Autophagy in Neurodegenerative Diseases: From Mechanism to Therapeutic Approach

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Autophagy in Neurodegenerative Diseases: From Mechanism to Therapeutic Approach

Jihoon Nah¹, Junying Yuan²*, and Yong-Keun Jung¹*

Autophagy is a lysosome-dependent intracellular degradation process that allows recycling of cytoplasmic constituents into bioenergetic and biosynthetic materials for maintenance of homeostasis. Since the function of autophagy is particularly important in various stress conditions, perturbation of autophagy can lead to cellular dysfunction and diseases. Accumulation of abnormal protein aggregates, a common cause of neurodegenerative diseases, can be reduced through autophagic degradation. Recent studies have revealed defects in autophagy in most cases of neurodegenerative disorders. Moreover, deregulated excessive autophagy can also cause neurodegeneration. Thus, healthy activation of autophagy is essential for therapeutic approaches in neurodegenerative diseases and many autophagy-regulating compounds are under development for therapeutic purposes. This review describes the overall role of autophagy in neurodegeneration, focusing on various therapeutic strategies for modulating specific stages of autophagy and on the current status of drug development.

INTRODUCTION

Eukaryotic cells use two major strategies for protein degradation, the ubiquitin-proteasome system and autophagy-lysosome pathway. The latter is a highly conserved, lysosome-dependent degradation process. Autophagy isolates cytosolic materials within a double membrane vesicle called an “autophagosome” which then fuses with lysosome to degrade isolated substrates (Mizushima et al., 2011). Until now, more than 30 autophagy-related (ATG) genes and three types of autophagy, macroautophagy, microautophagy, and chaperone-mediated autophagy (CMA), have been identified in yeast and mammalian systems (Boya et al., 2013; Nakatogawa et al., 2009). Moreover, various forms of selective autophagy have been characterized. Each is involved in the turnover of different cellular organelles (Komatsu and Ichimura, 2010). Defective regulation of the autophagy machinery and/or dysfunction of the lysosomal process can disrupt cellular homeostasis and lead to various disorders (Shintani and Klionsky, 2004). Three major autophagy deregulations have been observed in neurodegenerative diseases: (1) insufficient autophagy activation, (2) autophagy dysfunction due to reduced lysosomal function, and (3) autophagic stress related to pathologic activation of autophagy (Cherra and Chu, 2008). These observations indicate that autophagy generally plays an important neuroprotective role.

In this review, the overall mechanism of autophagy, the role of autophagy in neurodegeneration, and recent strategy for identifying therapeutic drugs will be described. Finally, the current strategies for targeting autophagy and the major issues in therapeutic autophagy for neurodegeneration treatment will be discussed.

SEQUESTRATION OF CYTOSOLIC MATERIALS AND AUTOPHAGOSOME MATURATION

For many years, autophagy was considered as a non-selective catabolic process to supply amino acids in response to starvation. Nowadays, however, autophagy is considered to recognize selective target proteins under various stress conditions, while a non-selective catabolic process is accepted in some cases (Filimonenko et al., 2010). Macroautophagy is the most widely studied and characterized autophagy process. The cytosolic substrates are sequestered within autophagosomes for lysosomal degradation. In microautophagy, the lysosomal or vacuolar membrane forms invaginations which then are differentiated into the autophagic tube to sequestrate portions of the cytosol for its degradation (Li et al., 2012). During CMA, heat shock cognate (HSC) 70, a chaperone protein, recognizes a pentapeptide, KFERQ, in its cytosolic substrate protein and then binds to a receptor on the lysosomal membrane, LAMP-2A. HSC70 finally unfolds these proteins and translocates directly to the lysosome (Kaushik and Cuervo, 2012).

Macroautophagy is initiated through the phosphorylation of the ULK1 complex (ULK1, ULK2, ATG13, FIP200, and ATG101). Depending on cellular energy level, phosphorylation of ULK1 (Ser 758) components, which are involved in the ULK1 complex inhibition, is regulated by mammalian target of rapamycin complex 1 (TORC1), while phosphorylation of ULK1 (Ser 317 and 377) components, involved in ULK1 complex activation, is regulated by AMP-activated protein kinase (AMPK) (Mizushima, 2010). Activated ULK1 complex recruits...
downstream ATG proteins to the autophagosome formation site and phosphorylates beclin 1 (BECN1) on Ser 14, promoting the activity of the VPS34 complex (PI3KCIII, BECN1, ATG14L, and VPS15) to initiate autophagosome formation (Russell et al., 2013). During the nucleation process, the VPS34 complex generates phosphatidylinositol 3-phosphate (PI3P). The accumulation of PI3P provides a platform to recruit PI3P-binding proteins (Shibutani and Yoshimori, 2014). BECN1, a protein essential for the VPS34 complex, has many binding partners such as ATG14L, UVRAG, and BCL2. Through these interacting partners, BECN1 finely regulates autophagy under different stress conditions (Abrahamsen et al., 2012). When the nucleation step starts, the autophagosome membrane is expanded.

In the elongation process, the autophagosome membrane around the cytoplasmic substrates requires two ubiquitin-like systems to extend the membrane. In the Atg5-Atg12 conjugation system, Atg12 was identified as the first ubiquitin-like protein (Ubl). Atg12 is activated by the E1-like enzyme, Atg7, and is transferred to the E2-like enzyme, Atg10, on its target protein Atg5. Finally, Atg5-Atg12 interacts with Atg16 to form a dimeric complex. The Atg5-Atg8-Atg16 complex targets the membranes depending on the formation of PI3P (Shpilka et al., 2012). The second ubiquitin-like conjugation system is the Atg8 conjugation system. Atg8 is cleaved by the cysteine protease, Atg4, and is processed by the ubiquitin-like enzymes, Atg7 and Atg3. The conjugated Atg8-phosphatidylethanolamine (PE) resides on the phagophore membrane and participates in cargo recruitment to the autophagosome (Weidberg et al., 2011). In the maturation process, the cargo sequestration is completed and the autophagosome fuses with lysosomes to degrade its cargo. Recent studies have implicated SNARE proteins, endosomal COPs, ESCRT II complex, small GTPase Rab proteins, cargo. Recent studies have implicated SNARE proteins, endo- and the autophagosome fuses with lysosomes to degrade its cargo (Weidberg et al., 2011). In the final step of autophagy, the cargo is broken down and degraded to cellular components and then released into the cytosol.

CHARACTERISTICS OF NEURONAL AUTOPHAGY

In general, the autophagic process has been reported to protect the neurons. Neuronal autophagy is essential for synaptic plasticity, anti-inflammatory function in glial cells, oligodendrocyte development, and myelinization process (Kesidou et al., 2013; Lee, 2012). The aggregate-prone proteins in neurons cannot be diluted by cell division because neurons are post-mitotic cells. Thus, neurons require well-regulated protein quality control systems. Accordingly, altered activity of the protein degradation system causes the accumulation of abnormal proteins and eventually leads to neuronal dysfunction such as deregulated transcription and impaired axonal transport (Millecamps and Julien, 2013). Increasing evidence shows that autophagy is implicated in neuronal disorders such as Alzheimer’s disease (AD), Parkinson’s disease (PD), Huntington’s disease (HD), Amyotrophic lateral sclerosis (ALS), and Multiple sclerosis (MS). In this review, we will summarize and discuss the relationship between autophagy and these neurodegenerative disorders.

AUTOPHAGY IN NEURODEGENERATION

Regulation of autophagy in Alzheimer’s disease (AD)

AD is the most common type of progressive dementia. In the early stages, the patient has difficulty in remembering recent events. As the disease advances, confusion, irritability, aggres-
degradation of abnormal proteins or organelles, is considered as an emerging therapeutic target. Moreover, autophagy plays a protective role against various stress stimuli and apoptotic insults (Giordano et al., 2014). Thus, the identification of autophagy inducers can be a good therapeutic strategy. Recently, non-toxic small-molecules that can restore basal-level of autophagy in neurons have been studied. Autophagy-inducing compounds show substantial therapeutic effects on various neurodegenerative disease animal models. Rapamycin, a selective inhibitor of TORC1, ameliorates Aβ and tau pathology in an AD mouse model (Caccamo et al., 2010). Latrepirdine, also known as dimebon, stimulates Atg5-dependent autophagy in the mouse brain and reduces Aβ neuropathology (Steele and Gandy, 2013). Protein phosphatase 2A (PP2A) agonist, metformin, inhibits tau hyperphosphorylation through inhibiting TORC1 and is currently tested in clinical trials for AD (Kickstein et al., 2010). In addition, SMER28, small-molecule enhancer of rapamycin 28, greatly decreases the level of Aβ peptide and APP-CTF in a gamma-secretase-independent manner (Tian et al., 2011).

Autophagy can also be induced by activating the ULK1 kinase, AMPK, in a TORC1-independent manner. Recently, various experimental and clinical trials have suggested that lithium may ameliorate AD pathogenesis through a combination of mechanisms that include AMPK activation and the regulation of autophagy (Forlenza et al., 2012). Resveratrol and its analogs, RSVA314 and RSVA405, have multiple molecular actions that show protective effect against AD (Vingtdeux et al., 2010). Nicotinamide prevents pathology and cognitive decline through enhancing lysosome/autophagosome acidification to reduce autophagosome accumulation in an AD mouse model (Liu et al., 2013). Even though the pathologic evidence is not sufficient in AD, virally packaged BECN1 and small-molecule BECN1 mimetics can also reduce the accumulation of toxic aggregates by targeting the early stage of autophagy (Shoji-Kawata et al., 2013). It appears that, in the early stage of AD, autophagy-inducing agents can prevent the accumulation of plaques and tangles by degrading these aggregates. However, autophagy promotion can aggravate the pathologies in the late stage of AD in which autophagosome/lysosome fusion or lysosomal function is impaired (Ching and Weihl, 2013). Therefore, new strategies that can enhance the fusion of autophagosome/lysosome and the lysosomal activity will be essential for advanced AD therapy.

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**Regulation of autophagy in Parkinson’s disease (PD)**

PD is a chronic and progressive movement disorder. PD symptoms develop gradually over time. PD results from the death of vital nerve cells in the brain and primarily affects the substantia nigra. During PD development, the amounts of dopaminergic neurons are decreased in the substantia nigra (Tan et al., 2014). PD is well-characterized by the accumulation of α-synuclein (SNCA) and ubiquitin into intracytoplasmic inclusions called Lewy bodies. Most PD cases are sporadic, meaning that they...
occur with unknown etiology and only 5% of PD cases are hereditary, due to mutations of at least 6 genes, SNCA, Parkin (PARK2), α-glucocerebrosidase (GBA), PTEN-induced putative kinase 1 (PINK1), DJ1, and leucine-rich repeat kinase 2 (LRRK2) (Alcalay et al., 2010).

The autophagic degeneration in melanized neurons of the substantia nigra in patients with PD provides evidence that autophagy is related to PD (Anglade et al., 1997). Emerging evidence then implicated that proteins encoded by PD-related genes can regulate autophagy pathways. Macroautophagy, chaperone-mediated autophagy (CMA), and mitophagy are involved in PD. SNCA level is a major determinant of its neurotoxicity and is related to the formation of Lewy bodies. Thus, SNCA degradation is essential in PD therapeutics. Recent studies indicate that deubiquitinated SNCA is mainly degraded by autophagy, while monoubiquitinated SNCA is preferentially removed by the proteasome (Pan and Yue, 2014). In SNCA transgenic mice, the ubiquitin-proteasome system is the main way responsible for SNCA under normal conditions, while autophagy is functional against increased intracellular SNCA degradation pathway for SNCA under normal conditions, while transgenic mice, the ubiquitin-proteasome system is the main way responsible for SNCA under normal conditions, while autophagy is functional against increased intracellular SNCA degradation.

Antioxidants such as thiols, ascorbic acid, and polyphenols, decreasing oxidative stress through autophagy can be an effective approach should consider the importance of selectivity without interfering with wild-type HTT. Mutant HTT forms perinuclear cytoplasmic aggregates and intracellular inclusions, which can be removed by autophagy. Previous studies have shown that autophagy induces the degradation of both aggregated and soluble forms of HTT and decreases toxicity in cell, fly, and mouse models of HD (Ravikumar et al., 2004). Moreover, autophagy alteration has been observed in various types of HD models such as primary striatal neurons from HD mice or lymphoblasts of patients with HD (Nagata et al., 2004). In general, inefficient macroautophagy is known to con-
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tribute to HD pathogenesis. More specifically, the ability of AVs to recognize cytosolic cargos is largely defective in HD cells. The autophagosome-lysosome pathway is normal or even increased in HD cells, but AVs fail to efficiently recognize and trap cytosolic cargos due to the interaction between mutant HTT and p62 (Martinez-Vicente et al., 2010). In addition, the accumulated mutant HTT can recruit cytosolic BECN1 and impairs BECN1 complex-mediated autophagy, causing the accumulation of mutant HTT and leading to neuronal toxicity in patients with HD (Shibata et al., 2006).

Increasing evidence supports the fact that HTT is also degraded by CMA. While CMA activity increases in response to macroautophagy dysfunction in the early stages of HD, this compensatory CMA activity continuously decreases with aging, leading to the onset of pathological symptoms (Koga et al., 2011). There is a KFERQ-like motif in the HTT fragment (1-552) (Qi and Zhang, 2014), raising the possibility that HTT can be affected by CMA. This notion is also in line with the observation that HSP70 and its co-chaperone, HSP40, modulates polyQ aggregation by partitioning monomeric conformations (Wacker et al., 2004). Accordingly, LAMP2A is involved in the chaperone/HTT clearance. A study showed that HTT is phosphorylated by IκB kinase and the phosphorylated HTT can be better cleared by LAMP2A and HSP70-mediated CMA through additional post-translational modifications (Thompson et al., 2004).

Regulation of autophagy in Amyotrophic lateral sclerosis (ALS)
ALS, often referred to as “Lou Gehrig’s disease” is a motor neuron disease that is caused by a selective loss of upper and lower motor neurons in the brain and spinal cord. The progressive degeneration of motor neurons in ALS eventually leads to death due to respiratory failure. The most common form of ALS is a sporadic type and only about 5-10% of all ALS cases are hereditary. Since 1993, several genes associated with both familial ALS and sporadic ALS, have been identified, including superoxide dismutase 1 (SOD1), RNA-binding protein FUS, and TAR DNA-binding protein 43 (TDP43) (Andersen and Al-Chalabi, 2011). Many factors such as oxidative stress, mitochondrial dysfunction, abnormalities of the immune system, and glutamate toxicity are known to cause sporadic ALS (Kieman et
Recently, dysfunction of the autophagic/lysosomal system was also shown to be tightly associated with ALS. The first evidence came from the SOD1G93A-expressing ALS mouse model. Although autophagy seems impaired in the ALS model, many researchers showed its induction in ALS mouse models (Mormoto et al., 2007; Song et al., 2012). In patients with sporadic ALS, the autophagy features were observed under electron microscopy in the cytoplasm of normal motor neurons and more frequently in degenerated motor neurons (Sasaki, 2011). However, this feature cannot ensure which factor causes the enhancement of LC3-II in ALS. Despite some controversy regarding the role of autophagy in ALS, many studies suggest that autophagic clearance of mutant SOD1 is beneficial to ALS (Li et al., 2008). Small heat shock protein B8 (HSPB8) decreases the aggregation of mutant SOD1 and increases its solubility and clearance by enhancing autophagy without affecting wild-type SOD1 turnover in the SOD1G93A ALS model mice (Crippa et al., 2010). A dramatic decrease in mutant SOD1 toxicity was also observed in X-box-binding protein-1 (XBP-1), a key molecule in unfolded protein response, deficient mice, correlating with the increased levels of autophagy and the reduced accumulation of mutant SOD1 aggregates in the spinal cord (Zhang et al., 2011). Additionally, functions of several ALS-related genes were reported to be associated with autophagy. ALS2/alsin is a guanine nucleotide exchange factor for the small GTPase Rab5 and is involved in micropinocytosis-associated endosomal fusion and trafficking. Recently, ALS2 loss has been shown to exacerbate SOD1H46R-mediated neurotoxicity by disturbing endosome-autophagosome trafficking (Hadano et al., 2010). Another protein mutation causing ALS is TDP43, a nuclear RNA-binding protein involved in several aspects of RNA processing. TDP43 turnover is known to be enhanced by autophagy activation and that autophagy-activating compounds improve TDP43 clearance and enhance survival in neuronal ALS models (Barmada et al., 2014). Unlike other neurodegenerative disorders, the identification of autophagy-regulating drugs as potential ALS therapeutic agents is not much studied. Like other diseases, however, autophagic degradation of mutant SOD1 and TDP43 is believed to be beneficial to ALS. Therefore, several autophagy enhancers such as rapamycin, lithium, and trehalose are expected to function as pathology reliever in ALS. In general, rapamycin plays a neuroprotective role in several neurodegenerations. However, in ALS models, it showed neuroprotective effects and delayed the disease onset and duration in the SOD1G93A ALS mouse model (Fornai et al., 2008). In contrast, under the same conditions, another group showed that lithium does not ameliorate disease progression in SOD1G93A mice (C57BL/6 or 129S2/Sv strains) (Pizzagolla et al., 2009). While this aspect needs to be further characterized using animal genetic models, combination strategies or modified autophagy enhancers may still be appropriate as ALS therapeutic approaches.

**CONCLUSION**

Accumulated evidence revealed that neuronal autophagy is essential for the healthy aging of neurons. Moreover, neuronal autophagy is the major process for the degradation of an abnormal protein aggregate, which is the major cause of most neurodegenerative diseases such as AD, PD, HD, and ALS. Increasing research in autophagy revealed several links connecting autophagy and neurodegenerative diseases. However, direct links and molecular mechanisms remain elusive and need to be further addressed. Impairment of lysosomal function or autophagosome/lysosome fusion is observed in most neurodegenerative disorders. Nevertheless, the recent attempts to treat the autophagic impairment in neurodegeneration have focused on the induction of initial autophagy. Therefore, it is critical to overcome lysosomal dysfunction when developing therapeutic strategies against neurodegenerative diseases. Research on autophagy as a potential therapeutic target for neurodegenerative disease treatment is only starting. Some compounds for the treatment of AD have been tested in human clinical trials. Other compounds for the treatment of the other neurodegenerative diseases are now in the preclinical phase. Despite research limitations, therapeutic approaches targeting autophagy are highly expected to contribute to the treatment of neurodegenerative diseases.

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**REFERENCES**


