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The "Janus-Faced Role" of Autophagy in Neuronal Sickness: Focus on Neurodegeneration

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Abstract The mature brain is a highly dynamic organ that constantly changes its organization by destroying and forming new connections. Collectively, these changes are referred to as brain plasticity and are associated with functional changes, such as memory, addiction, and recovery of function after brain damage. Neuronal plasticity is sustained by the fine regulation of protein synthesis and organelle biogenesis and their degradation to ensure efficient turnover. Thus, autophagy, as quality control mechanism of proteins and organelles in neurons, is essential to their physiology and pathology. Here, we review recent several findings proving that defects in autophagy affect neuronal function and impair functional recovery after brain insults, contributing to neurodegeneration, in chronic and acute neurological disorders. Thus, an understanding of the molecular mechanisms by which the autophagy machinery is finely regulated might accelerate the development of therapeutic interventions in many neurological disorders for which no cure is available.

Keywords Autophagosomes · Autophagic flux · Cell death · Acute brain damage · Neuronal recovery

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Introduction

Autophagy is the homeostatic and degradative cellular pathway that mediates the recycling of proteins and organelles, which are sequestered in the autophagosome—a double-membrane-bound vesicle—and delivered to the lysosome for degradation [1]. Autophagy is highly conserved from yeast to mammals and exists in three principal types—microautophagy, macroautophagy, and chaperone-mediated autophagy.

Of these classifications, macroautophagy, herein referred to as autophagy, has been well characterized and mediates the turnover of the cytosolic portions that contain organelles, sequestered in the autophagosome; based on the types of organelles that are degraded in lysosomes, there are subclassifications of autophagy, such as mitophagy, ribophagy, and reticulophagy [2].

Of the cell types that harbor an autophagy pathway, postmitotic neurons are more susceptible to the effects of dysfunctional autophagy, due to (1) the presence of specific cell compartments (axon, synapse, dendrite) that are characterized by high-energy demand and protein turnover, which are essential for neuronal function; and (2) the evidence that they do not replicate, a phenomenon that requires an efficient protein quality control system to avoid the accumulation of toxic proteins and damaged organelles that cannot be diluted during cell division.

Studies have demonstrated that autophagy is involved in many neurological disorders, such as Alzheimer disease, Parkinson disease, and Huntington disease, and in acute brain damage, in which autophagosomes accumulate abnormally. We review the recent progress in our understanding of the molecular mechanisms of autophagy in mammalian brain and discuss the implications of autophagy in neurodegenerative diseases.

The Autophagy Machinery

A comprehensive description of autophagic machinery has been extensively reviewed [3–5], and we shall describe only the main steps and components of autophagy to understand how pathological events interfere with this homeostatic degradation pathway. The initial steps of autophagy comprise the formation (vesicle nucleation) and expansion (vesicle elongation) of an isolation membrane, also called a phagophore (Fig. 1). The edges of the phagophore then fuse to form the autophagosome, a double-membraned vesicle that sequesters the cytoplasmic material. Next, the autophagosome fuses with a lysosome to generate an autolysosome, in which the captured material is degraded (Fig. 1).

Many autophagy regulatory genes (Atg) have been discovered and characterized in yeast [6, 7], and some of them are conserved in higher eukaryotes. In these multicellular organisms, specific Atg genes, together with their regulators, control different stages of autophagosome formation and maturation.

In mammals, the autophagy induction is initiated by activation of the ULK1,2 complex (the mammalian homolog to yeast ATG1) and the target of rapamycin (TOR)—specifically, TOR complex 1 (TORC1)—is an upstream negative regulator of this complex [5, 8].

ULK1,2 forms a stable complex with mammalian Atg13, FIP200 (a putative counterpart of yeast Atg17) and Atg10 (an Atg13-binding protein), irrespective of TORC1 activation. Under nutrient-rich conditions, the active TORC1 associates with the ULK1,2 complex, phosphorylates ULK1,2, and hyperphosphorylates Atg13, which inhibits the kinase activity of ULK1,2 and thus blocks autophagy induction. Under starvation conditions, TORC1 is inactivated and it dissociates from the ULK complex, preventing phosphorylation of Atg13 and ULK1,2 by TORC1 and leading to autophagy induction, whereas ULK1,2 still phosphorylates Atg13 and itself and hyperphosphorylates FIP200 [8–10]. ULK1,2 activation causes the activation of another complex that comprises (among other proteins) the class III PI3 kinase Vps34 and the protein Beclin-1 (a mammalian ortholog of yeast Atg6) [11–14]. The Vps34 complex can also be activated by proteins that interact with Beclin-1, including UV radiation resistance protein and AMBRA1 (activating molecule in Beclin-1-regulated autophagy protein 1) [11–14]. The activated Vps34 complex generates phosphatidylinositol 3-phosphate, an essential lipid component of the autophagosome, and activates the ATG proteins [11]. Evidence suggests that SNARE (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor) proteins mediate homotypic fusion of phagophore precursors and thereby increase the size of these structures. This fusion process contributes to elongation of the autophagosome precursor membranes and allows the recruitment of proteins that enable maturation into phagophores [15].

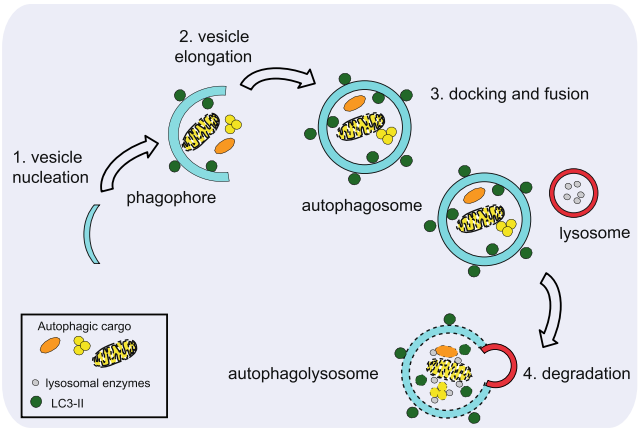

Fig. 1 The cellular steps during autophagy. Autophagic process follows distinct stages: *1* vesicle nucleation (formation of phagophore); *2* vesicle elongation (autophagosome formation); *3* vesicle completion (the edges of the phagophore then fuse to form the autophagosome, a double-membraned vesicle that sequesters the cytoplasmic material); *4* vesicle degradation: fusion of the autophagosome with a lysosome to form an autolysosome where cytosolic components are degraded

During the initiation stage, the autophagic membrane forms a phagophore, and then elongates: these autophagy steps are mediated by two specialized protein conjugation systems. The first stage involves conjugation of ATG12 to ATG5, whereas in the second stage, the LC3-I protein (an ortholog of yeast ATG8) is conjugated to the lipid phosphatidylethanolamine to form LC3-II, which localizes to the autophagosome membrane [16, 17] (Fig. 1). Consequently, autophagy can be examined biochemically (by assessing the generation of Atg8-PE or LC3-II) or microscopically (by observing the localization of fluorescently tagged Atg8 or LC3) [18]. These approaches must be coupled with ancillary measures to discriminate between two distinct situations: increased autophagic flux without impairment in autophagic turnover and impaired clearance of autophagosomes, which results in defects in autophagic catabolism.

Autophagosomes are transported along microtubules towards the microtubule-organizing center, where lysosomes are abundant [19]. A number of SNARE proteins, including VAMP8 and Vti1B, are believed to be involved in regulating heterotypic fusion between autophagosomes and the lysosomal compartment [20]. In mammals, proteins that function predominantly in lysosomes are required for fusion with autophagosomes, such as the lysosomal transmembrane proteins LAMP-2, and the degradation of autophagosomal contents, such as the lysosomal cysteine proteases cathepsins B, D, and L [21, 22].

Autophagy in Progressive Neurodegenerative Disorders

Although neurodegenerative diseases, such as Alzheimer, Huntington, and Parkinson diseases, have disparate etiologies,

they have disease-specific profiles of adult-onset neuronal loss and share a common pathological feature: the aggregation and deposition of abnormal proteins. Degenerating neurons of patients who suffer from neurodegenerative diseases accumulate autophagic vacuoles (AVs) abnormally. However, this accumulation might reflect a robust increase in their formation (Fig. 2(B)) or in autophagic flux or a reduction in AV degradation due to impairments in autophagic flux (Fig. 2(C)).

In both cases, the balance between the accumulation and degradation of AVs is compromised, but the pathogenic mechanisms of this disruption differ, necessitating disparate pharmacological strategies to combat these devastating diseases. We will briefly review the pathogenic mechanisms that are associated with impairments in autophagy pathway in neurodegenerative disorders.

Alzheimer Disease

Alzheimer disease (AD) is the most common cause of dementia, accounting for 50 to 60 % of all cases [23], and is characterized by age-related brain degeneration that leads to progressive cognitive and behavioral impairments. The most frequently validated histopathological feature of AD brains is the presence of neurofibrillary tangles—intracellular fibers that comprise hyperphosphorylated aggregates of the microtubule-associated protein tau and neuritic plaques, extracellular depositions that are composed of amyloid-β (Aβ) peptide, causing progressive, selective, and massive neuronal loss and primarily affecting the hippocampal, mesial temporal, and parietal cortices. Post-mortem electron microscopy analyses of AD brains have demonstrated the presence of AVs, particularly in dystrophic neuritis but also in perikarya that accumulate neurofibrillary tangles [24].

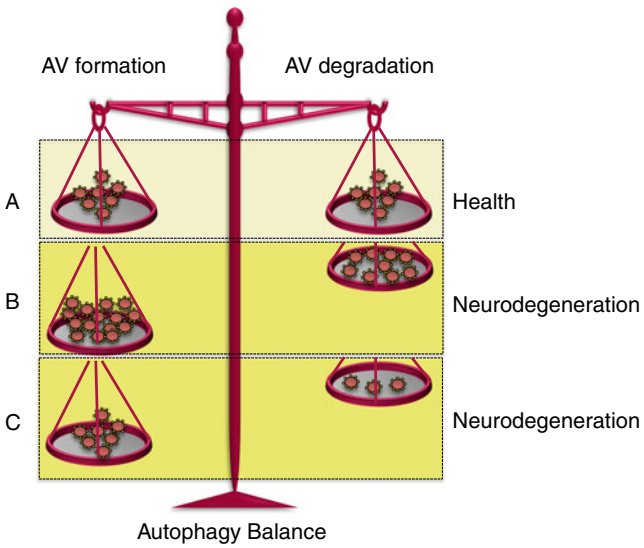

Fig. 2 The balance of autophagic flux in neuronal survival. Autophagic flux refers to the complete process of autophagy (Fig. 1), which begins with the formation of the phagophore, that expands around a portion of cytoplasm and fuses to form the autophagosome (*AV*). *A* In physiological condition, AV can be efficiently degraded in autolysosome and neurons maintain their homeostasis. In pathological conditions, the rate of AV formation might exceed the rate of AV degradation (*B*), or in other cases (*C*), late stage of autophagic process (AV degradation) might result impaired. In both cases, accumulation of AVs leads to neurodegeneration

Two types of autophagic dysfunction have been implicated in AD [25, 26]: (1) impaired autophagy initiation and (2) defective lysosomal clearance of autophagic substrates due to impaired transport of AVs to lysosomes. The former was derived from observations that brains of AD patients have reduced levels of Beclin-1, which is pivotal in the activation of autophagy machinery [27]. According to these findings, AD mouse models overexpressing mutated forms of APP and haploinsufficient for Beclin-1 accumulate intracellular Aβ and experience increased neurodegeneration compared with age-matched Beclin homozygous mice [27].

The function of impaired lysosomal degradation in the etiology of AD was highlighted in a study that used AD mice deficient in cystatin B, an endogenous inhibitor of lysosomal cysteine proteases [4]. In these mice, the lack of cystatin B stimulated protein turnover by lysosomes and enhanced the clearance of Aβ, rescuing memory function. This report and other seminal papers [28] demonstrated that targeting defective lysosomes increases the clearance of aggregated proteins and is a potential therapeutic strategy to combat or limit the progression of AD.

Huntington Disease

Huntington disease (HD) is an autosomal dominant movement disorder and is one of the most common polyglutamine diseases that are caused by a CAG trinucleotide expansion in the huntingtin (Htt) gene. Brains from HD patients experience neuronal loss in the striatum and cortex, and the hallmarks of altered autophagy were first described approximately 40 years ago [29]. In experimental models of HD, the overexpression of mutant Htt in striatal neurons activates the endosomal–lysosomal pathway and autophagy machinery and ultimately causes neuronal death. The mechanism of autophagic dysfunction in HD and the function of autophagy in the onset and progression of the disease are being studied.

Rubinsztein's group has demonstrated that mutant huntingtin is an autophagy substrate [30] and that inhibition of autophagosome formation by 3-methyladenine (3-MA) [31] or inhibition of autophagosome–lysosome fusion using bafilomycin A1 [32] prevents the clearance of mutant huntingtin and raises the levels of soluble and aggregate mutant huntingtin in experimental models of HD [30]. Conversely, rapamycin increases mutant huntingtin clearance and decreases the levels of soluble proteins and aggregates

[30]. Notably, autophagy clears only mutant huntingtin; by contrast, clearance of wild-type huntingtin is unaltered in cells treated with autophagy modulators [30, 33]. Collectively, these data suggest that autophagy is specifically involved in the clearance of only aggregate-prone mutant forms of huntingtin.

Another mechanism that links the accumulation of mutant Htt and autophagy machinery comes from the observation that mutant Htt recruits and sequesters Beclin-1, reducing the formation of autophagosome and promoting the accumulation of mutant Htt and disease progression, as demonstrated in Beclin-1 haploinsufficient mice [34]. Consistent with evidence that further stimulation and activation of autophagy enhance the clearance of mutant Htt—beneficial for HD patients—the enhancement of chaperone-mediated autophagy (CMA) by selective peptide targeting of mutant huntingtin (fusion molecules that bind mutant Htt) affects the degradation of toxic fragments and ameliorates the disease phenotype in HD animal models [35]. Thus, therapeutic induction or recovery of autophagy machinery might increase the clearance of mutant Htt and limit its toxicity in HD neurons.

Parkinson Disease

Parkinson disease (PD) is a late-onset neurodegenerative disease that is caused by the degeneration of dopaminergic neurons in the substantia nigra [36]. The hallmark pathology of PD is the accumulation of α-synuclein, which is visible as Lewy body inclusions in vulnerable neurons, and similar to AD brains, accumulation of autophagosomes is also evident in PD brains [3]. Autosomal-recessive PD is associated with mutations in two genes, PINK1 and PARK2 [37]. PINK1 encodes the serine–threonine kinase PINK, which is expressed in the outer mitochondrial membrane, and PARK2 encodes Parkin, an E3 ubiquitin ligase. PINK and Parkin are components of a signaling pathway that controls mitophagy, by which the neurons degrade damaged mitochondria [38]. Parkin is selectively recruited from the cytosol to damaged mitochondria (a process requiring PINK) and mediates the elimination of mitochondria by mitophagy. Parkin has been proposed to ubiquitinate membrane proteins on damaged mitochondria, which appears to constitute a degradation signal for these organelles [39–42].

Mutations that induce the overexpression of α-synuclein, such as gene duplications, are sufficient to cause PD [43]. Overexpression of α-synuclein impairs autophagy by inhibiting the small GTPase Rab-1A [44], which has an important role in autophagosome biogenesis, and an abundance of α-synuclein induces a mislocalization of an early acting part of the autophagy machinery called Atg9 and blocks the formation of putative autophagosome precursors known as omegasomes [44]. Because autophagy has many protective functions in neurons, the accumulation of α-synuclein might have pleiotropic pathological effects, such as increasing the levels of protein aggregation, preventing effective clearance of dysfunctional mitochondria, and increasing neuronal susceptibility to proapoptotic insults. Further, CMA is believed to be impaired by mutations in α-synuclein and by specific posttranslational modifications of wild-type α-synuclein that are associated with PD [45, 46]. Thus, mutations in α-synuclein might contribute to neuronal toxicity by modulating more than one type of autophagy.

Autophagy in Acute Brain Damage

Increased research on autophagy has identified it as a new participant in the control of neuronal cell fate in the pathogenesis of chronic diseases and many types of acute brain damage, such as traumatic brain injury, cerebral ischemia, spinal cord injury, and axonal damage [47]. In contrast to chronic neurodegenerative disorders, for which much effort has been made to characterize the molecular participants in the various steps of autophagy and the effects of autophagy dysfunction, the study of the biological effects and mechanisms of autophagy in acute brain damage remains in its infancy. A more expansive understanding of the autophagy machinery and its function in normal physiology and pathology might be helpful in identifying effective new treatments for acute brain neurological disorders.

Traumatic Brain Injury

Traumatic brain injury (TBI) is the leading cause of mortality in young persons and is one of the chief reasons for hospital admissions [48]. The mechanical disruption of neurons triggers a cascade of events that effect tissue edema, neuronal cell death, and impaired motor and cognitive functions after TBI [49].

Autophagic activity is significantly altered after TBI, as evidenced by ultrastructural changes in the neurons of affected brain regions. Levels of autophagosomes and autolysosomes that contain partially digested or digested materials begin to rise several hours after the injury in mice [50] and in rat models of TBI [51]. Several biochemical evidences have confirmed the activation of autophagy. ATG12–ATG5 conjugate, required for AP formation, with LC3-II [40] is upregulated, as is LC3-II alone, in various rodent models of TBI [50, 52].

The rapid appearance of autophagic hallmarks indicates that TBI is a powerful inducer of autophagy. Notably, although most studies on autophagy after TBI have used rodent brain trauma models, there is evidence of autophagy after TBI in humans [53]—by ultrastructural and biochemical analysis

of brain tissue of TBI patients, autophagosomes are present in the lesioned brain regions.

Although much evidence has implicated the autophagy in the response to TBI, the function of autophagy in TBI remains unknown. Several studies suggest that pharmacological modification of the mTOR-dependent pathway by rapamycin increases neuronal survival in the injured region and improves functional recovery in injured mice between 2 and 34 days following closed head injury [54].

Conversely, recent evidence has demonstrated that autophagy is part of the complex mechanism of the cellular response to TBI and can mediate neuronal death [55]. Pretreatment with 3-MA, a nonselective inhibitor of autophagy, attenuates TBI-induced cell death, lesion volume, and neurological deficits, implying that inhibition of autophagy is an efficacious therapeutic goal for the treatment of TBI. Although increased and reduced autophagy correlate with lower and higher neuronal cell death, respectively, whether autophagy is an active process that controls cell fate is unknown. These findings suggest that autophagy mediates TBI and that its modulation is an attractive strategy with regard to drug design in mitigating the neuronal damage that is associated with TBI.

Remote Degeneration After Focal CNS Damage

Marked activation of autophagy was recently observed in a paradigm of "remote cell death," induced by focal cerebellar lesion [56]. In this experimental model, neuronal cell death is caused by target deprivation and axonal damage of contralateral precerebellar neurons. Thus, neuronal death occurs in regions that are remote, but functionally connected, to the primary lesion site. Yet, until several decades ago, the mechanism of remote degeneration was considered irrelevant. Today, it is recognized as being critical to the clinical outcome in many acute CNS pathologies, such as multiple sclerosis, stroke, and spinal and brain trauma [57].

Recently, Viscomi and colleagues [58] demonstrated autophagy activation after acute brain lesion in remote neurodegenerative events. The accumulation of autophagosomes and autophagolysosomes in axotomized neurons at early time points after injury, as demonstrated by morphological, ultrastructural, and biochemical analyses, was considered by this group to be a reactive response in damaged neurons to engulf damaged mitochondria.

However, the lesion-induced autophagic response is insufficient due to the greater number of damaged mitochondria that has accumulated in axotomized neurons, which renders cells more vulnerable and causes cell death. This hypothesis was confirmed by findings of enhanced autophagy by rapamycin, which was associated with a significant increase in survival of axotomized neurons and greater functional recovery, consistent with the neuroprotective function of autophagy in remote degeneration after acute brain damage. Conversely, heterozygous deletion of Beclin-1 in mice decreased neuronal autophagy and resulted in greater cytochrome-*c* release from damaged mitochondria, increased neuronal death, and worse functional recovery. These findings suggest that, in this experimental paradigm, inadequate levels of autophagy result in a pronounced susceptibility to apoptosis.

The crosstalk between autophagy and apoptosis is a complex topic that is being debated. Under several circumstances, autophagy can prevent apoptotic cell death; in other scenarios, autophagy is an alternative pathway of cell death, and in certain cases, autophagic cell death and apoptosis occur in parallel and share some common regulatory mechanisms. These pathways have recently been reported to play a key role in remote cell death mechanisms. Specifically, it has been shown that in the axotomized neurons, autophagy occurs before caspase-3 activation—an irreversible sign of cells toward apoptosis—and after cytochrome-*c* release from damaged mitochondria, suggesting that cytochrome-*c* release triggers autophagy activation.

In conclusion, these data support the beneficial effect of autophagy in protecting axotomized neurons in the early stages of neuronal remote damage (Fig. 3). However, further study is required to determine how autophagy prevents remote cell death. These findings have therapeutic significance for several acute brain pathologies, such as stroke and spinal trauma, which would benefit tremendously if early autophagy events can be targeted.

Spinal Cord Injury

Acute traumatic spinal cord injury (SCI) is a physically disabling and psychologically devastating condition that affects approximately 2.5 million individuals globally for which there are no universally accepted treatments. Thus, defining the course of pathobiological changes that occur after SCI is paramount to identify novel therapeutic strategies.

Although marked autophagic activity after SCI has been demonstrated, there are few reports on this topic [59–61]. Recent studies have observed that after SCI, Beclin-1 expression increases significantly in damaged neural tissue, paralleling the kinetics of apoptosis—Beclin-1 expression apoptotic cell death—and autophagy has been associated with apoptosis after spinal cord injury [59].

In this context, Kanno and colleagues [60] reported clear biochemical, morphological, and ultrastructural evidence of autophagy in injured spinal cords. An increase in autophagosomes with double membrane structures and multilamellar bodies and autophagolysosomes was observed at the injured site after spinal hemisection. Additionally, strong LC3-immunopositive punctate and

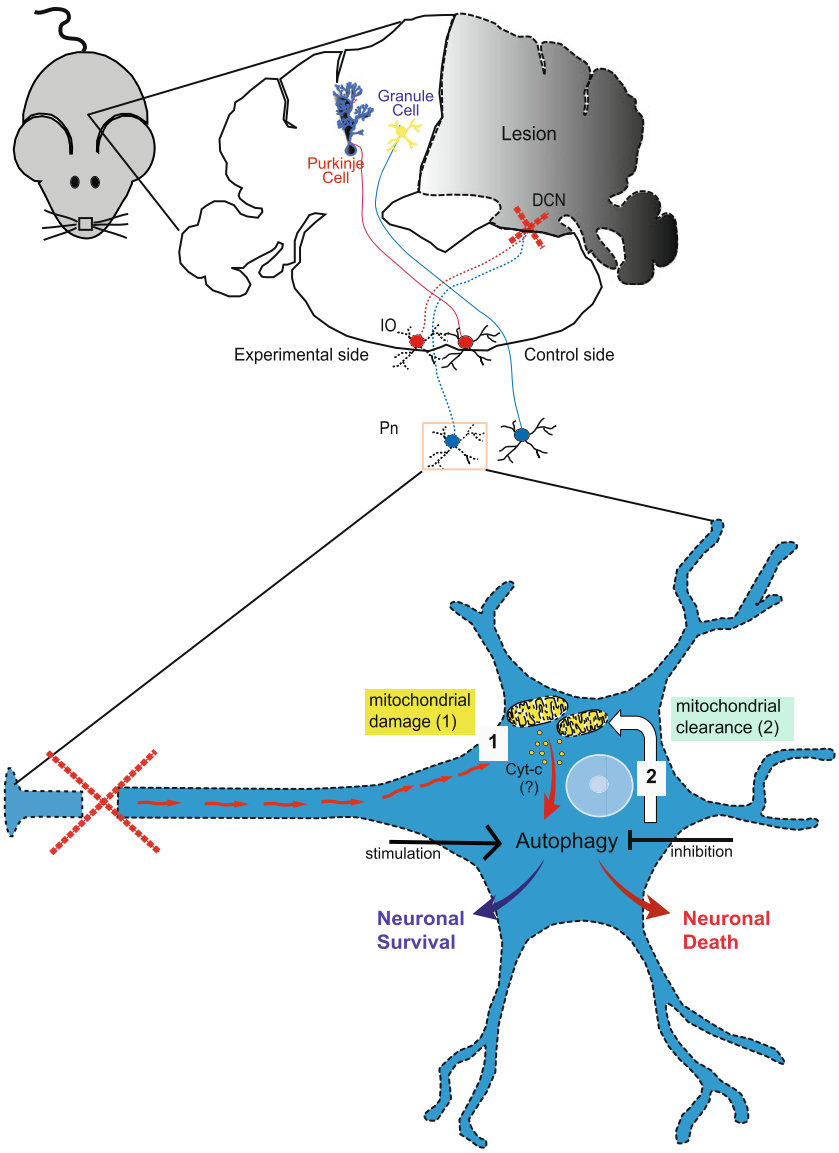

Fig. 3 Schematic diagram of the function of autophagy in remote cell death mechanism. Due to the crossed input–output organization of the cerebellar connections, unilateral lesion of a cerebellar hemisphere induces axonal lesions and subsequent degeneration of the contralateral (experimental side) inferior olive (*IO*) and pontine nuclei (*Pn*), with sparing of the IO and Pn on the ipsilateral side (control side). Further, the lesion activates autophagy in precerebellar axotomized neurons. It has been hypothesized that the autophagy machinery is activated in response to mitochondria sufferance. When damaged mitochondria release cytochrome-*c* (Cyt-*c*) into the cytosol (*1*), autophagy is activated possibly to engulf the suffering mitochondria (*2*), neutralizing pro-apoptotic factors release. By enhancing autophagy, it is possible to support neuronal survival while a reduction in autophagic activation is associated with increased cell death. *DCN* deep cerebellar nuclei, *icp* inferior cerebellar peduncle

conversion of the LC3-I level to LC3-II were observed in the injured side.

There is scant evidence on the function of autophagy in the pathophysiology of SCI. Recently, pharmacological modulation of the mTOR-dependent pathway by rapamycin was shown to reduce neuronal tissue damage and cell death and improve neurological recovery after SCI [61]. Although this study supported rapamycin-enhanced autophagy-mediated neurological recovery in the acute phase after SCI, the molecular and biochemical mechanisms by which autophagy is protective after spinal cord injury remain unknown.

In conclusion, because little evidence exists regarding the involvement of autophagy in the response to SCI, further study is needed to determine the function of autophagy in SCI. Addressing this question is paramount to understanding the pathophysiology of SCI and identifying novel targets for treatment strategies in patients with spinal cord injuries.

Cerebral Ischemia

Cerebral ischemia causes extensive neuronal death and is a major cause of morbidity and mortality in infants and adults. As in any pathological insult, several injury and adaptation pathways are activated after hypoxic–ischemic brain injury. Determining the positive and negative contributions of these pathways in neuronal cell loss is critical for the development of neuroprotective therapies.

A combination hypoxia–ischemia (HI) procedure in rodents has been used widely as a model to study cerebral ischemia. The activation of autophagy by HI has been studied extensively in the past several years [62–64].

Similar to TBI and SCI, autophagy is upregulated after cerebral ischemia in mice [65] and rat [66]. Morphological and biochemical analyses have shown that HI induces robust formation of autophagosomes in a short period after injury, demonstrating that HI is a powerful stimulus of autophagy in CNS neurons [65–69].

In addition, in this experimental model, the influence of development and sex on the autophagic response has been examined [70, 71]. Notably, after HI, the expression of autophagosome-related marker microtubule-associated protein 1 light chain 3-II (LC3-II) is more pronounced in younger brains (post-natal day 5 animals—PND 5) than in older brains (PND 60). Thus, similar to apoptosis, autophagy appears to be more predominant during the early stages of development, declining significantly at the older stages. In contrast, sex does not have any effect on the induction of autophagy after neonatal HI [71].

A recent study demonstrated that the degree of HI-triggered autophagy is brain region dependent and that its function after HI is region specific [72]. Specifically, in a neonatal HI model, enhanced autophagy preferentially affects cortical and hippocampal CA3 neurons rather than CA1 and dentate gyrus neurons. Further, whereas autophagy appears to be linked to apoptosis and may trigger it in the cortex, enhanced autophagy that affects CA3 neurons is apoptosis independent in the hippocampus. These results implicate enhanced autophagy in delayed neuronal death after HI and suggest that autophagy is differentially linked to apoptosis by the cerebral region [72]. These findings have clinical ramifications and might be valuable in developing effective therapeutic strategies.

Whether increased autophagy is beneficial, detrimental, or simply an epiphenomenon after HI is another topic of debate, for which conflicting results exist. Pharmacological treatment with rapamycin before unilateral carotid artery ligation in rat pups is associated with reduced apoptosis. Conversely, pretreatment with 3-MA or wortmannin, nonselective inhibitors of autophagy, has opposite effects on neuronal cells, suggesting that the activation of autophagy is part of complex and related signaling pathways that promote cell survival and that apoptosis and autophagy are intertwined phenomena that share key components [73].

Further, rapamycin provides neuroprotection against neonatal brain ischemia through the concomitant activation of autophagy, the PI3K/Akt–mTOR axis, and CREB. The interruption of the autophagic or PI3KAkt–mTOR pathway drives neurons toward necrotic cell death [74].

The crosstalk between autophagy and apoptosis in HI models has been addressed recently. Rapamycin pretreatment elicits antiapoptotic effects by decreasing the activation of the intrinsic apoptotic mitochondrial pathway. This effect is blocked by the autophagy inhibitor 3-MA, which, under these conditions, induces necrotic cell death in neurons [75]. In this situation, pretreatment with rapamycin appears to confer pharmacological preconditioning-like neuroprotection, and 3-MA blocks the adaptive mechanisms that are initiated by injury and pharmacological preconditioning.

Conversely, there are ample evidences that implicate autophagy as part of death mechanisms. Puyal and Clarke [69] blocked autophagosome formation by administering 3-MA 4 h after middle cerebral artery occlusion in PN12 (post-natal) rat pups, resulting in neuroprotection. Similarly, 3-MA pretreatment reduces infarct volume, brain edema, and motor deficits [66]. These results demonstrate that ischemic insults activate autophagy and that autophagic mechanisms contribute to ischemic neuronal injury. However, the mechanisms by which 3-MA elicits its dual effects after HI remain unknown. This disparity in outcomes suggests that differences in findings are due to the treatment, ischemia model, and age [75].

Further, the detrimental effects of autophagy in an HI model were supported by a study of conditional knockout mice that were deficient in brain Atg7, a gene that is essential for the induction of autophagy. Mice that lack Atg7 in neurons receive nearly complete protection from neonatal HI-induced cell death [76].

These discrepant results are additional evidence that autophagy contributes actively to cerebral ischemia and that autophagy is a potential target for a novel stroke therapy. Yet, because the contribution of autophagy remains unknown, more work is needed to determine its function in HI.

Concluding Remarks

The significance of autophagy in neurological disorders emerged several decades ago, and its importance in physiological and pathological situations has been established. Nevertheless, the factors that govern the neuroprotective/neurotoxic functions of autophagy and how dysfunctional autophagy machinery contributes to neuronal death are unknown. The activation of autophagy beyond a certain threshold may cause cell death by initiating the self-digestion of dying cells. Conversely, an insufficient autophagic response might render cells more vulnerable to stress conditions and induce cell death.

Studies on autophagy have revealed a complex relationship between autophagy and cell death pathways, and whether autophagy is a prosurvival strategy or a step in the cell death program requires further examination. Extensive evidence supports the hypothesis that the duality of autophagy is context dependent and depends on several factors, including the type and extent and duration of stress conditions and the intrinsic vulnerability of the brain areas that are affected.

Greater recognition of these factors might provide insight into the complex mechanism of autophagy and unravel the relationship between autophagy and various cell death pathways. Thus, a profound understanding of the neuronal autophagy process will ultimately aid the future development of therapeutic interventions in chronic and acute neuronal disorders.

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

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