lysosomes for degradation [16]. Depending on the mode of cargo delivery to lysosome, autophagy is commonly divided into three main subtypes, namely microautophagy, chaperone-mediated autophagy (CMA) and macroautophagy [17–19]. In this review, we will focus on macroautophagy (hereafter referred to as autophagy), the major type of autophagy–lysosomal pathway that eukaryotic cells use to degrade long-lived proteins and organelles (Fig. 1) [20]. In the case of this autophagic process, cytoplasmic cargos are sequestered into double-membrane vesicles known as autophagosomes, which are then delivered to the lysosomes for degradation [21]. Mechanistically, the autophagy–lysosomal pathway can be broken down into series of sequential steps: nucleation, elongation, maturation, docking, fusion and degradation [22]. In detail, it begins with initiation and nucleation, where cup-shaped membrane structures termed phagophores are formed [23]. Then, portions of cytoplasm, including organelles, are enclosed by phagophores to form autophagosomes [24]. Autophagosomes are thereafter trafficked to the lysosomes to form autolysosomes, where the captured substrates, together with the inner membrane, are degraded by lysosomal enzymes [25]. The resulting monomeric units (*e.g.* amino acids) are subsequently exported to the cytoplasm for reuse.

Importantly, several hetero-oligomeric protein complexes that contain autophagy-related (Atg) proteins exert a critical role at different stages of autophagy [26]. As an example, the Unc-51 like autophagy activating kinase (ULK) complex, consisting of ULK1/2, Atg13, FIP200 (focal adhesion kinase family–interacting protein of 200 kD) and Atg101, is essential for the initiation of autophagy [27]. Under normal nutritional conditions, the serine/threonine kinase mammalian target of rapamycin (mTOR) complex 1 targets the ULK complex and inactivates it by phosphorylation of ULK1/2 and Atg13 [28]. Kim *et al.* reported that high mTOR activity prevents ULK1 activation by phosphorylating ULK1 Ser 757 when nutrients are plentiful [29]. In contrast, when nutrients are depleted, the mTOR activity is inhibited and phosphorylation of ULK1 Ser 757 is decreased, and subsequently ULK1 can interact with and be phosphorylated by AMP-activated protein kinase (AMPK) on Ser 317 and Ser 777, which resulting in activation of ULK1 kinase and autophagy induction [27, 29]. This evidence indicates that different phosphorylation events have distinct functions in autophagy initiation. Downstream of the ULK complex, the class III phosphatidylinositol 3-kinase (PI3K) complex, composed of class III PI3K, Beclin-1, p150 and barkor (Beclin-1-associated autophagy-related key regulator), is required for the nucleation and assembly of the initial phagophore membrane [30–32]. The elongation and closure of phagophores depends on two ubiquitination-like reactions. In the first of the reactions, the ubiquitin-like protein Atg12 is covalently tagged to Atg5 to form Atg12–Atg5 conjugate, with the help of the E1-like enzyme Atg7 and the E2-like enzyme Atg10 [33]. The Atg12–Atg5 then conjugates with Atg16L (Atg16-like protein) to form an ~800-kDa protein complex, which serves as a platform for stimulating the microtubule-associated protein 1 light-chain 3 (LC3)-PE (phosphatidylethanolamine) conjugation [34, 35]. In the second ubiquitin-like reaction, the precursor LC3 is cleaved at its COOH terminus by the protease Atg4B, resulting in the cytosolic isoform LC3-I. LC3-I is conjugated to PE to form LC3-II with the action of the E1-like enzyme Atg7 and the E2-like enzyme Atg3 [36, 37]. Thus, the conversion of LC3-I to LC3-II is a well-known marker of autophagy induction [38]. More importantly, the lipidated form of LC3, namely LC3-II, mediates membrane tethering and hemifusion that essential for the expansion and closure of phagophores to form autophagosomes during autophagy [39].

Because of its pathophysiological significance in cellular self-cannibalism, the autophagic process must be tightly regulated. In mammalian cells, multiple signalling cascades, including mTOR-dependent and mTOR-independent pathways, participate in the regulation of autophagy in response to numerous environmental and cellular stimuli [40]. As aforementioned above, the classical mTOR pathway acts as a major negative regulator of autophagy through blocking the ULK complex [29, 41]. Apart from the classical mTOR pathway, the mTOR-independent pathways, such as the cAMP-Epac-phospholipase C (PLC)-ε-inositol 1,4,5-trisphosphate ($IP_3$) pathway and the $Ca^{2+}$-calpain-G-stimulatory protein α ($G_s\alpha$) pathway, can also regulate autophagy in mammalian systems [42, 43]. As an example, elevation of intracellular cAMP levels by adenylate cyclase (AC) activates Epac, and activated Epac in turn activates a small G protein Rap2B, leading to PLC-ε-mediated hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2) to generate IP3, which eventually inhibits autophagy [44]. Besides, an increase in cytosolic $Ca^{2+}$ activates calpains, and activated calpain activates Gsα, resulting in enhanced AC activity that generates cAMP to suppress autophagy [43]. Additionally, starvation-induced activation of c-Jun N-terminal protein kinase 1 phosphorylates Bcl-2, which allows Bcl-2 to dissociate from the autophagy-inhibitory Beclin-1–Bcl-2 complex, thereby promoting the formation of the autophagy-initiating Beclin-1–Vps34 complex to drive autophagy [45, 46]. In contrast, molecular mechanisms underlying the processes of autophagosome transport, autophagosome–lysosome fusion, autolysosomal degradation and reutilization of degradation products, are just beginning to be understood and warranted to be further investigated.



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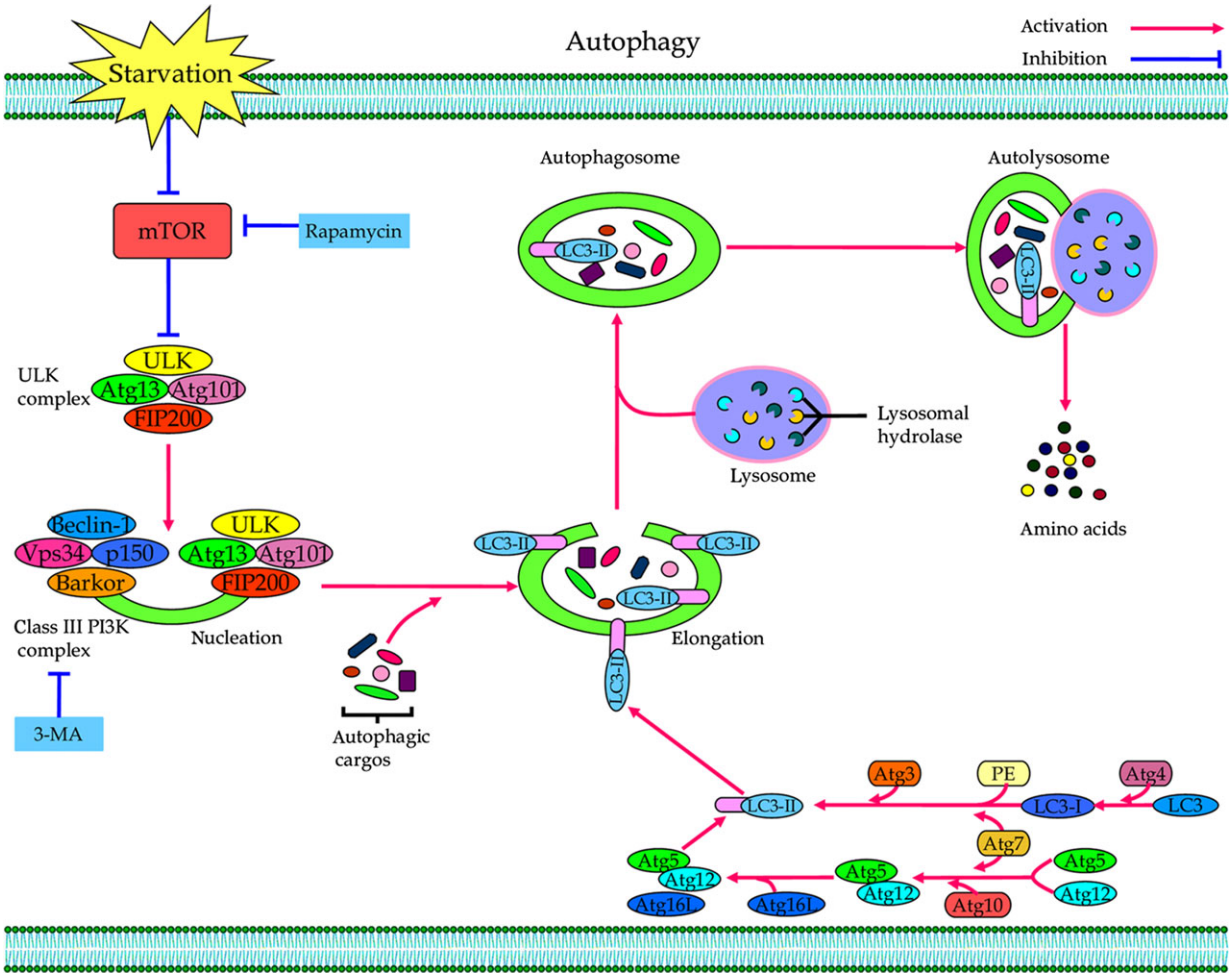


**Fig. 1** Overview of the cellular and molecular events of autophagy–lysosomal pathway. The autophagy–lysosomal process consists of a series of sequential steps: nucleation, elongation, maturation, docking, fusion and degradation. Several hetero-oligomeric protein complexes that contain autophagy-related (Atg) proteins play a critical role at different stages of autophagy–lysosomal process. Multiple signalling pathways, including mTOR dependent and independent, participate in the regulation of autophagy–lysosomal cascades in response to numerous environmental and cellular stimuli.

## The autophagy–lysosomal system in subarachnoid haemorrhage: potential targets for therapeutic intervention

Subarachnoid haemorrhage is a complex, multisystem and multifaceted disorder which involves several ongoing pathological processes [47]. EBI and DBI have been recognized as the important determinants of morbidity and mortality as well as worsened clinical outcome for SAH patients [48]. EBI was coined to describe the acute pathophysiological events occurring in the brain within the first 72 hrs after an SAH [49]. Early pathological changes, such as acute global ischaemia, mechanical and biochemical alterations, impaired ionic homeostasis, excitotoxicity, oxidative stress, inflammation and apoptosis, are all clinically relevant to the poor outcome of SAH patients [50]. By contrast, DBI is designated to demonstrate a host of critical, interrelated pathological events arising in the late phase (3–14 days) of SAH [48]. Cerebral vasospasm (CVS) is conceived as a major cause of delayed cerebral ischaemia and plays a crucial role in the pathogenesis of DBI following SAH [51]. It is noteworthy that molecular mechanisms leading to EBI and DBI are not mutually exclusive. Instead, multiple pathological pathways deleterious to brain activate after the initial haemorrhage, evolve with time and eventually contribute to overall outcome of SAH. More importantly, the autophagy–lysosomal pathway is activated and involved in the pathophysiologic process of SAH (Fig. 2). In consideration of the importance of autophagy–lysosomal system for neuronal survival, its

pathological significance and underlying mechanisms are discussed below.

## Autophagy and brain injury

The autophagy–lysosomal system is a catabolic process that allows the degradation and recycling of intracellular components to ensure cell homeostasis and survival. In a modified endovascular perforation rat model of SAH, Lee *et al.* demonstrated that the autophagy–lysosomal pathway is activated in the ipsilateral frontobasal cortex following SAH and lasts during the entire phase of EBI (up to 3 days) [15]. For electron microscopy, numerous double- or multiple-membrane autophagic vesicles are predominantly observed in neurons at 24 hrs following SAH, demonstrating enhanced autophagy–lysosomal pathway activity in neuronal cells after SAH [15]. By applying Rapamycin (an autophagy inducer targeting mTOR) or 3-Methyladenine (3-MA) (an autophagy inhibitor) to manipulate the autophagic activity, the potential beneficial effect of autophagy on EBI was examined in rat endovascular perforation models of SAH [52, 53]. Autophagy activation reduces translocation of Bax, a pro-apoptotic member of Bcl-2 family, from the cytosol to the mitochondrial membrane [52]. As a consequence, Bax-mediated mitochondrial outer membrane permeabilization is alleviated, the subsequent cytochrome c release into the cytosol is decreased, and the mitochondrial apoptotic pathway is eventually inhibited [54]. Interestingly, melatonin, a hormone secreted by the pineal gland in the brain, stimulated autophagy to suppress apoptotic death of neural cells and ameliorated neurological deficits after SAH [55]. The anti-apoptotic effect of melatonin-enhanced autophagy is associated with prevention of mitochondrial release of cytochrome c to mediate caspase-dependent apoptotic cascades [55]. Also, Shao *et al.* demonstrated that trichostatin A, a pan-

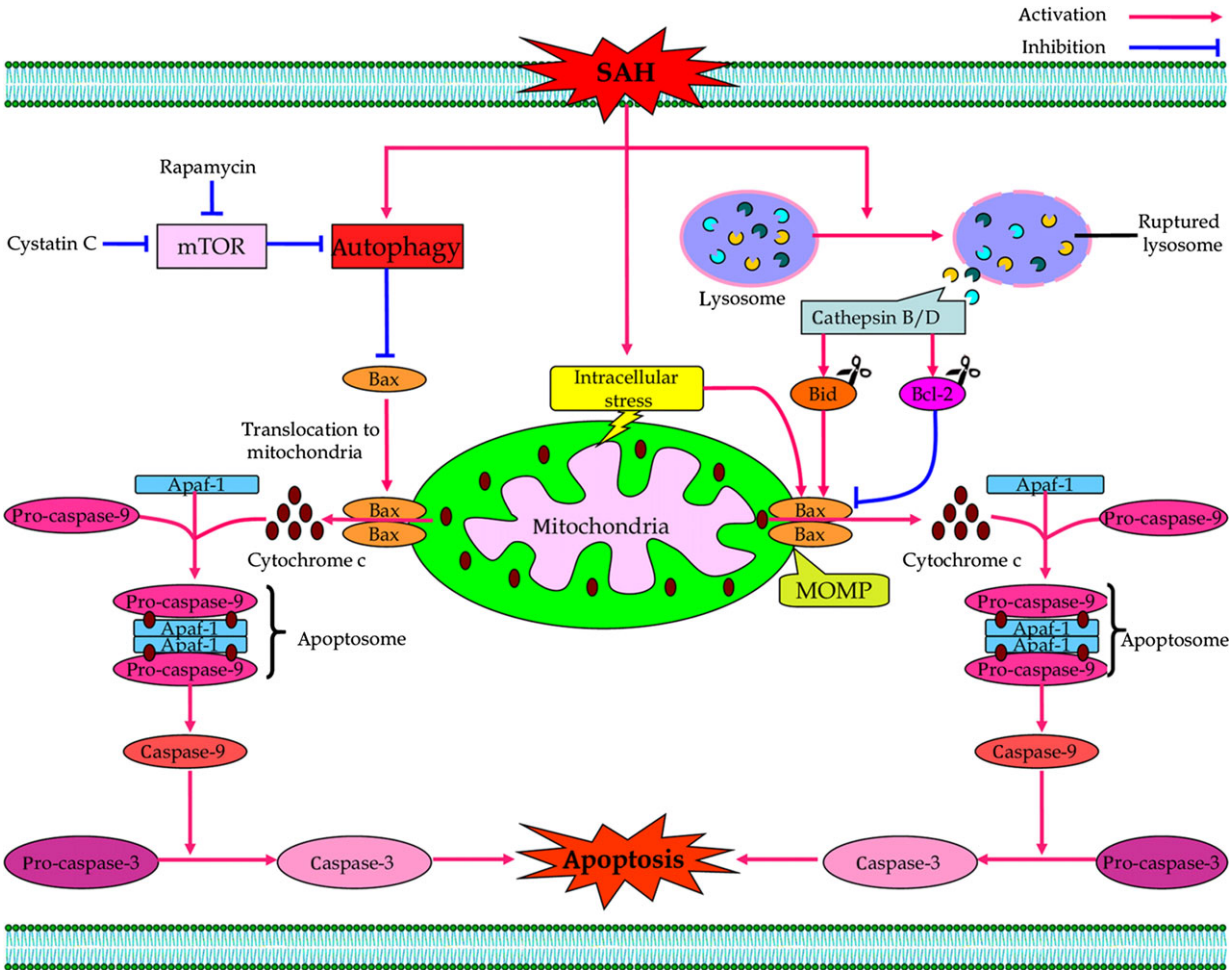


**Fig. 2** Scheme of the role of autophagy–lysosomal system in the pathophysiology of SAH. The autophagy–lysosomal pathway plays a vital role in the pathophysiological process of SAH. Appropriate autophagy–lysosomal activity acts as a pro-survival mechanism in SAH, while excessive self-digestion of autophagy results in cell death after SAH.



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histone deacetylase inhibitor, significantly increased the levels of Beclin-1 and LC3-II/LC3-I ratio, while decreased the expression of Bax and cleaved caspase-3 in the cortex at 24 hrs after experimental SAH [56]. Simultaneously, SAH-induced neuronal apoptosis in the ipsilateral basal cortex was significantly inhibited, and neurological deficits are largely attenuated after TSA application [56]. However, it remains to be investigated whether TSA and other histone deacetylase inhibitors could correct potentially low levels of histone acetylation status following SAH, thus facilitate Atg genes transcription through increasing the accessibility of their promoters to related transcription factors [57]. Additionally, autophagy activation in response to endoplasmic reticulum stress is protective in prevention of EBI in the rat endovascular perforation SAH model [58]. Inhibition of autophagy with 3-MA promoted apoptotic cascades, aggravated neurological deficits and naturalized the endoplasmic reticulum stress-induced beneficial effect after SAH [58].

Similar to the data from endovascular perforation SAH models, Wang *et al.* found that the expression of LC3 and Beclin-1 was significantly increased in the cortex and peaked at 24 hrs after prechiasmatic cistern blood injection, indicating the activation of autophagy–lysosomal system in the brain post-SAH [59]. The autophagy activator Rapamycin can up-regulate the expressions of LC3 and Beclin-1, down-regulate cortical apoptosis, ameliorate blood–brain barrier (BBB) permeability and alleviate clinical behaviour function impairment caused by SAH [59]. Conversely, the autophagy inhibitor 3-MA can decrease the expressions of LC3 and Beclin-1, increase cortical apoptosis, promote BBB permeability and ultimately aggravate clinical behaviour function impairment induced by SAH [59]. Liu *et al.* demonstrated that pre-treatment with a cysteine protease inhibitor Cystatin C with low or medial dosages promotes the autophagic process within neurons, inhibits BBB impairment and ameliorates brain oedema formation, which contributes to alleviation of secondary learning deficits in the prechiasmatic cistern SAH injection model [60]. Cystatin C-mediated inhibition of mTOR-signalling pathway may contribute to autophagy activation under those stress conditions [61, 62]. Taken together, these findings suggested the protective contribution of autophagy–lysosomal pathway in the pathogenesis of EBI during experimental SAH.

## Autophagy and cerebral vasospasm

Cerebral vasospasm is one of the most common and devastating sequelae for patients who have sustained SAH. It is involved in the development of delayed cerebral ischaemia and contributes to DBI after SAH [51]. The contribution of autophagy–lysosomal system in the pathogenesis of CVS following SAH has also been investigated. In a rat cisterna magna single-injection model of SAH, Liu *et al.* demonstrated that the autophagic pathway is activated in the spastic basilar arteries after SAH [63]. Interestingly, Cystatin C promotes the activation of autophagy in the walls of basilar arteries and ameliorates the degree of CVS in this SAH model [63]. However, the exact anti-vasospasm mechanisms of autophagy remain unknown and are warranted to be clarified.

## Lysosome and cathepsin in subarachnoid haemorrhage

Lysosomes, the cytoplasmic membrane-enclosed organelles that contain hydrolytic enzymes, are the key degradative compartments of the cell that control the intracellular turnover of macromolecules [64]. The lysosomal hydrolases including cathepsins, which are enclosed in the lysosomes, play a crucial role in the degradation of heterophagic and autophagic material [65]. It is important to note that lysosomes participate in cellular iron metabolism and recycling [66]. Because of this, most lysosomes contain relatively large amounts of redox-active iron [67]. These iron-rich lysosomes are unusually susceptible to destabilization in response to oxidative challenge, resulting in the release of hydrolytic enzymes (*i.e.* cathepsin B/D) into the cytoplasm, which in turn trigger the lysosomal pathway of apoptosis through cleavage of the pro-apoptotic Bcl-2 family member Bid and the degradation of the anti-apoptotic Bcl-2 members such as Bcl-2, Bcl-xL and Mcl-1 [68].

In the setting of SAH, lysosomes may become iron overload and is particularly prone to destabilization, resulting in lysosomal membrane rupture and the release of hydrolytic enzymes into cytoplasm [69, 70]. Alternatively, overactivation of autophagy may lead to the accumulation of enlarged and unstable acidic vesicles, which would contribute to lysosomal permeabilization and hydrolytic enzymes released from destabilized autolysosomes [71, 72]. However, whether these events are key mediators in SAH-induced hydrolytic enzymes up-regulation deserve further investigation. During the acute phase of SAH, the levels of cathepsin B/D and caspase-3 were up-regulated in the neuron of rat cortex soon after blood injection, which peaked at 48 hrs post-SAH, suggested that the lysosomal membrane of neuron was damaged after SAH [70]. The disruption of lysosomal membrane allows lysosomal proteases (*i.e.* cathepsin B/D) to be released into the cytoplasm to activate caspase-dependent apoptotic pathway [70, 73, 74]. Intraperitoneal administration of deferoxamine, an iron chelator, down-regulates expression of cathepsin B/D and prevents up-regulation of caspase-3 in the cortex 48 hrs after SAH, which contributes to attenuate apoptotic cell death, BBB permeability, brain oedema and motor deficits after SAH [70]. More recently, Wang *et al.* also demonstrated that lysosomes were impaired and cathepsin B/D was up-regulated in the cerebral cortex of affected rats under SAH conditions [75]. By contrast, α-lipoic acid-plus, an amine derivative of α-lipoic acid, can provide neuroprotective effects against EBI *via* targeting lysosomes and chelating intra-lysosomal iron in this prechiasmatic cistern SAH model [75]. Treatment with α-lipoic acid-plus reduces oxidative stress and decreases iron deposition in the cortex of brain, alleviates lysosomal membrane permeabilization and prevents lysosomal rupture following SAH [75, 76]. As a result, the protein levels of cathepsin B/D in the cytoplasm of neurons are decreased and the ensuing Bax-induced apoptotic cell death is reduced, which is protective for amelioration of BBB disruption, brain oedema and neurological behaviour impairment after experimental SAH [75, 77].

Additionally, an imbalance between cysteine cathepsin enzymes and their inhibitor Cystatin C in the arterial walls may exert a prominent role in the progression and rupture of cerebral aneurysms [78,

**Table 1** Main findings of the autophagy–lysosomal system in the pathogenesis of subarachnoid haemorrhage

| Model | Stage | Main findings | Reference |
|---|---|---|---|
| Modified endovascular perforation rat model | EBI | Activation of autophagy–lysosomal pathway | Lee *et al.*, [15] |
| Prechiasmatic blood injection rat model | EBI | Activation of autophagy–lysosomal pathway<br>Inhibition of EBI | Wang *et al.*, [59] |
| Endovascular perforation rat model | EBI | Activation of autophagy–lysosomal pathway<br>Inhibition of EBI<br>Anti-apoptotic effect | Jing *et al.*, [52] |
| Endovascular perforation rat model | EBI | Activation of autophagy–lysosomal pathway<br>Inhibition of EBI<br>Anti-apoptotic effect | Zhao *et al.*, [53] |
| Endovascular perforation rat model | EBI | Melatonin-induced autophagy activation<br>Inhibition of EBI<br>Anti-apoptotic effect | Chen *et al.*, [55] |
| Prechiasmatic blood injection rat model | EBI | Cystatin C-induced autophagy activation<br>Inhibition of EBI | Liu *et al.*, [60] |
| Endovascular perforation rat model | EBI | Endoplasmic reticulum stress-induced autophagy activation<br>Inhibition of EBI<br>Anti-apoptotic effect | Yan *et al.*, [58] |
| Endovascular perforation rat model | EBI | Trichostatin A-induced autophagy activation<br>Inhibition of EBI<br>Anti-apoptotic effect | Shao *et al.*, [56] |
| Cisterna magna blood injection rat model | CVS | Cystatin C-induced autophagy activation<br>Inhibition of CVS | Liu *et al.*, [63] |
| Prechiasmatic blood injection rat model | EBI | Deferoxamine-mediated protection of lysosomal membrane<br>Decreased release of cathepsin B/D<br>Inhibition of EBI<br>Anti-apoptotic effect | Yu *et al.*, [70] |
| Prechiasmatic blood injection rat model 3 | EBI | $\alpha$-Lipoic acid-plus-mediated chelation of intralysosomal iron<br>Decreased release of cathepsin B/D<br>Inhibition of EBI<br>Anti-apoptotic effect | Wang *et al.*, [75] |

79]. When compared with the control cerebral arterial walls, cathepsin B, K and S were highly expressed in the intima and media of aneurysmal walls [78]. In contrast, Cystatin C was lowly expressed in the endothelial cell layer and the media of arterial wall of cerebral aneurysm [78]. Increased expression of cathepsins and decreased expression of Cystatin C causes excessive degradation of extracellular matrix in the aneurysmal walls, which will lead to the progression and rupture of cerebral aneurysm [78, 80, 81]. Treatment with NC-2300, a selective inhibitor for cysteine cathepsins, decreased the activity of cathepsin B, K and S, inhibited the degradation of extracellular matrix in aneurysmal walls and prevented the progression of cerebral aneurysms [78]. It is noteworthy that research on the role of cathepsins in the progression of cerebral aneurysms is still limited, and further investigations are anticipated, which may reveal new therapeutic avenues in preventing aneurysmal progression and rupture.

Taken together, accumulating lines of evidence indicate that the autophagy–lysosomal system is deeply involved in the pathophysiology of SAH. Thus, pharmacological modulation of the autophagy–lysosomal system may represent a potential therapeutic strategy to limit brain injury after SAH. Currently, several pharmacological agents that are able to modulate the autophagy–lysosomal system have been identified, such as mTOR inhibitors, AMPK modulators, calcium lowering agents and lysosome inhibitors [82–84]. These modulators of the autophagy–lysosomal system could be tested in the treatment of SAH in future, many of whom appear to have high potential to be efficient.

## Perspective

Subarachnoid haemorrhage is a complex, multifaceted event that involves multiple ongoing processes contributing to its final pathogenesis. Despite great advances have been made in diagnostic methods, surgical and endovascular repair of ruptured aneurysms and



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management of medical complications, outcome for patients with SAH remains poor. Early brain injury and DBI, two major pathological mechanisms, are recognized as dominant contributors to the prognosis of SAH. The autophagy–lysosomal system is activated and plays a role in the pathogenesis of EBI and CVS after SAH (Table 1). It is significant to note that proper functioning of autophagy–lysosomal pathway acts as a pro-survival mechanism to combat apoptotic cell death following SAH [56, 85]. However, if SAH-induced stress gets too high to deal with, lysosomal membranes would become destabilized so that hydrolytic enzymes would escape into the cytosol to trigger apoptotic cell death [70]. Consequently, it is imperative to maintain the most appropriate threshold of autophagic activity for neuronal survival in the context of SAH, which would be beneficial for patient outcome after SAH.

It is noteworthy that even though knowledge of autophagy–lysosomal system in SAH pathology, the precise roles and underlying mechanisms of autophagy–lysosomal pathway in the setting of SAH remain vague. Indeed, 'self-eating' autophagy and 'self-killing' apoptosis crosstalk with each other extensively in the pathophysiological conditions [86]. Core directors, such as Beclin-1, caspase family proteases and p53, play a crucial role in directing molecular switches between these two intimately connected processes [85, 87]. Therefore, future investigating the role of those core directors will help to elucidate the interrelationship between autophagy and apoptosis in the setting of SAH. In addition, autophagy extensively communicates with other subtype of autophagy (*i.e.* CMA), as well as the ubiquitin-proteasome system during the protein degradation process [88]. Also, autophagy is intricately interlinked with necroptotic cell death [89]. It has been shown that autophagy can either promote or suppress necroptosis under certain conditions [90, 91]. However, the crosstalk between autophagy and necroptosis in SAH pathology remains largely unclarified. And a better knowledge of the interconnection between these degradation pathways is of great significance, with the goal of developing effective strategies to manipulate them for optimizing the therapeutic approaches for SAH.

## Conclusion

The autophagy–lysosomal system exerts critical roles in maintaining intracellular homeostasis in the brain under SAH conditions. Appropriate autophagy functions as protective mechanisms for cell survival after SAH, while excessive 'self-eating' autophagy may lead to cell death. Therefore, approaching molecular mechanisms of autophagy–lysosomal system in the setting of SAH is anticipated, which may ultimately allow to develop effective therapeutic strategies for SAH patients through regulating this pathway.

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## Conflict of interest

No potential conflicts of interest were disclosed.

## References

1. **Rubinsztein DC, Marino G, Kroemer G.** Autophagy and aging. *Cell*. 2011; 146: 682–95.
2. **Nixon RA.** The role of autophagy in neurodegenerative disease. *Nat Med*. 2013; 19: 983–97.
3. **Rubinsztein DC, Codogno P, Levine B.** Autophagy modulation as a potential therapeutic target for diverse diseases. *Nat Rev Drug Discov*. 2012; 11: 709–30.
4. **Qu X, Yu J, Bhagat G, *et al.*** Promotion of tumorigenesis by heterozygous disruption of the beclin 1 autophagy gene. *J Clin Invest*. 2003; 112: 1809–20.
5. **Cooney R, Baker J, Brain O, *et al.*** NOD2 stimulation induces autophagy in dendritic cells influencing bacterial handling and antigen presentation. *Nat Med*. 2010; 16: 90–7.
6. **Komatsu M, Waguri S, Chiba T, *et al.*** Loss of autophagy in the central nervous system causes neurodegeneration in mice. *Nature*. 2006; 441: 880–4.
7. **Hong Y, Shao A, Wang J, *et al.*** Neuroprotective effect of hydrogen-rich saline against neurologic damage and apoptosis in early brain injury following subarachnoid hemorrhage: possible role of the Akt/GSK3beta signaling pathway. *PLoS ONE*. 2014; 9: e96212.
8. **van Gijn J, Kerr RS, Rinkel GJ.** Subarachnoid haemorrhage. *Lancet*. 2007; 369: 306–18.
9. **Sehba FA, Hou J, Pluta RM, *et al.*** The importance of early brain injury after subarachnoid hemorrhage. *Prog Neurobiol*. 2012; 97: 14–37.
10. **Fujii M, Yan J, Rolland WB, *et al.*** Early brain injury, an evolving frontier in subarachnoid hemorrhage research. *Transl Stroke Res*. 2013; 4: 432–46.
11. **Chen S, Wu H, Tang J, *et al.*** Neurovascular events after subarachnoid hemorrhage: focusing on subcellular organelles. *Acta Neurochir Suppl*. 2015; 120: 39–46.
12. **Shao AW, Wu HJ, Chen S, *et al.*** Resveratrol attenuates early brain injury after subarachnoid hemorrhage through inhibition of NF-kappaB-dependent inflammatory/MMP-9 pathway. *CNS Neurosci Ther*. 2014; 20: 182–5.
13. **He Z, Ostrowski RP, Sun X, *et al.*** CHOP silencing reduces acute brain injury in the rat model of subarachnoid hemorrhage. *Stroke*. 2012; 43: 484–90.
14. **Cahill J, Calvert JW, Marcantonio S, *et al.*** p53 may play an orchestrating role in apoptotic cell death after experimental subarachnoid hemorrhage. *Neurosurgery*. 2007; 60: 531–45; discussion 45.
15. **Lee JY, He Y, Sagher O, *et al.*** Activated autophagy pathway in experimental subarachnoid hemorrhage. *Brain Res*. 2009; 1287: 126–35.

16. **Boya P, Reggiori F, Codogno P.** Emerging regulation and functions of autophagy. *Nat Cell Biol*. 2013; 15: 713–20.
17. **Mizushima N, Komatsu M.** Autophagy: renovation of cells and tissues. *Cell*. 2011; 147: 728–41.
18. **Uttenweiler A, Schwarz H, Mayer A.** Microautophagic vacuole invagination requires calmodulin in a $Ca^{2+}$-independent function. *J Biol Chem*. 2005; 280: 33289–97.
19. **Cuervo AM, Stefanis L, Fredenburg R, *et al.*** Impaired degradation of mutant alpha-synuclein by chaperone-mediated autophagy. *Science*. 2004; 305: 1292–5.
20. **Mizushima N.** Autophagy: process and function. *Genes Dev*. 2007; 21: 2861–73.
21. **Levine B, Kroemer G.** Autophagy in the pathogenesis of disease. *Cell*. 2008; 132: 27–42.
22. **Levine B, Klionsky DJ.** Development by self-digestion: molecular mechanisms and biological functions of autophagy. *Dev Cell*. 2004; 6: 463–77.
23. **He C, Baba M, Cao Y, *et al.*** Self-interaction is critical for Atg9 transport and function at the phagophore assembly site during autophagy. *Mol Biol Cell*. 2008; 19: 5506–16.
24. **Axe EL, Walker SA, Manifava M, *et al.*** Autophagosome formation from membrane compartments enriched in phosphatidylinositol 3-phosphate and dynamically connected to the endoplasmic reticulum. *J Cell Biol*. 2008; 182: 685–701.
25. **Webb JL, Ravikumar B, Rubinsztein DC.** Microtubule disruption inhibits autophagosome-lysosome fusion: implications for studying the roles of aggresomes in polyglutamine diseases. *Int J Biochem Cell Biol*. 2004; 36: 2541–50.
26. **Ravikumar B, Sarkar S, Davies JE, *et al.*** Regulation of mammalian autophagy in physiology and pathophysiology. *Physiol Rev*. 2010; 90: 1383–435.
27. **Jung CH, Jun CB, Ro SH, *et al.*** ULK-Atg13-FIP200 complexes mediate mTOR signaling to the autophagy machinery. *Mol Biol Cell*. 2009; 20: 1992–2003.
28. **Hosokawa N, Hara T, Kaizuka T, *et al.*** Nutrient-dependent mTORC1 association with the ULK1-Atg13-FIP200 complex required for autophagy. *Mol Biol Cell*. 2009; 20: 1981–91.
29. **Kim J, Kundu M, Viollet B, *et al.*** AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. *Nat Cell Biol*. 2011; 13: 132–41.
30. **Sun Q, Fan W, Chen K, *et al.*** Identification of Barkor as a mammalian autophagy-specific factor for Beclin 1 and class III phosphatidylinositol 3-kinase. *Proc Natl Acad Sci USA*. 2008; 105: 19211–6.
31. **Jaber N, Dou Z, Chen JS, *et al.*** Class III PI3K Vps34 plays an essential role in autophagy and in heart and liver function. *Proc Natl Acad Sci USA*. 2012; 109: 2003–8.
32. **Sun Q, Fan W, Zhong Q.** Regulation of Beclin 1 in autophagy. *Autophagy*. 2009; 5: 713–6.
33. **Yamaguchi M, Noda NN, Yamamoto H, *et al.*** Structural insights into Atg10-mediated formation of the autophagy-essential Atg12-Atg5 conjugate. *Structure*. 2012; 20: 1244–54.
34. **Romanov J, Walczak M, Ibiricu I, *et al.*** Mechanism and functions of membrane binding by the Atg5-Atg12/Atg16 complex during autophagosome formation. *EMBO J*. 2012; 31: 4304–17.
35. **Mizushima N, Kuma A, Kobayashi Y, *et al.*** Mouse Apg16L, a novel WD-repeat protein, targets to the autophagic isolation membrane with the Apg12-Apg5 conjugate. *J Cell Sci*. 2003; 116: 1679–88.
36. **Satoo K, Noda NN, Kumeta H, *et al.*** The structure of Atg4B-LC3 complex reveals the mechanism of LC3 processing and delipidation during autophagy. *EMBO J*. 2009; 28: 1341–50.
37. **Tanida I, Ueno T, Kominami E.** LC3 conjugation system in mammalian autophagy. *Int J Biochem Cell Biol*. 2004; 36: 2503–18.
38. **Klionsky DJ, Abdalla FC, Abeliovich H, *et al.*** Guidelines for the use and interpretation of assays for monitoring autophagy. *Autophagy*. 2012; 8: 445–544.
39. **Kabeya Y, Mizushima N, Ueno T, *et al.*** LC3, a mammalian homologue of yeast Apg8p, is localized in autophagosome membranes after processing. *EMBO J*. 2000; 19: 5720–8.
40. **Harris H, Rubinsztein DC.** Control of autophagy as a therapy for neurodegenerative disease. *Nat Rev Neurol*. 2012; 8: 108–17.
41. **Ravikumar B, Vacher C, Berger Z, *et al.*** Inhibition of mTOR induces autophagy and reduces toxicity of polyglutamine expansions in fly and mouse models of Huntington disease. *Nat Genet*. 2004; 36: 585–95.
42. **Sarkar S, Ravikumar B, Floto RA, *et al.*** Rapamycin and mTOR-independent autophagy inducers ameliorate toxicity of polyglutamine-expanded huntingtin and related proteinopathies. *Cell Death Differ*. 2009; 16: 46–56.
43. **Williams A, Sarkar S, Cuddon P, *et al.*** Novel targets for Huntington's disease in an mTOR-independent autophagy pathway. *Nat Chem Biol*. 2008; 4: 295–305.
44. **Sarkar S, Floto RA, Berger Z, *et al.*** Lithium induces autophagy by inhibiting inositol monophosphatase. *J Cell Biol*. 2005; 170: 1101–11.
45. **Wei Y, Pattingre S, Sinha S, *et al.*** JNK1-mediated phosphorylation of Bcl-2 regulates starvation-induced autophagy. *Mol Cell*. 2008; 30: 678–88.
46. **Sarkar S.** Regulation of autophagy by mTOR-dependent and mTOR-independent pathways: autophagy dysfunction in neurodegenerative diseases and therapeutic application of autophagy enhancers. *Biochem Soc Trans*. 2013; 41: 1103–30.
47. **Chen S, Li Q, Wu H, *et al.*** The harmful effects of subarachnoid hemorrhage on extracerebral organs. *Biomed Res Int*. 2014; 2014: 858496.
48. **Chen S, Feng H, Sherchan P, *et al.*** Controversies and evolving new mechanisms in subarachnoid hemorrhage. *Prog Neurobiol*. 2014; 115: 64–91.
49. **Caner B, Hou J, Altay O, *et al.*** Transition of research focus from vasospasm to early brain injury after subarachnoid hemorrhage. *J Neurochem*. 2012; 123 (Suppl. 2): 12–21.
50. **Sehba FA, Pluta RM, Zhang JH.** Metamorphosis of subarachnoid hemorrhage research: from delayed vasospasm to early brain injury. *Mol Neurobiol*. 2011; 43: 27–40.
51. **Macdonald RL.** Delayed neurological deterioration after subarachnoid haemorrhage. *Nat Rev Neurol*. 2014; 10: 44–58.
52. **Jing CH, Wang L, Liu PP, *et al.*** Autophagy activation is associated with neuroprotection against apoptosis *via* a mitochondrial pathway in a rat model of subarachnoid hemorrhage. *Neuroscience*. 2012; 213: 144–53.
53. **Zhao H, Ji Z, Tang D, *et al.*** Role of autophagy in early brain injury after subarachnoid hemorrhage in rats. *Mol Biol Rep*. 2013; 40: 819–27.
54. **Chipuk JE, Bouchier-Hayes L, Green DR.** Mitochondrial outer membrane permeabilization during apoptosis: the innocent bystander scenario. *Cell Death Differ*. 2006; 13: 1396–402.
55. **Chen J, Wang L, Wu C, *et al.*** Melatonin-enhanced autophagy protects against neural apoptosis *via* a mitochondrial pathway in early brain injury following a subarachnoid hemorrhage. *J Pineal Res*. 2014; 56: 12–9.
56. **Shao A, Wang Z, Wu H, *et al.*** Enhancement of autophagy by histone deacetylase inhibitor trichostatin a ameliorates neuronal apoptosis after subarachnoid hemorrhage in rats. *Mol Neurobiol*. 2016; 53: 18–27.
57. **Shein NA, Shohami E.** Histone deacetylase inhibitors as therapeutic agents for acute central nervous system injuries. *Mol Med*. 2011; 17: 448–56.




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58. **Yan F, Li J, Chen J, *et al.*** Endoplasmic reticulum stress is associated with neuroprotection against apoptosis *via* autophagy activation in a rat model of subarachnoid hemorrhage. *Neurosci Lett*. 2014; 563: 160–5.
59. **Wang Z, Shi XY, Yin J, *et al.*** Role of autophagy in early brain injury after experimental subarachnoid hemorrhage. *J Mol Neurosci*. 2012; 46: 192–202.
60. **Liu Y, Li J, Wang Z, *et al.*** Attenuation of early brain injury and learning deficits following experimental subarachnoid hemorrhage secondary to Cystatin C: possible involvement of the autophagy pathway. *Mol Neurobiol*. 2014; 49: 1043–54.
61. **Tizon B, Sahoo S, Yu H, *et al.*** Induction of autophagy by cystatin C: a mechanism that protects murine primary cortical neurons and neuronal cell lines. *PLoS ONE*. 2010; 5: e9819.
62. **Watanabe S, Hayakawa T, Wakasugi K, *et al.*** Cystatin C protects neuronal cells against mutant copper-zinc superoxide dismutase-mediated toxicity. *Cell Death Dis*. 2014; 5: e1497.
63. **Liu Y, Cai H, Wang Z, *et al.*** Induction of autophagy by cystatin C: a potential mechanism for prevention of cerebral vasospasm after experimental subarachnoid hemorrhage. *Eur J Med Res*. 2013; 18: 21.
64. **Repnik U, Stoka V, Turk V, *et al.*** Lysosomes and lysosomal cathepsins in cell death. *Biochim Biophys Acta*. 2012; 1824: 22–33.
65. **Boya P, Kroemer G.** Lysosomal membrane permeabilization in cell death. *Oncogene*. 2008; 27: 6434–51.
66. **Kurz T, Eaton JW, Brunk UT.** The role of lysosomes in iron metabolism and recycling. *Int J Biochem Cell Biol*. 2011; 43: 1686–97.
67. **Kurz T, Gustafsson B, Brunk UT.** Intralysosomal iron chelation protects against oxidative stress-induced cellular damage. *FEBS J*. 2006; 273: 3106–17.
68. **Droga-Mazovec G, Bojic L, Petelin A, *et al.*** Cysteine cathepsins trigger caspase-dependent cell death through cleavage of bid and antiapoptotic Bcl-2 homologues. *J Biol Chem*. 2008; 283: 19140–50.
69. **Lee JY, Keep RF, He Y, *et al.*** Hemoglobin and iron handling in brain after subarachnoid hemorrhage and the effect of deferoxamine on early brain injury. *J Cereb Blood Flow Metab*. 2010; 30: 1793–803.
70. **Yu ZQ, Jia Y, Chen G.** Possible involvement of cathepsin B/D and caspase-3 in deferoxamine-related neuroprotection of early brain injury after subarachnoid haemorrhage in rats. *Neuropathol Appl Neurobiol*. 2014; 40: 270–83.
71. **Gonzalez P, Mader I, Tchoghandjian A, *et al.*** Impairment of lysosomal integrity by B10, a glycosylated derivative of betulinic acid, leads to lysosomal cell death and converts autophagy into a detrimental process. *Cell Death Differ*. 2012; 19: 1337–46.
72. **Degtyarev M, De Maziere A, Orr C, *et al.*** Akt inhibition promotes autophagy and sensitizes PTEN-null tumors to lysosomotropic agents. *J Cell Biol*. 2008; 183: 101–16.
73. **Johansson AC, Steen H, Ollinger K, *et al.*** Cathepsin D mediates cytochrome c release and caspase activation in human fibroblast apoptosis induced by staurosporine. *Cell Death Differ*. 2003; 10: 1253–9.
74. **Guicciardi ME, Deussing J, Miyoshi H, *et al.*** Cathepsin B contributes to TNF-alpha-mediated hepatocyte apoptosis by promoting mitochondrial release of cytochrome c. *J Clin Invest*. 2000; 106: 1127–37.
75. **Wang Y, Gao A, Xu X, *et al.*** The neuroprotection of lysosomotropic agents in experimental subarachnoid hemorrhage probably involving the apoptosis pathway triggering by cathepsins *via* chelating intralysosomal iron. *Mol Neurobiol*. 2015; 52: 64–77.
76. **Blomgran R, Zheng L, Stendahl O.** Cathepsin-cleaved Bid promotes apoptosis in human neutrophils *via* oxidative stress-induced lysosomal membrane permeabilization. *J Leukoc Biol*. 2007; 81: 1213–23.
77. **Oberle C, Huai J, Reinheckel T, *et al.*** Lysosomal membrane permeabilization and cathepsin release is a Bax/Bak-dependent, amplifying event of apoptosis in fibroblasts and monocytes. *Cell Death Differ*. 2010; 17: 1167–78.
78. **Aoki T, Kataoka H, Ishibashi R, *et al.*** Cathepsin B, K, and S are expressed in cerebral aneurysms and promote the progression of cerebral aneurysms. *Stroke*. 2008; 39: 2603–10.
79. **Abisi S, Burnand KG, Waltham M, *et al.*** Cysteine protease activity in the wall of abdominal aortic aneurysms. *J Vasc Surg*. 2007; 46: 1260–6.
80. **Sadek M, Hynecek RL, Goldenberg S, *et al.*** Gene expression analysis of a porcine native abdominal aortic aneurysm model. *Surgery*. 2008; 144: 252–8.
81. **Shi GP, Sukhova GK, Grubb A, *et al.*** Cystatin C deficiency in human atherosclerosis and aortic aneurysms. *J Clin Invest*. 1999; 104: 1191–7.
82. **Fleming A, Noda T, Yoshimori T, *et al.*** Chemical modulators of autophagy as biological probes and potential therapeutics. *Nat Chem Biol*. 2011; 7: 9–17.
83. **Mihaylova MM, Shaw RJ.** The AMPK signalling pathway coordinates cell growth, autophagy and metabolism. *Nat Cell Biol*. 2011; 13: 1016–23.
84. **Sciarretta S, Zhai P, Volpe M, *et al.*** Pharmacological modulation of autophagy during cardiac stress. *J Cardiovasc Pharmacol*. 2012; 60: 235–41.
85. **Wu HJ, Pu JL, Krafft PR, *et al.*** The molecular mechanisms between autophagy and apoptosis: potential role in central nervous system disorders. *Cell Mol Neurobiol*. 2015; 35: 85–99.
86. **Maiuri MC, Zalckvar E, Kimchi A, *et al.*** Self-eating and self-killing: crosstalk between autophagy and apoptosis. *Nat Rev Mol Cell Biol*. 2007; 8: 741–52.
87. **Wu H, Che X, Zheng Q, *et al.*** Caspases: a molecular switch node in the crosstalk between autophagy and apoptosis. *Int J Biol Sci*. 2014; 10: 1072–83.
88. **Wu H, Chen S, Ammar AB, *et al.*** Crosstalk between macroautophagy and chaperone-mediated autophagy: implications for the treatment of neurological diseases. *Mol Neurobiol*. 2015; 52: 1284–96.
89. **Nikoletopoulou V, Markaki M, Palikaras K, *et al.*** Crosstalk between apoptosis, necrosis and autophagy. *Biochim Biophys Acta*. 2013; 1833: 3448–59.
90. **Bonapace L, Bornhauser BC, Schmitz M, *et al.*** Induction of autophagy-dependent necroptosis is required for childhood acute lymphoblastic leukemia cells to overcome glucocorticoid resistance. *J Clin Invest*. 2010; 120: 1310–23.
91. **Farkas T, Daugaard M, Jaattela M.** Identification of small molecule inhibitors of phosphatidylinositol 3-kinase and autophagy. *J Biol Chem*. 2011; 286: 38904–12.