**Mini Review**

# Autophagy in neurodegeneration: two sides of the same coin

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**Autophagy is a bulk lysosomal degradation process important in development, differentiation and cellular homeostasis in multiple organs. Interestingly, neuronal survival is highly dependent on autophagy due to its post-mitotic nature, polarized morphology and active protein trafficking. A growing body of evidence now suggests that alteration or dysfunction of autophagy causes accumulation of abnormal proteins and/or damaged organelles, thereby leading to neurodegenerative disease. Although autophagy generally prevents neuronal cell death, it plays a protective or detrimental role in neurodegenerative disease depending on the environment. In this review, the two sides of autophagy will be discussed in the context of several neurodegenerative diseases. [BMB reports 2009; 42(6): 324-330]**

## INTRODUCTION

Although first described about 40 years ago, most of the research on autophagy has occurred within the past decade (1). Recent studies show extensive involvement of the autophagy pathway in several biological processes under both normal and pathological conditions (2, 3).

Autophagy is an evolutionarily conserved pathway that mediates cellular degradation through the action of lysosomes. Autophagy being self-degradative has been also considered an alternative pathway for cell death (type II cell death), which is defined by the massive accumulation of autophagosomes without nuclear condensation (4). A growing body of evidence shows that autophagy is involved in stress-induced adaptation as well as cellular development, differentiation and survival. Moreover, dysfunction of the autophagy pathway often promotes human diseases such as cancer, heart disease, infection, liver disease and several neurodegenerative disorders (3, 5). Autophagy is paradoxical in that it can both protect and impair cell survival depending on the physiological and pathological environment. Thus, regulation of autophagy determines the fate of cells in multiple organs. One of the main concerns of

autophagic regulation is the significance of cell-type or tissue specificity. Specifically, neurons could be vulnerable to an accumulation of abnormal components such as cytosolic proteins or organelles that are damaged regarding their post-mitotic nature. Therefore, the regulation of neuronal autophagy in a healthy or diseased environment is most likely context-dependent. Understanding the molecular mechanisms underlying autophagic regulation is an essential first step in treating neurodegenerative diseases associated with the autophagy pathway. In this review, I'll provide a brief overview of the general and neuronal autophagy pathways, and then discuss their beneficial or detrimental effects on neurodegeneration.

## Core molecular machinery of autophagy

Three types of autophagy have been identified: microautophagy, chaperone-mediated autophagy and macroautophagy (2) (Fig. 1). Microautophagy is the removal of cytoplasmic proteins or organelles through the direct uptake of cytoplasm into lyso-
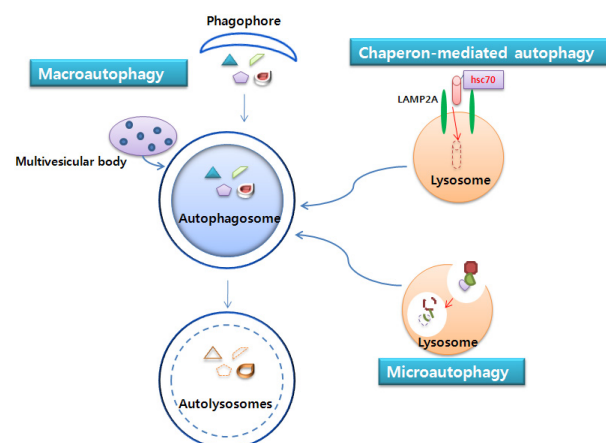


**Fig. 1.** Three types of autophagy. Autophagy is a conserved lysosomal degradation pathway. However, autophagy can differ in the ways it delivers cargo to lysosomes. In microautophagy, cytoplasmic cargo can be directly invaginated into lysosomes. In chaperone-mediated autophagy (CMA), cargo is recognized by a chaperone (hsc70) and is then delivered into lysosomes for degradation. In macroautophagy, double- or multi-membrane-bound autophagosomes are formed which are elongated from the isolation membrane (also called phagophore). These autophagosomes can fuse with endosomes or lysosomes and cause the degradation of cytosolic cargo.

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somes. In chaperone-mediated autophagy, Hsc70, which recognizes substrates containing a KFERQ motif, mediates the degradation of cytosolic proteins in lysosomes. However, in macroautophagy, autophagosomes, which are double or multi-membrane bound structures, sequester cytoplasmic components followed by fusion with lysosomes to degrade abnormal cytosolic materials. The formation of autophagosomes and their fusion with endosomes or lysosomes to create autolysosomes are unique features of macroautophagy (hereafter referred to as "autophagy"). Therefore, the balance between these processes determines the success of autophagic regulation, benefiting autophagy in terms of cellular function.

Autophagy generally undergoes three processes: 1) formation of autophagosomes (induction, nucleation, cargo recognition, expansion and completion of the autophagosomes), 2) maturation and fusion of autophagosomes with lysosomes, and 3) degradation of the cargo in autolysosomes (1). Several autophagy-related genes involved in these processes have

been characterized in yeast and mammals and identified as highly conserved (3, 6).

Autophagy is either constitutively active at basal levels or upregulated under certain conditions such as starvation, development or stress. It has been shown that autophagy is induced by mTOR-dependent or mTOR-independent pathways (4, 7). Once autophagy is induced, isolation membranes (also called phagophores) are formed (vesicle nucleation) that initially sequester organelles. These become the autophagosomes, which mature into autolysosomes by fusing with endosomes or lysosomes. Finally, cytosolic components sequestered by these autolysosomes are degraded (Fig. 2). During vesicle nucleation, Ulk1/2 (Yeast Atg1), a serine/threonine kinase, works cooperatively with Vps34/PI3K complexes, which are composed of Beclin1, ambra1 and UVRAG (1, 8). The other core molecular machinery is comprised of two ubiquitin-like conjugation systems that mediate elongation of autophagomes: One is the conjugation of atg5 with atg12 and the other is the covalent
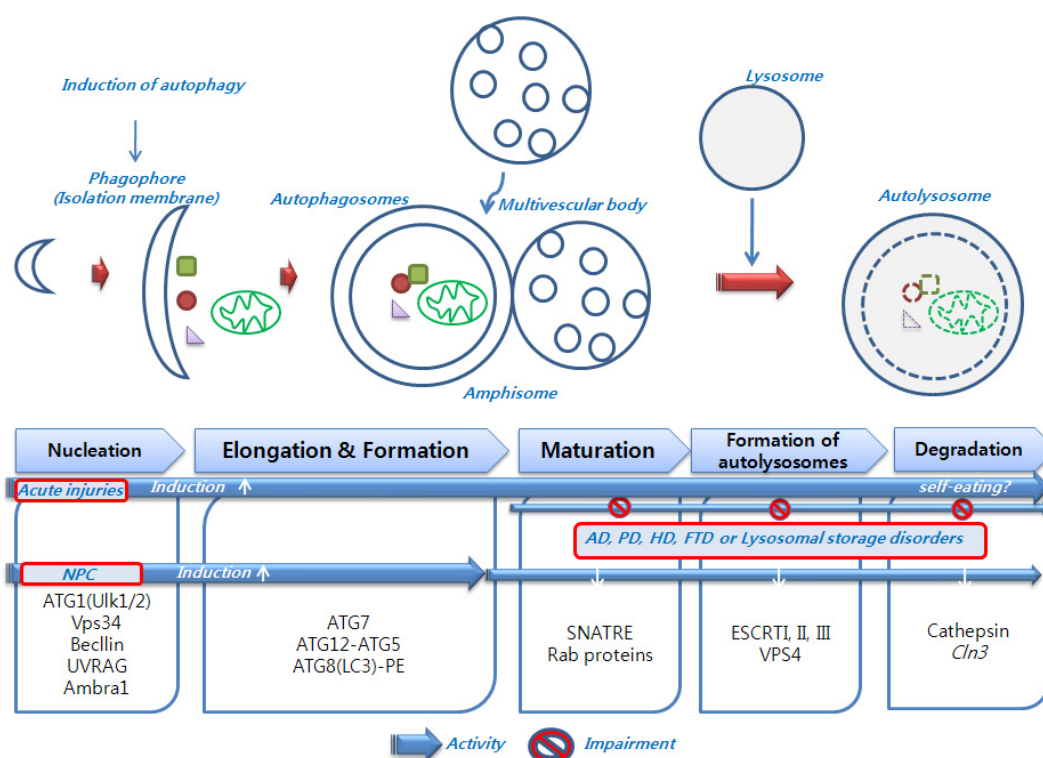


**Fig. 2.** The steps of autophagy and alteration of the autophagic process in neurodegeneration. Autophagy undergoes several processes: nucleation, elongation, formation of autophagosomes, maturation, formation of autolysosomes and degradation of cargo. Autophagy-related genes have been characterized in genetic and biochemical studies on yeast and mammals. A number of genes are involved in the specific processes of autophagy activation. Important molecular machinery is indicated in Fig. 2. Steps are not mutually exclusive and therefore a balance between these processes determines the fate of the cell. Fig. 2 shows that neurodegenerative disease is associated with abnormal autophagic processes. For example, defects in autophagosomal maturation, autophagosomal fusion with lysosomes and digestion by lysosomal hydrolyses are all associated with neurodegenerative disease (AD, PD, HD, FTD and lysosomal storage disorders). For NPC, autophagy insufficient and causes autophagic stresses. In contrast, acute injuries can highly activate autophagy, leading to self-degradation and neuronal cell loss. AD: Alzheimer's disease, HD: Huntington's disease, FTD: Frontotemporal dementia, NPC: Niemann-Pick type C disease.

linkage of atg8 to phosphatidylethanolamine (PE). In particular, LC3 (Microtubule-associated Light Chain1), a mammalian homolog of yeast *atg8*, is used as a marker of autophagosomes based on the fact its lipidated form is specifically localized to autophagosomes. After autophagosomes are formed they undergo a maturation step by fusing with endosomes or multivesicular bodies (9), followed by another fusion event with lysosomes to become autolysosomes. SNARE, Rab proteins, ESCRT complexes, Vps4 and Fab1 mediate these fusion events (10) (Fig. 2). Finally, autolysosomal degradation of cytosolic proteins and organelles is mediated by lysosomal hydrolases. Therefore, each step in the autophagy pathway is tightly regulated and crucial for efficient execution; any disruption can impair autopahgy and lead to disease.

## Neuronal autophagy; the role of basal autophagy

Neurons differ from other cell types in that they are post-mitotic and highly dependent on the endo-lysosomal pathway for active signaling in the axons and dendrites. Due to these features, neurons require effective protein degradation as a quality control for cell survival, especially under disease conditions for the removal of toxic components. Any alteration of protein degradation can cause the accumulation of abnormal proteins, leading to cellular toxicity and ultimately neurodegeneration. There have been at least three effective protein degradation pathways identified in the CNS (central nervous system): UPS (ubiquitin-proteasome system), endo-lysosomal degradation system and the autophagy pathway (11). UPS is responsible for the degradation of polyubiquitinated cytosolic proteins. However, membrane proteins and receptors are downregulated and degraded through the endo-lysosomal pathway. Autophagy uses lysosomes to remove cytosolic proteins as well as organelles in a non-specific manner, contrary to the other degradation pathways. While the UPS and lysosomal degradation systems have been extensively studied in neurons as well as other cell types (12), little is known of the role of autophagy in the nervous system.

Previous studies using the autophagosome marker GFP-LC3 *in vitro* and *in vivo* indicate that autophagy is highly and distinctly regulated in neurons (13). More recent studies also show the differences in basal autophagy between non-neuronal and neuronal cells (14). For example, GFP-LC3-positive autophagosomes are hardly detected in healthy neurons, whereas massive accumulation of autophagic vacuoles is observed under pathological conditions. However, recent reports using mutant mice lacking the autophagy-related genes *atg5* or *atg7* indicate basal autophagy has an important role in neurons (15, 16). The mutant mice showed growth retardation, progressive motor deficits and prominent neurodegeneration, indicating a crucial role for autophagy in neuronal homeostasis. On a molecular level, autophagy deficiency caused an accumulation of ubiquitin-positive protein aggregates in neurons as well as neuronal loss in the cerebral cortex, hippocampus and cere-

bellum. Therefore, the autophagy pathway regulates cellular homeostasis by controlling degradation of cytosolic components in healthy neurons. Interestingly, impairment of the UPS can activate a compensatory role for the autophagy pathway in which cytotoxic materials are removed in order to maintain cellular homeostasis (17, 18). In addition to macroautophagy, a previous study also shows that chaperone-mediated autophagy is essential for the quality control of proteins. CMA normally degrades α-Synuclein, which contains a KRERQ domain. However, the mutant form of α-Synuclein inhibits CMA in a diseased state. Therefore, the activation of macroautophagy by CMA disruption shows the link between CMA and macroautophagy.

Another physiological role for neuronal autophagy was shown using mutant mice that display a specific loss of *atg7* in Purkinje neurons (19, 20). Autophagy-deficient Purkinje neurons in the cerebellum show the cell-autonomous accumulation of aberrant membrane-bound organelles and membrane structures in dystrophic axonal terminals. This study highlights a specialized role for autophagy in the homeostasis of membranes in axonal terminals, and therefore raises numerous interesting questions. What are the substrates for autophagy in the axonal terminal? How does autophagy regulate membrane homeostasis in the axonal terminal? Are there any differences in autophagy between soma and axonal terminals? If so, what are the differences? Neuronal autophagy in axonal terminals may play an important role in the homeostasis of synaptic vesicles or membrane-bound structures abundant in axons. In particular, synaptic vesicles are interesting candidate organelles because they are highly regulated in axonal terminals by synaptic activity. Autophagy in axonal terminals may also be involved in growth cone remodeling during axonal development or regenerative processes after injury. Future works that explore the role of neuronal autophagy in axonal terminals will enable us to further define neuronal autophagy as well as diseases associated with dysfunctional autophagy.

## Autophagy in neurodegeneration

The autophagy pathway has been also implicated in several neurodegenerative diseases such as Alzheimer's disease, Huntington's disease, Parkinson's disease and Frontotemporal dementia, as well as acute injuries (21, 22). A growing body of evidence shows an accumulation of autophagosomes in diseased brains, an event highly associated the progression of many neurological disorders. Despite research suggesting an association between neurodegeneration and autophagy, many issues remain to be addressed: How is the autophagy pathway regulated in each neurodegenerative disease? Is autophagy disrupted or activated? If disrupted, which molecular pathway is impaired? If not, is autophagy simply a consequence of disease progression?

Like two sides of the same coin, autophagy can be protective of or detrimental to neuronal cell survival. Thus, under-

standing the detailed mechanism of autophagy in specific disease conditions will help identify therapeutic targets for neurodegenerative diseases. In this section, I'll focus on how the autophagy pathway is associated with neurodegeneration based on recent studies.

## Neurodegeneration associated with defects in autophagy machinery

As described earlier in this review, autophagic processes are composed of several steps. A number of studies have shown that alteration of any of these steps can impair autophagy and lead to neurodegeneration. In this section, the phenotypes observed in animal models upon loss of autophagic machinery will be described.

**The disruption of autophagosome formation:** Autophagosome formation was disrupted following the impairment of autophagy by the loss of genes involved in induction or vesicle formation. Disruption of ULK1 (ATG1) leads to impairment of endocytosis, abnormal axonal branching and growth in mice and *c.elegans* (23, 24). Knockout mice deficient in the tumor suppressor BECN1 show neurodegeneration and lysosomal abnormality (25). Recent work by Fimia *et al.* (26) shows that loss of *Ambra1* induces cell death and neural tube defects. The depletion of *atg5* or *atg7* can cause accumulation of ubiquitinated proteins and ultimately neuronal cell loss (15, 16).

**The disruption of autophagosomal maturation:** Autophagosomes with defects in maturation rapidly accumulate in the cytosol due to their inability to be degraded. Interestingly, the ESCRT (Endosomal sorting complex required for transport) has been presented as a key regulator of autophagosome maturation (27). Loss of CHMP4b (a component of ESCRT-III) results in the accumulation of ubiquitin-positive proteins as well as neuronal cell loss in flies and mice (27). Interestingly, mutation of CHMP2B, another ESCRT-III subunit, is associated with chromosome 3 (FTD3)-linked frontotemporal dementia. Based a cell culture study using primary cortical neurons, the mutant form of CHMP2B can also cause neurodegeneration by disrupting the fusion of autophagosomes with lysosomes, which is one possible explanation of FTD-3 pathogenesis (27, 28). A recent study showed that loss of Hrs (a component of ESCRT-I) caused neurodegeneration and abnormal protein accumulation, thereby providing evidence that ESCRT components play essential roles in autophagy (29). However, further investigation is needed to identify how ESCRT regulates the fusion process.

**The disruption of autophagosomal clearance:** The final step of autophagy is the digestion of sequestered materials in autolysosomes. A defect in autophagic clearance is also observed in many neurological diseases whose main feature is a deficiency of lysosomal hydrolases. Loss of *cln3* or *cathepsinD* presents neuronal ceroid lipofuscinosis (30-32). Moreover, loss of *cathepsinB*/*cathepsinL* causes severe brain atrophy and enhanced apoptosis, indicating their crucial roles in autophagic degradation (31, 33).

## Neurodegenerative diseases associated with autophagy

Protein aggregates or inclusion bodies caused by toxic proteins are hallmarks of age-dependent neurodegenerative disease. To improve cell survival, stresses produced by protein accumulation cause the degradation of toxic disease proteins by autophagy. Here, the association of the autophagy pathway with several neurodegenerative diseases is briefly summarized.

**Alzheimer's disease:** A massive accumulation of autophagosomes in the dystrophic neuritis was observed in an animal model of Alzheimer's disease (AD) and in postmortem brains from AD patients. Here, autophagy seems to be abnormal due to alteration of the endo-lysosomal pathway, which impairs fusion of autophagosomes with lysosomes (34). In addition, a recent report details the contribution of mitophagy, a specialized form of autophagy that removes damaged mitochondria in AD (35). Interestingly, activation of autophagy can promote degradation of APP/Aβ and reduce tau pathology (34). Therefore, the autophagy pathway is considerably more complex in AD because it is simultaneously induced and impaired. APP/Aβ can be generated in autophagosomes at low levels in a physiological environment; however, during disease conditions autophagosomes rapidly accumulate in the cell body and axonal terminals due to either impaired autophagic flux or a defected endosomal pathway.

**Parkinson's disease:** Intriguingly, at least three types of autophagy have been reported in Parkinson's disease: macroautophagy, mitophagy and chaperone-mediated autophagy (36, 37). Macroautophagy and mitophagy both appear abnormal in animal and cell culture models of PD, as evidenced by massive autophagosome accumulation. Interestingly, while α-Synuclein is degraded by CMA, mutated α-Synuclein actually inhibits CMA, leading to accumulation of mutant α- Synuclein. A more recent study shows that mutant UCH-L1 (Ubiquitin carboxyl-terminal hydrolase L1) strongly binds to LAMP2A, thereby blocking the interaction of normal substrates with LAMP2A and leading to inhibition of CMA (38). Impairment of CMA by mutant UCH-L1 may explain one of the pathologic mechanisms of PD. Specifically, CMA inhibition activates macroautophagy in order to compensate for the impaired cellular degradation.

**Huntington's disease:** PolyQ-expanded huntington aggregates are shown to be degradable by autophagy (39-41). Therefore, the most important issues in HD related to autophagy are the induction and activation of the autophagy pathway in order to digest toxic components. HDAC and microtubules reportedly contribute to the effective clearance of polyQ aggregates by autophagy (42). Therefore, the autophagy pathway in HD becomes an attractive drug target.

**NPC (Niemann-Pick type C disease) and other lysosomal storage disorders:** In these specific diseases, the autophagic machinery is insufficient for proper degradation, imparing autophagic flux and causing autophagic stress. The impairment of autophagic flux has been also reported in lysosomal storage dis-

orders such as mucolipidosis type IV (43), Pompe disease (44), multiple sulfates deficiency and mucopolysaccharidosis type IIIA (45). These disorders have defects in the fusion of autophagosomes with lysosomes, which cause accumulation of autophagosomes and impair the autophagic degradation pathway. NPC1 deficiency in mice elevated the levels of autophagy and autophagic flux (46). However, the underlying mechanism should still be examined.

**Acute injuries:** Hypoxia/ischemia, trauma and pharmacological injury are all associated with autophagy (47, 48). Acute injuries can activate autophagy through the formation of autophagosomes. The role of autophagy in acute injuries and several chronic neurological diseases remains very controversial. Inhibition of autophagy in injury models seems to protect or delay neurodegeneration. Therefore, there is a possible role for autophagy in promoting neuronal cell death.

Nonetheless, further studies should be performed in time- and context-dependent manners in order to understand the contribution of autophagy in injuries and chronic neurodegenerative diseases.

## Conclusion and future perspectives

Autophagy is generally protective against neuronal cell death under physiological and pathological conditions. Dysfunction of basal autophagy can lead to neuronal cell loss, indicating how it benefits cell survival in the central nervous system (Fig. 3). Moreover, autophagy can be induced and activated for neuronal cell survival and for the removal of toxic components in several neurodegenerative diseases. Under these disease conditions, a balanced level of autophagic flux can be induced, which contributes to cell survival. In contrast, an in-
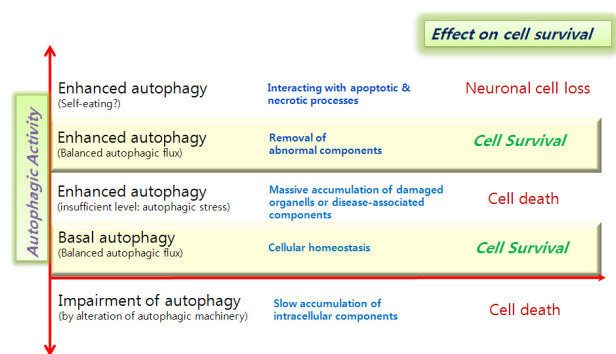


**Fig. 3.** Autophagic flux on neuronal cell survival. Autophagy plays an important role in cellular homeostasis at the basal level. Under disease conditions, autophagy can be upregulated if the autophagic flux is balanced, which benefits cell survival. In contrast, if regulation of autophagic flux fails, neurodegeneration can be a result. Moreover, even though the role of autophagy in neuronal cell death is controversial, inhibition of autophagy under certain conditions such as acute injury can contribute to cell survival, thereby suggesting a possible detrimental role for autophagy.

sufficient level of autophagy can cause an abnormal level of flux and lead to neurodegeneration. However, under specific condition, autophagy may be highly active and promote cellular reconstruction by digesting components. Therefore, understanding the molecular mechanism of neuronal autophagy as well as the specific role autophagy plays in neurodegenerative disease will be an important step in developing therapeutic approaches that depend upon modulating the autophagy pathway.

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