Role of autophagy in prion protein-induced neurodegenerative diseases

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Prion diseases, characterized by spongiform degeneration and the accumulation of misfolded and aggregated PrPSc in the central nervous system, are one of fatal neurodegenerative and infectious disorders of humans and animals. In earlier studies, autophagy vacuoles in neurons were frequently observed in neurodegenerative diseases such as Alzheimer’s, Parkinson’s, and Huntington’s diseases as well as prion diseases. Autophagy is a highly conserved homeostatic process by which several cytoplasmic components (proteins or organelles) are sequestered in a double-membrane-bound vesicle termed ‘autophagosome’ and degraded upon their fusion with lysosome. The pathway of intercellular self-digestion at basal physiological levels is indispensable for maintaining the healthy status of tissues and organs. In case of prion infection, increasing evidence indicates that autophagy has a crucial ability of eliminating pathological PrPSc accumulated within neurons. In contrast, autophagy dysfunction in affected neurons may contribute to the formation of spongiform changes. In this review, we summarized recent findings about the effect of mammalian autophagy in neurodegenerative disorders, particularly in prion diseases. We also summarized the therapeutic potential of some small molecules (such as lithium, rapamycin, Sirtuin 1 and resveratrol) targets to mitigate such diseases on brain function. Furthermore, we discussed the controversial role of autophagy, whether it mediates neuronal toxicity or serves a protective function in neurodegenerative disorders.

Keywords autophagy; prion protein; neurodegenerative disease

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Introduction

Prion diseases, also known as transmissible spongiform encephalopathies (TSEs), are infectious neurodegenerative disorders that can affect humans and several species of animals [1]. Prions are the infectious agents responsible for a group of fatal diseases, the most prevalent prion disease in animals are nature scrapie [2,3] in sheep and goat, bovine spongiform encephalopathy (BSE) [4] in cattle, and chronic wasting disease [5,6] in elk and deer. In recent years, it was found that domestic cats can be infected with prions, known as feline spongiform encephalopathy [7]. Examples for humans are kuru [8,9], Creutzfeldt–Jakob disease (CJD) [10], Gerstmann–Sträussler–Scheinker syndrome [11], and fatal familial insomnia [12]. Beside these, the newly appeared variant CJD (vCJD and secondary vCJD) [13] is associated with the consumption of BSE-contaminated food and iatrogenic blood transfusion, which raises high concern about a possible transmission from human to human [14].

Unlike conventional infectious microorganisms, the TSEs are caused by the conversion of the host cellular PrP to misfolded form of PrPSc with a β-sheet-rich conformation. According to the central idea of the Prion Hypothesis raised by Stanley B. Prusiner in 1982 [15], prion infectious agents did not have any nucleic acid [16]. Interestingly, PrPSc has the ability to replicate in the body of infected individuals by propagating its misfolding to normal PrP [17], then these newly formed prions can go on to convert more PrP into PrPSc [18], which results in the accumulation of misfolded and aggregated PrPSc in the brain. Similar to prion diseases, aggregates can be seen in other neurodegenerative diseases, such as amyloid precursor protein or the microtubule-associated protein Tau in Alzheimer’s disease (AD), polyglutamine-containing proteins in Huntington’s disease (HD), and α-synuclein in Parkinson’s disease (PD) [19].

Prion diseases are characterized by long incubation periods and central nervous system (CNS) spongiosis. This spongiosis is associated with neuronal loss and vacuolation [1], which disrupt the normal brain tissue structure. Other histological changes include strong astrogliosis, mild microglia activation, and the absence of an inflammatory reaction [20]. The disease develops rapidly once symptoms appear, while incubation period generally lasts for years to decades, leading to brain damage and individual inevitable death usually in a few months. Neurodegenerative symptoms may
include convulsions, dementia, ataxia, and behavioral or personality changes.

In earlier findings, it has been reported that the number of autophagosomes and other pre-lysosomal vesicles presence significantly increase in neurodegenerative disorders [21]. Some evidence showed that autophagic dysfunction is linked to aging and diseases, including cancer and neurodegenerative disorders [22]. To clarify in detail the correlation between autophagy and prion infection, we illustrated the recent findings on the molecular mechanism and regulation pathways of autophagy, and methods to monitor autophagy. Here we aim to summarize the current knowledge about the effects of mammalian autophagy in neurodegenerative disorders, particularly in prion diseases. Furthermore, we discussed whether autophagy mediates neuronal toxicity or serves a protective function in neurodegenerative disorders.

**Autophagy**

Autophagy, or autophagocytosis, is a highly conserved homeostatic process for the insulation and degradation of cytosolic macromolecules, damaged organelles, and some pathogens. In 1962, Ashford and Porter observed the intracytosolic macromolecules, damaged organelles, and some homeostatic process for the insulation and degradation of Autophagy, or autophagocytosis, is a highly conserved autophagy signaling system, accompanied by the activated ULK/Atg1-Atg13-FIP200/Atg17 complex [33], and then several kinases are involved in initiating autophagosome formation. In addition, recent studies have revealed that a novel mTOR-independent induction of autophagy can also occur involving Beclin-1 and the class III phosphatidylinositol 3-kinase (PI3KC3) Vps34 [34,35]. The above process is the initiation stage of autophagy. Once autophagy is activated, it begins with the formation of isolation membrane or phagophore characterized as a small crescent-shaped structure. Autophagosome formation proceeds with nucleation of the isolation membranes to generate vesicular structures [36]. The Beclin 1/PI3KC3 (Vps34) complex in this phase is essential for association of other Atg proteins as it is responsible for the formation of PI3P (phosphatidylinositol 3-phosphate) to which these proteins are recruited. The activation of PI3KC3 (Vps34) complex finally generate PI3P. Beclin-1 is an autophagic positive regulatory protein, other examples being ambra-1, UVRAG, and Bif-1; while negative regulatory proteins include Bcl-2 and Bcl-XL [37]. These specific components play a coordinated role within the Beclin 1/PI3KC3 (Vps34) complex to drive nucleation of isolation membranes. Numerous studies have shown that cytosolic proteins, protein aggregates, and intracellular pathogens for degradation, and then the region of cytoplasm form a double-membrane-bound vesicle called ‘autophagosome’. Autophagosome receive hydrolases by fusing with either lysosomes to form autophagolysosome, or late endosomes to form amphisomes. Efficient digestion of substrates within these compartments yields lysosomes containing mainly acid hydrolases with little residual cytoplasm.

**Molecular Mechanism of Autophagy and Its Regulation**

Autophagy is a conserved catabolic pathway from yeast to human [32]. In homeostasis, autophagy plays its housekeeping role in cytoplasmic and protein turnover, while during stress, cells can be protected from dying by elimination of harmful organelles and aggregates. Autophagy involves a series of molecular regulators to initiate autophagosome formation, as well as the nucleation, elongation, maturation, and degradation of autophagosomes (Fig. 1). Therefore, a complicated and precise autophagy regulation mechanism is required to prevent undesired removal of cytoplasmic contents.

Autophagy occurs under normal physiological conditions but can be up-regulated by numerous factors, including nutrient deprivation, and the inhibition of mTOR kinase (mammalian target of rapamycin). The mTOR is important in negative regulation of autophagy and can be inhibited in response to cellular stress such as starvation or treatment with rapamycin. Downstream of mTOR activates the autophagy signaling system, accompanied by the activated ULK/Atg1-Atg13-FIP200/Atg17 complex [33], and then several kinases are involved in initiating autophagosome formation. In addition, recent studies have revealed that a novel mTOR-independent induction of autophagy can also occur involving Beclin-1 and the class III phosphatidylinositol 3-kinase (PI3KC3) Vps34 [34,35]. The above process is the initiation stage of autophagy. Once autophagy is activated, it begins with the formation of isolation membrane or phagophore characterized as a small crescent-shaped structure. Autophagosome formation proceeds with nucleation of the isolation membranes to generate vesicular structures [36]. The Beclin 1/PI3KC3 (Vps34) complex in this phase is essential for association of other Atg proteins as it is responsible for the formation of PI3P (phosphatidylinositol 3-phosphate) to which these proteins are recruited. The activation of PI3KC3 (Vps34) complex finally generate PI3P. Beclin-1 is an autophagic positive regulatory protein, other examples being ambra-1, UVRAG, and Bif-1; while negative regulatory proteins include Bcl-2 and Bcl-XL [37]. These specific components play a coordinated role within the Beclin 1/PI3KC3 (Vps34) complex to drive nucleation of isolation membranes. Numerous studies have shown
the inhibition of PI3KC3 by treatment with 3-methyladenine (3-MA), wortmannin or LY294002 can block the autophagy pathway at a very early stage, preventing the formation of mature autophagosomes [38–40]. Among them, 3-MA is the most commonly used inhibitor of autophagy by suppressing DNA fragmentation and cytoplasmic degradation.

Consequently, other Atg proteins which mediate vesicle membrane elongation are recruited. Two ubiquitin-like protein systems are involved in the extension and expansion of autophagy membrane. Firstly, an Atg12-Atg5-Atg16L homology tetramer complex is necessary for the formation of vesicle curvature. In another ubiquitin-like protein system, LC3 (Atg8) is lipidated by binding to phosphatidylethanolamine (PE) located on the surface of autophagic membrane, which is in contrast to the cytoplasmic LC3 [41].

Following the elongation step, autophagosomes fuses with lysosomes for degradation of these contents, which is called autophagosome maturation. It is widely believed that autophagosome maturation and degradation require the action of late marker protein Rab7 and LAMP-2 (lysosomal membrane protein 2).

Although the process of autophagy has already been known for over 50 years, a complete picture of autophagy regulation is not yet available. Important progress in the field was achieved by the breakthrough in yeast genetics and the identification of genes involved in autophagy and autophagy-related processes. Several aspects of regulation mechanisms have recently been reviewed in great detail [42].

**Monitoring Autophagy in Neurodegenerative Diseases**

With the rapidly advancing research in the autophagy field, a range of useful and reliable morphological and molecular biological methods have been developed to monitor autophagy. The general approaches to investigate neurodegeneration include postmortem brain tissue, the use of transgenic and gene deletion mouse models, cultured cell lines, and primary cortical neurons. Comparative analysis of brain regions that are pathologically affected and those are not detectably affected can lead to the understanding of the underlying pathophysiological mechanisms of autophagy in etiologically relevant process. The presence of increased number of autophagic vacuoles in neurons is a frequent observation in many neurodegenerative diseases. A variety of genetically modified mice with mutations in autophagy genes (e.g. mice deficient in the genes for Atg5, Atg7 [22]) have been generated in the past several years. In addition, manipulating cellular pathways of cell models (e.g. presenilin gene-ablated murine blastocysts, most used models related to AD [43]) to induce a pathologic condition enable specific aspects of autophagy to be monitored more easily.
Nowadays, more superior technologies are available to characterize the autophagic vacuoles, evaluation of autophagosome, autolysosome formation, and autolysosomal clearance, such as standard histochemical stains for neuro-pathology, immunocytochemistry, electron microscopy, and fluorescence probes. At the same time, new powerful molecular tools such as RNAi silencing were developed in autophagy studies, which made it feasible to study the molecular role of autophagy in neurodegenerative diseases.

**Autophagy in Neurodegenerative Disorders**

As mentioned above, neuronal autophagy plays a critical role at basal physiological levels in controlling intracellular quality and maintaining nervous system health, presumably by removing aggregated proteins [44]. At first, it is believed that neuronal autophagy is relatively inactive, but in recent years genetic research using mouse models emphasized the importance of autophagy in the non-proliferating cells, particularly in neurons. Mice deficient in Atg7 and Atg5 specifically in CNS tissue have ubiquitin aggregates in neurons and massive loss of cerebral and cerebellar cortical neurons, resulting in neurodegeneration and short life span [45,46]. These mice develop progressive motor deficits and display abnormal reflexes. Compared with other organ systems, CNS autophagosomes are in low levels under normal conditions and even suffered starvation. Studies also demonstrated that intracytosolic degradation of cellular contents by autophagy is indispensable, even without any disease-associated mutant proteins expression [21,47].

Neurodegenerative disorders in the nervous system shares a common pathological symptom: aggregation of misfolded mutant proteins in neurons in specific areas of the brain [19]. For instance, polyglutamine (polyQ) expansion mutations in HD and point mutations α-synuclein in some familial forms of PD make the mutant proteins more aggregate-prone. Genetically ameliorating the impaired autophagic and lysosomal degradation in an AD mouse model reduces Aβ accumulation and memory deficits [48]. Consistent with these observations, autophagy is required for the removal of the protein aggregates that are toxic especially for postmitotic neurons [22].

Enhanced autophagy in animal models of these diseases improves clearance of the aggregated proteins and reduces the symptoms of neurodegeneration [49]. Recent studies showed that resveratrol, a type of phytoalexin and powerful anti-oxidant, attenuates neurodegeneration by inducing autophagy in animal models of AD and PD associated with the neuronal accumulation of Aβ and α-synuclein, respectively [50,51]. Similarly, enhanced autophagy can scavenge mutant Huntingtin proteins [52], reduce the amount of aggregates, and decrease related programmed cell death. Then with the regulation effect of autophagy, a neuronal protection both in patients with HD and HD mice model was established [53]. The results of abnormal proteins-induced autophagy can be explained by the decrease of mTOR activity [54]. Autophagy degrades expanded polyglutamine-containing disease proteins found in HD and spinocerebellar ataxias, as well as PD-associated A53T α-synuclein proteins [55].

Autophagy plays an important role in neuroprotection, through multiple key molecular regulation of autophagy pathway. Rapamycin may have a cytoprotective effect by inhibiting mTOR kinase and up-regulating the level of autophagy. In polyglutamine disease models in Drosophila and mice [54], rapamycin can fight against neurodegenerative diseases through degradation of mutant aggregation proteins. Examples showed that α-synuclein overexpression reduces the level of Atg7 and impairs autophagy, while infusion of rapamycin increases autophagy and ameliorates neurodegenerative phenotypes in mice overexpressing α-synuclein [56,57]. Beclin-1, another critical protein controlling nucleation stage of autophagy, can be detected in AD brains with a decreased expression level, whereas Beclin-1-deficient mice display decreased autophagy but increased neurodegeneration [58]. In view of the above, autophagy has the ability to protect against neurodegeneration, and it is reasonable to assume that autophagy could be a treatment target of these maladies, by using various molecules to regulate the pathological process.

Autophagy has a crucial pro-survival function for cells in stressful conditions and for maintenance of cellular homeostasis [59]. On the other hand, increasing evidence indicated that autophagy machineries are deregulated in diverse human diseases, including neurodegenerative disorders, cancers, and inflammatory disorders [22,60–62]. Deregulated autophagy contributes to neurite degeneration and neuronal cell death, as shown in the in vitro and in vivo systems. From a histopathological perspective, through a clinical study on the cortical biopsy specimens from AD, Nixon et al. [21] found that the number of autophagosomes and other pre-lysosomal vesicles presents a significant increase. Further studies showed that those γ-secretase and Aβ peptides are both located in the neuronal autophagosomes, and autophagy induction causes toxic Aβ to increase. These data provide strong evidence that Aβ is generated in autophagic vacuoles [63]. Under normal circumstances, Aβ is degraded along with the fusion of autophagosome and lysosome. Once this process is blocked, increased autophagosomes would be positive for Aβ deposition [64], which eventually gives rise to the appearance of related clinical symptoms. Similarly, pathological increases in autophagy vacuoles (AVs) and lysosomes are observed in PD, HD, and prion diseases [55,65,66]. These results suggest that excessive activation of autophagy or defects in autophagosomes maturation and degradation in these disorders are closely associated with disease pathology [36]. Further research is needed for the specific
mechanisms of autophagy dysfunction in neurodegenerative disorders.

Whether autophagy mediates neuronal toxicity involved in the accumulation of disease proteins or serves a protective mechanism for the clearance of such proteins remains controversial. Therefore, systematically clarifying the related molecular control is urgently needed for analyzing the contribution of autophagy in neurodegenerative diseases.

**Autophagy in Prion Diseases**

In the case of autophagy, the presence of AVs in neurons is observed frequently in TSEs. In 1991, Boellaard et al. [67] first reported the formation of giant AVs in neurons with experimental scrapie in hamsters. Moreover, the number of AVs increased through the incubation period in the 263K strain scrapie model. AVs develop not only in the neuronal perikarya but also in neuronal processes, eventually replacing the whole cross-section of affected neurites [68]. In neuroblastoma cells transfected in vitro with three different prion protein mutants (V203I, E211Q, and Q212P), all three protein mutants were converted into PrPSc-like form and accumulated in aggresomes [69]. Autophagy, as a conserved host defense response to infection, plays a protective role in prion diseases by degrading aggregate-prone proteins accumulated within endosomal/lysosomal vesicles.

Recent studies provided the first direct evidence that induction of autophagy results in degradation of cellular PrPSc. When autophagy is suppressed by pharmacological interference or siRNA gene-silencing of essential members of the autophagic machinery, the capacity of compound-induced autophagy in reducing cellular levels of PrPSc is impaired [70]. An example to support the protective effect of autophagy was that small molecules enhance autophagy in an mTOR-independent manner [71,72], which may suppress IMPase (Myo-inositol monophosphatase) to reduce inositol and IP3 (myoinositol-1,4,5-triphosphate). In addition, rapamycin also reduced the level of PrPSc in an mTOR-dependent manner. Of note, small molecules in both mTOR-independent and -dependent manners can induce autophagy. This suggested the possibility of using them in combination for therapeutic purposes. More detailed studies in vivo further elaborate the prospects of small molecules on the treatment of prion diseases. Compared with mock-treated control mice, rapamycin treatment can prolong the incubation period of prion-infected mice [72]. Trehalose treatment was demonstrated with a delayed appearance of PrPSc in the spleen [71]. Therefore, small molecules-based therapy may have the potential to prevent progression of the pathology associated with prions.

Mitochondrial failure caused by aggregation of misfolded proteins is a key mechanism of neurodegenerative disorders, including prion disease [73–75]. An interesting correlation between prion diseases and autophagy was observed in recent studies on Sirtuin 1 (Sirt1), a class III histone deacetylase. Activating Sirt1 induces autophagy, and activated autophagy protects neurons against prion diseases by regulating mitochondrial homeostasis. The underlying mechanism is associated with a decrease of the mitochondrial membrane potential value, and a reduction for PrP fragment (106–126)-induced Bax translocation to the mitochondria and cytochrome c release into the cytosol, as Sirt1 overexpression is mediated by adenoviral vector [76]. Further study proved that resveratrol, the Sirt1 activator, is important in attenuating cellular injury and oxidative stress that prevents PrP (106–126)-induced neuronal cell death, and blocks neurotoxicity by activating autophagy through the autophagy-lysosome pathway [77]. In addition, a new finding was reported that the inhibitory effects of resveratrol was associated with increased expression and activation of SIRT1, which regulates Bcl-XL, p21, phospho-p38, cleaved caspase-3 by enhancing the deacetylation of p53 and p65 [78]. The above results suggested that Sirt1 may be involved in the pathogenesis and, as such, may be a valid therapy target for prion-related neurodegenerative diseases.

Reduced autophagy in combination with endosomal/lysosomal dysfunction may contribute to the development of prion disease. Galectin-3, a multifunctional protein participating in mediation of inflammatory reactions, was overexpressed noticeably in prion-infected brain tissue. It was found that in prion-infected galectin-3−/−-mice, LAMP-2 were markedly reduced, and lower mRNA levels of Beclin-1 and Atg5 indicated an impairment of autophagy, although autophagosome formation was unchanged [79].

Given the role of autophagy in prion disease, it has been reported that autophagic flux impairment is thought to contribute to formation of spongiform changes, one of the pathological hallmarks in PrPSc-infected brain [80]. The PrP homologue Doppel (Dpl) is an N-glycosylated protein with glycosylphosphatidylinositol anchor like PrP. The ectopic expressed of Dpl in neurons caused progressive cerebellar Purkinje cell death in prion protein-deficient Ngsk mice (NP0/0). It was demonstrated that before and during neuronal loss, neighboring cell surface-bound Dpl activates death receptors, leading to the production of ROS in a calcium-dependent mechanism, then several autophagy-related molecules such as the Serg1 (scrapie-responsive gene one), LC2-II, and p62 were increased without showing any changes in mRNA expression [81]. These results suggest that Dpl toxicity might impair the ultimate steps of autophagic degradation in NP0/0 Purkinje cell, which may trigger the apoptotic cascade by a progressive dysfunctional autophagy.
Further detailed studies of Dpl-induced autophagy dysfunction will be important in understanding the mechanism of pathological PrP\(^{Sc}\) in prion diseases. Meanwhile, the interplay between these two multiple pathways of programmed cell death may provide information for the development of new therapeutic approaches.

Unlike those neurodegenerative disorders of humans (such as AD, HD, and PD), studies and discoveries showed that prion diseases may be more harmful to mammalian animal population. Only sporadic cases of CJD in humans were reported. Based on this, the relationships between autophagy and prion diseases have not got enough attention, so we summarized the latest and related research in this section. Much deeper research on the systematic analysis of autophagy in prion infection is required.

Conclusion

Autophagy is an evolutionarily conserved catabolic pathway involved in the turnover of proteins, protein complexes, and organelles through lysosomal degradation. Basal levels of autophagy ensure the maintenance of intracellular homeostasis through sequestering proteins and organelles into specialized double-membraned vesicles, termed autophagosomes. Due to the fact that neurons are postmitotic and do not replicate in general, they are heavily dependent on basal autophagy compared with non-neuronal cells, as misfolded proteins inside neurons cannot be diluted through cell division [83].

The common characteristic shared by neurodegenerative disorders is the accumulation of misfolded mutant proteins deposit in neurons in specific areas of the brain, such as AD, PD, amyotrophic lateral sclerosis, prion diseases, and polyglutamine disorders, including HD and various spinocerebellar ataxias. In this review, we were particularly concerned about prion protein-induced neurodegenerative disorders in humans and mammalian animals, which are collectively referred to as TSEs.

In recent decades, along with great strides achieved in the studies of autophagic molecular mechanisms, the role of autophagy in the pathogenesis of neurodegenerative diseases has been revealed by many researches. From what is found so far, there is good experimental evidence from in vivo and in vitro studies demonstrating that autophagy, as a housekeeper in prion diseases, when induced by chemical compounds, can play a protective role in the clearance of pathological PrP\(^{Sc}\) accumulated within neurons. When medicines promote autophagy by activating both mTOR-
dependent and -independent pathways simultaneously, it showed more significant than the maximum effect of one pathway alone. In contrast, defective autophagy may lead to the occurrence of neurodegenerative diseases, and contribute to the formation of spongiform changes. Therefore, autophagy may become a new target for the therapy of prion diseases.

The exact molecular mechanisms are still incompletely understood, in particular what kinds of effect does autophagy have on prion disorders associated with neurons survival? Does autophagy degrade abnormal proteins and protect neurons involved, or promote neurons programmed cell death to accelerate the progress of diseases? Or there is another signal transduction pathway? Autophagy may act as neuroprotection for PrPSc clearance at first, while PrPSc accumulation exceed a certain threshold value, autophagy becomes dysfunctional and contributes to prion infection. More work is needed to elucidate whether autophagy mediates neuronal toxicity or fights against the accumulation of disease proteins in neurodegenerative disorders.

In summary, plenty of evidence has shown the importance of autophagic pathway in neurodegenerative disorders. A better understanding of neurons autophagy will promote a broad view of deficient autophagy that underlies neurodegenerative disorders and ultimately helps to develop potential therapeutic interventions targeting autophagic dysregulation.

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**References**