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Autophagy in Neurodegenerative disorders: Pathogenic Roles and Therapeutic Implications

Rebecca Banerjee[1], **M. Flint Beal**[1], and **Bobby Thomas**[1,*]

[1] Department of Neurology and Neuroscience, Weill Medical College of Cornell University, 525 East 68th Street, A-501, New York, NY 10065

Abstract

Autophagy is a highly conserved intracellular pathway involved in the elimination of proteins and organelles by lysosomes. Known originally as an adaptive response to nutrient deprivation in mitotic cells, autophagy is now recognized as an arbiter of neuronal survival and death decisions amongst neurodegenerative diseases. Studies using postmortem human tissue, genetic and toxin- induced animal and cellular models indicate that many of the etiological factors associated with neurodegenerative disorders can perturb the autophagic process. The emerging data support the view that dysregulation of autophagy may play a critical role in the pathogenesis of neurodegenerative disorders. In this review, we highlight the pathophysiological roles of autophagy and its potential therapeutic implications in debilitating neurodegenerative disorders, including Amyotrophic lateral sclerosis, Alzheimer's, Parkinson's and Huntington's disease.

Introduction

Autophagy or "self-eating" is a lysosome-mediated degradation process for non-essential or damaged cellular constituents. It plays an important homeostatic role in cells preserving the balance between synthesis, degradation and subsequent recycling of cellular components[1]. During autophagy cytoplasmic constituents (including misfolded or aggregated proteins), damaged organelles (such as mitochondria, endoplasmic reticulum (ER) and peroxisomes), as well as intracellular pathogens are sequestered into double membrane autophagosomes and their contents degraded by lysosomal hydrolases. This machinery has been implicated in multiple physiological processes including protein and organelle turnover, stress response, cellular differentiation, programmed cell death, in addition to various pathological settings[2]. While basal levels of autophagy ensure the physiological turnover of old and damaged organelles as a garbage removal process, significant accumulation of autophagosomes is thought to represent either an alternative pathway of cell death or an ultimate survival attempt for cells in response to stress[3].

Autophagosomes in neurons have been recently observed to be abundant in a growing number of neurodegenerative disorders. Such observations have provoked controversial viewpoints about whether these structures foster neuronal cell death or render neuroprotection. It is often

*Corresponding author: Bobby Thomas PhD, Department of Neurology and Neuroscience, Weill Medical College of Cornell University, 525 East 68th Street, A-501, New York, New York 10065. Tel: 212-746-5341, Fax: 212-746-8276, bot2003@med.cornell.edu.

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debated whether accumulation of autophagosomes in neurons leads to an increase in autophagic activity, or rather is a consequence of impaired autophagic degradation creating a buildup of autophagosomes. Whether autophagic induction in neurons leads to cell death (traditionally mediated via conserved canonical pathways like apoptosis and necrosis[4]) or if it simply occurs as a process alongside these other pathways is a controversial issue. Thus, it is crucial to address this bewilderment about the opposing views in defining its fundamental role in neuronal physiology[5].

It is becoming increasingly evident that autophagy may contribute differently to cell death induction according to the type and degree of the environmental changes or stress stimuli. A cell's response may shift gradually from elimination of damaged proteins/organelles by autophagy which leads to its recovery, to the induction of apoptotic pathways determining cellular demise. However, in the context of neurodegenerative disorders an emerging consensus is the view that induction of autophagy is a neuroprotective response and that inadequate or defective autophagy, rather than excessive autophagy, promotes neuronal cell death in most of these disorders[6]. In this review, we provide an overview of current understanding on the putative role of autophagic mechanisms in common neurodegenerative disorders such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD) and Amyotrophic lateral sclerosis (ALS). Furthermore, we discuss implications for how the prevailing knowledge about this degradation machinery could be harnessed for designing tangible therapeutic approaches.

The autophagic process

Autophagy was first described by Christian De Duve in 1963, as a cellular process in which a double membrane vesicle, the autophagosome, delivers cytoplasmic constituents to lysosomes for degradation and recycling[7]. Our understanding towards the genetic basis of this physiological process remained ambiguous until a decade ago with the discovery of over 30 autophagy related genes (ATG) and identification of their roles in the regulation and execution of autophagy at the molecular level[8]. This machinery is orchestrated based on the induction and presentation of target substrates such as proteins and organelles to the lysosomes via three major distinct autophagic processes: macroautophagy, microautophagy and chaperone-mediated autophagy (CMA).

Macroautophagy

Macroautophagy is a bulk degradation pathway potentially capable of degrading large protein aggregates or damaged organelles. This process of autophagy starts with the derivation of phagophore (or isolation membrane) which is likely derived from the lipid bilayer of the plasma membrane, ER and/or trans-Golgi, endosomes or mitochondria[9–12]. This phagophore expands to engulf intracellular cargo, such as protein aggregates, organelles and ribosomes, thereby sequestering the cargo in a double membranous autophagosome[1]. Autophagosomes are formed randomly in the cytoplasm and are then trafficked along microtubules in a dynein-dependent fashion towards the microtubule-organizing center. Once at this center, they mature through fusion with multivesicular bodies (MVB), early and/or late endosomes, before fusing with lysosomes, promoting the degradation of autophagosomal contents by lysosomal acid proteases. Once degraded by lysosomes, the lysosomal permeases and transporters export amino acids and other by-products of degradation back out to the cytoplasm, to be recycled for building macromolecules and for metabolism[1] (Figure 1). The only known mammalian protein that specifically associates with the autophagosome membrane (as opposed to other vesicles) is microtubule associated protein 1 light chain 3 (MAP1 LC3), which is post-translationally modified into cytosolic LC3-I, which conjugates with phosphatidylethanolamine upon autophagy induction to form autophagosome-associated LC3-II[13].

Microautophagy

Microautophagy is somewhat similar to macroautophagy, but far less is understood about it in mammalian systems compared to yeast. Here, the lysosomes directly engulf cytoplasm bypassing the need for autophagosome and autophagolysosome formation. It involves the pinocytosis of small quantities of cytosol directly by lysosomes[14]. Microautophagy is active in the resting state and is responsible for the removal of selective organelles and continuous turnover of intracellular constituents. It is not activated by nutritional deprivation or stress. Microautophagy and macroautophagy are both able to engulf large structures through selective and non-selective mechanisms (Figure 1).

Chaperone-mediated autophagy

Chaperone-mediated autophagy (CMA) is quite distinct from macroautophagy and microautophagy processes, in terms of selectivity and mechanism of degradation. CMA is responsible for the selective degradation of 30% of all soluble cytosolic proteins harboring a unique pentapeptide motif (KFERQ). This amino acid sequence is specifically recognized by a cytosolic chaperone, heat-shock cognate 70 (hsc70) and its co-chaperones, which selectively target these proteins to lysosomes for degradation. The substrate/chaperone complex moves to the lysosomes, where the CMA receptor lysosome-associated membrane protein type-2A (LAMP-2A) recognizes the protein, which is then unfolded and translocated across the lysosome membrane assisted by the lysosomal hsc70 inside the lysosomes for degradation[15, 16] (Figure 1). CMA differs from macroautophagy and microautophagy since the substrates are transported across the lysosomal membrane on a one-by-one basis, where as in the macroautophagy and microautophagy, the substrates are engulfed or sequestered in bulk. CMA is active under basal conditions but stress can enhance this pathway. While macroautophagy can be induced due to nutritional scarcity for a brief period, induction of CMA may happen over prolonged periods of nutrient deprivation[15, 16].

Regulation of autophagy in common neurodegenerative disorders

Cytoplasmic, nuclear and extracellular inclusions composed of aggregated and ubiquitinated proteins comprise key pathological hallmarks of numerous neurodegenerative diseases. Since protein aggregation is believed to contribute to organelle damage, synaptic dysfunction and neuronal degeneration, intracellular protein aggregate clearance pathways have been suggested as a potential therapeutic approach for such disorders. It is now apparent that aggregate-prone proteins and damaged organelles seen among a variety of neurodegenerative disorders are eliminated more efficiently via the autophagy-lysosome pathways in contrast to the ubiquitin-proteasome machinery. Here, we provide defining characteristics of autophagic pathways and their contributions to the underlying mechanisms of disease pathogenesis amongst common neurodegenerative disorders (Table 1) and describe potential targets for therapeutic modulation (Table 2).

Alzheimer's disease

AD is the most common neurodegenerative disorder characterized by the pathogenic accumulation of β-amyloid (Aβ) peptides, generated by the proteolytic cleavage of amyloid precursor protein (APP) via β- and γ-secretases that are localized in secretory and endocytic compartments of affected cells. Autophagosomes and endosomes within neurons actively form in synapses and along neuritic processes. Efficient trafficking of autophagosomes along microtubules is essential for autophagic degradation in neurons given the distances from neuronal processes, where endosomes and autophagosomes are formed, and the neuronal soma where the lysosomes are concentrated. In AD there is massive accumulation of autophagosomes within large swellings along dystrophic and degenerating neurites, primarily

due to deficits in the maturation of autophagosomes and their retrograde transport towards the neuronal cell body[17].

In general, autophagy is considered to be activated in AD primarily because of impaired clearance of autophagosomes that contain both APP and its processing enzymes, thereby increasing the propensity to generate toxic Aβ peptides[18]. Although most Aβ formed during autophagy is normally degraded within lysosomes via macroautophagy, Aβ also accumulates within the large pool of autophagosomes in dystrophic neurites and becomes a major intracellular reservoir of toxic peptides in AD brains. It is well established that Aβ can disrupt autophagosome membranes and trigger the release of hydrolytic enzymes into the cytoplasm. It is suggested that local accumulation of autophagosomes in dystrophic neurons may contribute to Aβ generation within plaques and that increased autophagy in the neuropil could be a major source of Aβ [17, 19]. This is achieved by increased turnover of APP together with an enrichment of the γ-secretase complex inside autophagosomes that cleaves APP to Aβ. In fact, autophagosome membranes are rich in presenilin 1, a component of the APP cleaving γ-secretase complex which is encoded by the presenilin gene whose mutations lead to early onset forms of autosomal dominant AD. Presenilin 1 mutant human AD brains show accumulation of extensive lysosomal pathology, together with amyloid pathology and neurodegeneration[20]. It was recently demonstrated that neurons and blastocysts from presenilin 1 hypomorphic mice and fibroblasts from presenilin 1 mutant AD patients show loss of macroautophagy, resulting in increased Aβ accumulation due to impaired maturation of the V0a1 subunit of the bimodular v-type H^+-ATPase proton pump that acidifies the lysosome[21]. Furthermore, levels of beclin 1 (or ATG6), required for the formation of autophagosomes, have been consistently found to be significantly reduced in postmortem AD brains[22]. In a transgenic mouse model of AD that expresses human APP, genetic reduction of beclin 1 expression stimulated formation of Aβ accumulation and neurodegeneration due to a decrease in autophagy[22]. Conversely, increased expression of beclin 1 in APP-transgenic mice significantly reduced amyloid pathology and neurodegeneration.

If reduced induction of autophagy is suggested to be the underlying mechanism for the observed higher levels of Aβ peptides and aggregates, then these results are consistent with the findings that the maturation of autophagosomes may be impaired in AD[22]. However, Aβ levels are known to be affected by both production and clearance[23]. Therefore, defects in autophagosome maturation could enhance Aβ production, whereas reduced autophagy through beclin 1 deficiency may inhibit Aβ clearance by altering APP metabolism[24]. Studies indicate ultrastructural alterations in mitochondrial morphology, such as reduced size, broken cristae and abnormalities in mitochondrial dynamics in AD[25, 26]. Sporadic AD patient fibroblasts[27] and cells overexpressing the Swedish variant of APP[28] demonstrate an imbalance in mitochondrial fission/fusion proteins, either via post-translational modification such as S-nitrosylation[29], or by alterations in their expression[26, 28], leading to an increase in mitochondrial fission. It is suggested that increased mitochondrial fission is probably an attempt to segregate and eliminate damaged mitochondria by a macroautophagic process known as “mitophagy”, which is consistent with a reduction in the level and size of mitochondria observed in human post-mortem tissue [25].

Alterations in the mammalian Target Of Rapamycin (mTOR) pathway, which is known to play a central role in signaling induced by nutrients and growth factors, is suggested to support autophagic failure in AD. Accumulation of Aβ peptides has been shown to increase signaling of the mTOR pathway, whereas decreasing mTOR signaling has been shown to reduce Aβ levels[30, 31]. In a mouse model of AD, pharmacological inhibition of mTOR signaling via rapamycin (an FDA-approved drug) rescues cognitive deficits and ameliorates amyloid and tau pathology by increasing autophagy[31]. Rapamycin-induced inhibition of mTOR led to increased neuronal autophagy in the mutant AD mice, suggesting that the reduction in Aβ levels

and the improvement in cognitive function are due in part to increased autophagy via mTOR inhibition. Considering that age is an important risk factor for the development of AD and many other neurodegenerative disorders rapamycin administration in animals was recently shown to extend lifespan in multiple studies[32, 33]. This effect of rapamycin on lifespan due to increased autophagy further reinforces the benefits of promoting autophagy in age-related neurodegenerative disorders. Thus, autophagy induction may serve as a potential therapeutic target for blocking AD pathogenesis.

Parkinson's disease

PD is a chronic neurodegenerative disorder characterized by progressive and relentless degeneration of dopaminergic neurons, especially within the substantia nigra[34]. Representing the multifactorial causes of PD, the pattern of neurodegeneration is complex, having features of necrosis, apoptosis[35] and accumulation of autophagosome like structures[36]. The first evidence in support of a significant role of autophagy in PD came from the demonstration that α-synuclein, which is a major constituent of Lewy bodies found in PD, is degraded by macroautophagy and CMA[37–39]. Inhibition of CMA has been shown to cause formation of high molecular weight and detergent-insoluble species of α-synuclein[39], suggesting that clearance of α-synuclein by CMA is crucial for limiting the oligomerization of α-synuclein. Importantly, A53T and A30P mutants of α-synuclein, which cause autosomal dominant PD, were found to inhibit CMA through exhibiting a higher binding affinity for the lysosomal marker LAMP2A compared with wild-type α-synuclein[38]. However, mutant α-synuclein is poorly internalized into lysosomes, and degraded by macroautophagy instead of CMA. Accompanying the inhibition of CMA by α-synuclein mutants is a compensatory activation of macroautophagy[38], although the physiological significance of this event is incompletely understood. Nonetheless, these observations suggest that A53T and A30P α-synuclein mutants may induce α-synuclein aggregation through inhibition of CMA, thereby leading to impaired clearance of these proteins.

Several posttranslational modifications, including monoubiquitination of α-synuclein, impair degradation of this protein by CMA further, but not degradation of other substrates[38, 40]. A modified version of α-synuclein is suggested to be responsible for neuron toxicity via a non-covalent interaction of α-synuclein and oxidized dopamine[41]. Dopamine-modified α-synuclein is not only poorly degraded by CMA, but also blocks degradation of other substrates, thereby increasing cellular vulnerability to stressors[41]. This is significant since oxidized metabolites of dopamine such as dopamine quinone derivatives are thought to play a pivotal role in the degeneration of nigrostriatal dopaminergic neurons[41] and further reveal that inhibition of CMA-mediated degradation of α-synuclein may constitute an important pathogenic mechanism for PD. Interestingly, it was recently demonstrated that α-synuclein may also contribute to neuronal death in PD by inhibiting CMA-mediated degradation of MEF2D (survival factor myocyte specific enhancer factor 2D)[42]. Both wild type and A53T mutant α-synuclein interfered with the binding of MEF2D to the chaperone hsc70 in the CMA degradation machinery. Consistent with this observation, MEF2D levels were elevated in A53T α-synuclein transgenic mice, and also in postmortem brain tissue from PD patients[42]. Furthermore, over-expression of both wild type and A53T mutant forms of α-synuclein inhibited MEF2 activity, leading to neurodegeneration[42]. These observations reveal that in addition to α-synuclein, impaired CMA may also trigger neuronal death through inefficient degradation of other proteins. These findings collectively raise the interesting possibility that preservation of CMA functions may serve as an effective treatment strategy against PD.

Besides the degradation of α-synuclein, the autophagic pathway is also involved in the turnover of mitochondria in cells. Mitochondrial dysfunction represents one of the crucial pathogenic mechanisms in PD[43]. Mutations in genes such as parkin and PINK1 are known to cause

autosomal recessive forms of PD and have been implicated in the control of mitochondrial morphology and function[43]. How parkin and PINK1 affects mitochondrial functionality remains poorly understood. Recently, parkin was found to facilitate macroautophagy of impaired mitochondria, by mitophagy[44]. Parkin is selectively targeted to functionally impaired and depolarized mitochondria, which are then eliminated by mitophagy[44]. The selective targeting of impaired mitochondria for mitophagy is brought about via a functional parkin-mediated ubiquitination of impaired mitochondria that serves to recruit ubiquitin-binding histone deacetylase, HDAC6, and p62, which assemble the autophagic machinery for efficient degradation of the impaired mitochondria[45]. It has been suggested that mitophagy could protect neurons by eliminating dysfunctional mitochondria, which may produce toxic reactive oxygen species that damage neurons[46]. In support of a role for autophagy in the clearance of defective mitochondria in PD, knockdown of PINK1 expression induces mitochondrial fragmentation, followed by activation of autophagy/mitophagy[47]. Moreover, it was recently shown that targeting of parkin to impaired mitochondria during mitophagy relies on the expression of wild type PINK1, but not PD-associated PINK1 mutants[48]. This occurs through the phosphorylation-dependent regulation of the ubiquitin E3 ligase activity of parkin[49], suggesting a pathogenic role of PINK1 and parkin loss-of-function mutations in PD. These observations suggest that the autophagic pathway is essential for the turnover of dysfunctional mitochondria in PD. Failure to activate efficient mitophagy may serve as an important pathogenic mechanism of cell death in PD.

Huntington's disease

HD is a progressive, autosomal dominant, neurodegenerative disorder caused by the expansion of CAG trinucleotide repeats (> 35 repeats) in the huntingtin (htt) gene, which is translated into an expanded polyglutamine tract in the N-terminus of the htt protein. Mutant htt toxicity is believed to be expressed after it is cleaved to form N-terminal fragments comprising the first 100–150 residues with the expanded polyglutamine tract. Although HD and other polyglutamine disorders are associated with intraneuronal aggregates, it is debatable whether these aggregates are toxic or protective[50]. Several lines of evidence have implicated the preaggregate oligomers as the most toxic species[51]. However, studies indicate that induction of autophagy results in the decrease of both aggregated and soluble 'monomeric' htt species, and results in decreased toxicity in various *in vitro* and *in vivo* models of HD[52]. Postmortem brains of patients with HD have endosomal and/or lysosomal organelles, and multivesicular bodies, characteristic features of autophagy[53]. Increased numbers of autophagosomes have been also found in lymphoblasts of HD patients in comparison to control lymphoblasts[54]. Mutant htt is known to induce endosomal and/or lysosomal activity[55] and mouse clonal striatal cells transiently transfected with truncated and full length human wild-type and mutant htt show the presence of both normal and mutant proteins in dispersed and perinuclear vacuoles[56]. Strong support for the involvement of autophagy in HD is provided by examining mTOR which is sequestrated into aggregates of mutant htt in cell models, transgenic mice and human brains, leading to decreased mTOR activity fostering the induction of autophagy to clear htt aggregates [57,58]. Numerous studies also suggest that pharmacological modifiers of mTOR-dependent and independent autophagic pathways can provide neuroprotection in various models of HD by inhibiting polyglutamine aggregation (Table 2).

Htt protein has been shown to modulate autophagy through its stress sensitive ER membrane association domain[59]. In response to ER stress, htt releases from membranes and rapidly translocates into the nucleus. However, this membrane release is inhibited when htt contains the polyglutamine expansion seen in HD, leading to perturbed ER function and an increase in autophagosomes. Notably, expression of htt with a deleted polyglutamine tract in a knockin mouse (Hdh (140Q/+)) model of HD showed upregulated autophagic markers and increased lifespan[60]. A recent study has also shown that despite the formation of autophagosomes at

enhanced rates in HD cells there is a failure to efficiently trap cytosolic cargo in the lumen[61]. This phenomenon might explain how inefficient engulfment of cytosolic components by autophagosomes is responsible for the slower turnover of htt, leading to htt accumulation in HD[61].

Another mechanism for increased accumulation of htt is via sequestration of beclin 1 into neuronal intranuclear inclusions. This has been demonstrated to occur in patients with HD, leading to reduced autophagic clearance of mutant huntingtin[62]. Expression of beclin 1 has been shown to decrease in an age-dependent fashion in human brains[62]. As the beclin 1 gene is haploid insufficient in regulating autophagosome function, it is proposed that an age-dependent decrease in beclin 1 expression may lead to a reduction in autophagic activity during aging, that in turn promotes accumulation of mutant htt and disease progression. The significance of autophagy in HD pathogenesis is further supported by the recent finding that age of onset of HD is modified by V471A polymorphism in ATG7[63]. A general consensus that has emerged from these studies is that blocking autophagy will reduce cell viability and increase the number of cells bearing mutant htt aggregates, whereas stimulating autophagy promotes htt degradation. A recent report provides strong evidence that stimulating autophagy is indeed beneficial for HD. Enhancement of the CMA was achieved by expressing a fusion molecule comprising polyglutamine binding peptide 1 (QBP1) and hsc70-binding motifs in cellular and mouse models of HD. This was found to result in the selective targeting of mutant htt for degradation and ameliorated the disease phenotype in a HD mouse model[64]. QBP1 is known to bind only the expanded polyglutamine tract seen in mutant htt and not wild-type htt. Such novel strategies using similar adaptor molecules comprising hsc70-binding motifs fused to an appropriate structure-specific binding agent(s) may have therapeutic potential for treating diseases caused by misfolded proteins other than those with expanded polyglutamine tracts. Thus taken together, autophagy may represent an initial attempt of HD neurons to eliminate mutant htt protein, however, over the course of the disease the autophagy processes become overloaded, ineffective and dysfunctional, eventually resulting in neurodegeneration. Induction of autophagy enhances the clearance of both soluble and aggregated forms of mutant htt, and protects against toxicity caused by these mutations in several disease models of HD.

Amyotrophic lateral sclerosis

In ALS, motor neurons degenerate leading to respiratory failure and fatality. A potential link between autophagy and ALS was initially based on morphological findings from postmortem spinal cords of sporadic and familial ALS (fALS) patients and subsequently from various genetic models of the disease exhibiting autophagic abnormalities[65–67]. The presence of abundant autophagosomes and associated increases in autophagy proteins suggested that autophagy activation is detrimental for the survival of motor neurons. In fact, it is quite common to find evidence of increased recruitment of the autophagic machinery to compensate for an increase in misfolded proteins and dysfunctional organelles in motor neurons[68]. However, other studies have found an increase in the LC3II macroautophagy marker protein and a decreased ratio of phosphorylated mTOR-positive motor neurons suggestive of defective autophagy associated with motor neuron loss[65, 67]. Similarly, defective autophagy is also suggested in the accumulation of ubiquitinated TAR DNA-binding protein 43kD (TDP-43) inclusions in ALS[69] and in motor neuron degeneration seen in ALS due to mutations in endosomal sorting complexes required for transport subunit III (ESCRT III) and CHMP2B[70, 71].

Two recent studies suggest that autophagic clearance of mutant superoxide dismutase 1 (SOD1) is beneficial for motor neuron loss in ALS. The heat-shock protein HspB8 increases mutant SOD1 clearance by promoting autophagy in an ALS mouse model[72]. Furthermore, the unfolded protein response transcription factor X-box binding protein 1 (XBP-1) deficiency in

mice has been shown to protect against ALS by autophagic induction and the subsequent degradation of mutant SOD1 [66]. While the recruitment of the autophagy system in ALS has been well documented, its significance (either detrimental or beneficial) for neuronal survival has been largely speculative up till now. A plausible explanation could be that mutant SOD1 in motor neurons may impair the autophagic machinery to promote neurodegeneration whereas autophagic clearance of SOD1 aggregates could be beneficial for the survival of motor neurons. The recent realization that autophagic induction may be an important therapeutic possibility for ALS has lead to clinical trials to assess the merits of autophagy inducers like lithium and rapamycin for neuroprotection in ALS patients[73]. Notwithstanding this, in-depth studies of the precise role of autophagy in ALS- associated motor neuron death remain to be performed.

Concluding remarks

Protein aggregation and organellar dysfunction are characteristic features of many late-onset neurodegenerative diseases. The ensuing accumulation of damaged proteins and organelles result in a mass of toxic debris within the afflicted neurons that results in neuronal dysfunction and may ultimately cause cell death. Accumulating evidence suggests that accelerating the removal of toxic accumulation of damaged membranes, organelles, and proteins may be a tractable therapeutic strategy for neurodegenerative disorders. The ubiquitin-proteasome system and the autophagy-lysosome pathways are the major routes of clearance for toxic proteins. The ubiquitin-proteasome pathway is mainly responsible for the breakdown and degradation of short-lived proteins with low-medium molecular weights; the narrow proteasome barrel precludes the entry of oligomers and high molecular weight protein aggregates commonly seen in neurodegenerative disorders. These latter proteins and aggregates, as well as cytoplasmic contents such as damaged membranes and organelles, can undergo bulk degradation in an efficient manner via distinct types of autophagy-lysosome pathways. Hence, in neurodegenerative diseases where autophagic pathway(s) is dysregulated, therapeutic interventions that activate autophagy may be beneficial in the prevention of neuronal cell death. However, in the present scenario, the consequences of manipulating the autophagic process are likely complex. In this regard, just as down-regulation or partial inhibition of autophagy could provoke or aggravate neurodegeneration, excessive activation of autophagy may invoke a similar response such as “self-cannibalism” in neurons. Thus, it is probable that this critical biological process will require an accurate titration to ensure that its activity is carefully controlled within physiological settings.

One may suggest that a beneficial or detrimental contribution of autophagy in the pathogenesis of neurodegenerative disorders could strictly rely on precisely when (the temporal aspect), where (the spatial feature), and how much (the quantity) activation. Even if one ensures that engulfment of protein aggregates or organelles by autophagosomes during autophagy is well-controlled, another crucial factor will be the efficient transport of these autophagosomes through the long processes of neurons to the site of lysosomes (predominantly in the neuronal soma). Future avenues of research (Box 1) should focus on understanding the intricate relationships between temporal, spatial and quantitative aspects and factors regulating the transport of autophagosomes during this degradation process. One can only hope that uncovering these finer details of this complex machinery will not only yield insights to pathophysiological mechanisms underlying common neurodegenerative disorders but also help us determine unique interventional targets for therapies that can be modulated to treat these disorders that presently lack effective treatments.

Box 1

Outstanding questions

- How are the different autophagic processes regulated in neurons both during physiological conditions and in the context of specific neurodegenerative disorders? Are these processes prevalent in all neurons that undergo degeneration and follow common regulatory mechanisms?
- What leads to a decline in autophagy during aging and in age-related neurodegenerative disorders? Does an aging-associated decline in autophagy lead to inability of a neuron to clear undesired cytosolic components?
- What is the precise relationship between the autophagy and the ubiquitin proteasome systems? Do these systems work in a collaborative manner? What determines this cross-talk between proteolytic systems and other signaling pathways? Are ubiquitinated proteins turned over via autophagy, or does ubiquitination occur in proteins that cannot be properly eliminated from the cytosol through other systems of elimination?
- What factors control cargo recognition and transport of autophagosomes to sites of lysosomes in a cell during autophagy? What kinds of signals trigger mitophagy? Is it selective to damaged mitochondria? What factors tag damaged mitochondria for degradation? How do alterations in the mitochondrial fission/fusion machinery impact this process? Is mitophagy universal for all age-related neurodegenerative disorders that demonstrate mitochondrial dysfunction?
- Can the activation of autophagic pathways be accurately titrated within the physiological range to allow this strategy to be effectively used for therapeutic intervention in neurological disorders?

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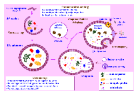

Figure 1. Steps involved in the autophagic process and their role in common neurodegenerative disorders

The autophagic process involves- 1) Formation of pre-autophagosomal structures- a membrane source provides lipid bilayers for formation of a phagophore by a process known as "nucleation". 2) Phagophore/Isolation membrane formation - here a double membranous phagophore or isolation membrane derived from pre-autophagosomal structures sequester portions of cytosol, including organelles that leads to 3) the formation of autophagosomes. 4) Maturation phase- the completed autophagosomes during this step undergo maturation, which involves steps such as fusion with multivesicular bodies or endosomes to form an amphisome. 5) Docking and fusion- during this step the inner membrane compartment fuses with a lysosome and its contents are degraded by lysosomal hydrolases. The three major forms of autophagy prevalent amongst common neurodegenerative disorders are macroautophagy, microautophagy and chaperone-mediated autophagy (see text for the detailed description of the three types of autophagy and how they are associated with pathogenesis of common neurodegenerative disorders).

Table 1

Proteins involved in autophagy and their disease modifying role in common neurodegenerative disorders.

Autophagy Signaling Factors	Cellular function(s)	Clinical Relevance	Disease Model
Beclin-1.	Involved in autophagosome formation	Reduced beclin-1 expression in postmortem AD brains[22]. Beclin-1 sequestration into neuronal intranuclear inclusions in HD patients- reduced beclin-1 function lead to impaired autophagic clearance of mutant htt[62]. Beclin-1 induction in sporadic ALS spinal cord – believed to promote autophagy[66]	Beclin-1 deficiency in APP transgenic AD mice leads to Aβ accumulation due to reduced autophagy. Beclin-1 over- expression attenuates this pathological phenotype[22]. Beclin-1 gene transfer activated autophagy and attenuated neuropathology in α-synuclein transgenic PD mice[74]. Induction of Beclin-1 in SOD1 ALS mice[66].
mTOR (Phosphatidyl kinase-related kinase)	Negatively regulates autophagy	mTOR levels are dramatically increased in AD brains correlating with tau pathology[30].	mTOR inhibition induced autophagy with neuroprotective effects in AD mice and in HD flies and mice[30, 31, 58]. Elevated mTOR levels are observed in α-synuclein transgenic mice[75].
LC3	Conversion of LC3I to LC3II is indicative of autophagosome formation.	Marked induction of LC3 in sporadic and familial ALS spinal cords – suggestive of enhanced autophagy[67].	LC3-II levels increased in symptomatic SOD1 ALS mice[67].
p62/Sequestosome 1	Autophagic adaptor that interacts with LC3.	p62 immunoreactivity observed in neurofibrially tangles of postmortem AD brains[76].	p62-dependent PINK1/parkin-mediated autophagy in non neuronal and neuronal cells[77]. p62-mediated recognition and targeting of mutant SOD1 for autophagic degradation[78].
Dynein	Autophagosome-lysosome fusion.	Dynein mutations – familial ALS[79].	Dynein-loss-of function caused premature mutant huntingtin aggregate formation in HD flies and mice, increased LC3II in cell and mice models[80].
Parkin	An E3 ligase that facilitates mitophagy.	Parkin loss-of-function mutations – Autosomal recessive juvenile PD[81].	Parkin mutations fail to ubiquitinate defective mitochondria causing mitophagic deficits[44, 45].
PINK1	A mitochondrial serine-threonine kinase involved in mitochondrial fission, mitochondrial quality control, and mitophagy.	PINK1 loss-of-function mutations – Autosomal recessive PD[82].	PINK1 loss-of-function promoted mitophagy as a compensatory response[47]. PINK1 mutants impaired parkin-mediated ubiquitin signaling and recruitment to mitochondria for mitophagy[48, 49, 83, 84].

Table 2

Autophagy modulators and their disease modifying action in common neurodegenerative disorders.

Autophagy Modulators	Mode of Action	Disease Pathology Modifier
Rapamycin CCI-779 (rapamycin analog) Glucose transporter (GLUT1)	Inhibit mTOR activating autophagy Raise intracellular glucose or glucose-6-phosphate	Clearance of mutant α-synuclein in cells[37]. Reduces Aβ levels and associated cognitive deficits[31]. Decreased mutant aggregate-prone tau proteins in flies[85]. Reduced toxicity of polyglutamine expansion in fly and mouse HD models[58, 86, 87].
Small molecule enhancers (SMERs)	mTOR-independent autophagic inducers	Mutant htt clearance[86]. Mutant A53T α-synuclein reduction[86].
Lithium, Sodium valproate, carbamazepine, L-690, 330.	Inositol lowering agents – inhibit inositol monophosphatase (IMPase) mTOR-independent autophagic inducer	Increased survival in ALS mouse[88]. Improved outcome in ALS clinical trial[89]. Mutant Htt aggregation/toxicity reduction[90].
Trehalose disaccharide, Minoxidil, Clonidine	mTOR-independent autophagic inducer	Increased autophagic flux, clearance of mutant htt and α-synuclein mutants[91, 92]. Ameliorates dopaminergic and tau pathology[93].
Combination Therapy: Rapamycin + Trehalose/ Calpastatin/SMERs	mTOR-dependent and mTOR-independent targets	Additive effect-enhanced autophagic clearance of protein aggregates[91, 92].