REVIEW

Autophagy in neurodegenerative diseases: pathogenesis and therapy

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Abstract

The most prevalent pathological features of many neurodegenerative diseases are the aggregation of misfolded proteins and the loss of certain neuronal populations. Autophagy, as major intracellular machinery for degrading aggregated proteins and damaged organelles, has been reported to be involved in the occurrence of pathological changes in many neurodegenerative disorders, including Alzheimer's disease, Parkinson's disease, Huntington's disease and amyotrophic lateral sclerosis. In this review, we summarize most recent research progress in this topic and provide a new perspective regarding autophagy regulation on the pathogenesis of neurodegenerative diseases. Finally, we discuss the signaling molecules in autophagy-related pathways as therapeutic targets for the treatment of these diseases.

INTRODUCTION

Cellular aggregations of misfolded proteins are the most common pathological hallmark of many neurodegenerative diseases, including Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD) and amyotrophic lateral sclerosis (ALS) (3). The pathological abnormalities of various neurodegenerative diseases are often associated with corresponding protein aggregations that reside in different cellular environment and subcellular compartments. Some of them are resulted from specific genetic mutations that cause autosomal recessive or dominant familial type of neurodegenerative diseases, while diverse mechanisms leading to impaired proteostasis contribute to the protein aggregations in neurodegenerative diseases.

Autophagy is one of the major intracellular machinery to eliminate misfolded proteins and maintain proteostasis. Dysregulated autophagy is increasingly considered to play key roles in most neurodegenerative diseases, and the regulation of autophagy is therefore proposed as a potential therapeutic avenue for these diseases (73, 74, 108). Macroautophagy (referred as autophagy) literally means "self-eating" in Greek that is responsible for removal of long-lived proteins and damaged organelles which are too large for proteasome to degrade. Autophagy not only plays a vital role in development, cell differentiation, apoptosis, pathogen infection and starvation, but also contributes to cancer, immune diseases and neurodegenerative diseases (17, 47, 78). Many studies have shown that autophagy is closely linked with neurodegenerative diseases. For example, the amount of autophagic vacuole, an intermediate vesicular compartment in the process of autophagy, is much more in the brains of neurodegenerative diseases than in health controls, suggesting impaired maturation of autophagosome to autolysosome (35). Without any other causing factors, the depletion of key autophagy-related genes (such as Atg5, Atg7) can lead to neurodegeneration in mouse central nervous system (54, 76).

Autophagy exerts a key role in degrading aggregate-prone proteins, which have been implicated in the pathogenesis of various neurodegenerative diseases, such as mutant α-synuclein in PD, mutant huntingtin in HD and mutant TAR DNA-binding protein 43 (TDP-43) in ALS. Once autophagy is inhibited, the clearance of these substrates is impeded. On the contrary, activation of autophagy may lead to enhanced clearance of those toxic proteins.

Lysosomal dysfunction in neurons is closely tied to neurodegeneration and cell death mechanisms (88). Growing genetic and biochemical evidence implicates the dysfunction of endosomal–lysosomal and autophagic lysosomal pathways during the pathogenesis of many neurodegenerative diseases, including AD, PD and ALS (31, 128). The therapeutic efficacy of autophagy/lysosome modulators in animal models of these diseases (88, 128) further underscores the significance of lysosomal impairments to the pathogenesis of neurodegenerative diseases.

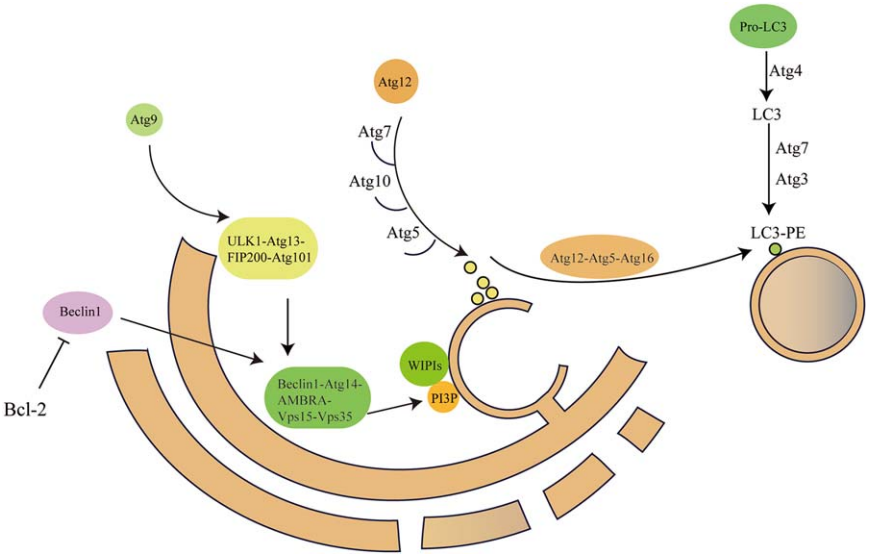

Figure 1. Autophagy induction and autophagosome formation. This diagram shows the process of autophagy induction and roles of Atg related proteins in autophagosome formation.

In this review, we summarize recent research findings showing that the dysregulated autophagy contributes to protein aggregation, organelle impairment and neuronal loss, eventually leads to neurodegenerative diseases. Autophagy modulation can prevent the occurrence and progression of these diseases (132). Even though various factors underlie the pathology of these diseases, we aim at providing an interaction between autophagy and the cause/progression of neurodegenerative diseases. We also review autophagy-inducing agents, both mTOR-dependent and -independent, and evaluate their effectiveness in disease models *in vitro* and *in vivo*.

AUTOPHAGY MECHANISM

Autophagy includes three subtypes: macroautophagy, microautophagy and chaperon mediated autophagy. Although subtypes of autophagy differ from cargo recognition, mechanism of molecular chaperon, they share lysosome as the unique place for cargo digestion and products recycling. An intact autophagy process is depicted as autophagic flux including autophagosome formation, fusion of autophagosome and lysosome, and cargo degradation in lysosome (15, 77). Firstly, misfolded proteins and damaged organelles are enwrapped by newly formed membrane termed as phagophore that is potentially derived from plasma membrane, Golgi, mitochondria or endoplasmic reticulum (ER) (99, 106, 134). Phagophore gradually sequesters cargoes through elongation till forming a closed autophagosome. By means of cytoskeletal microtubule systems, autophagosome traffics to lysosome and fuses with lysosome to form autolysosome. In autolysosome, cargoes are digested by lysosomal enzymes and recycled for reuse (2, 53, 55, 64).

Autophagy is a multi-stage process containing numerous proteins, including several autophagy-related proteins identified in mammals (79, 118). Autophagy is initiated by two major complexes UN51-like Ser/Thr kinases (ULK) complex and the class III phosphatidylinositol-3-kinase (PI3K), which are recruited to the phagophore assembly site (PAS) (2, 91). The ULK complex contains ULK1/2 family, FAK family kinase interacting protein of 200 kDa (FIP200) and ATG13 (121). The other complex PI3K, also named Beclin1 complex, consists of vacuolar protein sorting 34 (Vps34), p15 (VPS15), Beclin1 (ATG6) and Barkor (ATG14) (27). Notably, Beclin1 which localizes on ER membrane is regulated by anti-apoptotic dimer BCL-2 and BCL-XL. When autophagy is activated, Beclin1 will be dissociated from BCL-2 complex to coordinate with Vps34 (36, 69, 95). Subsequently, bulk phosphatidylinositol 3-phosphate [PI (3) P] will concentrate on the surface of phagophore (89, 99).

The extension and closure of autophagosome are exerted by two ubiquitin-like complexes. At the first, with the interaction of Atg7, Atg5 links with Atg12 covalently (114). Then the covalent complex links with Atg16 to form Atg5–Atg12–Atg16 complex, responsible for elongating phagophore. Atg9 which binds Atg2 and Atg18 is essential for trafficking between the Trans-Golgi-network, endosomes and newly formed autophagosomes. In another ubiquitin-like complex, microtubule-associated protein 1 light chain 3 (LC3) is cleaved by Atg4B to generate LC3-I (122). The Atg5–Atg12–Atg16 complex assists the transformation of LC3-I to phosphatidylethanolamine (PE)-conjugated LC3-II. Since LC3-II mainly resides on autophagosome, it is viewed as the significant marker for autophagosome (44).

Subsequently, maturated autophagosome needs kinesin and motor proteins to move along microtubules (101). Meanwhile, autophagosome fuses with lysosome in which multiple membrane proteins complexes such as the soluble NSF attachment protein receptor (SNAREs) are recruited (40). After the formation of autolysosome is completed, cargoes carried by autophagosomes are degraded by proteolysis. A detailed illustration of autophagy process is shown in Figure 1.

Autophagy plays an essential role in protein degradation and recycling. Though both of ubiquitin proteasome system and autophagy could clear ubiquitinated substrates, compared with proteasome, autophagy is the only one way to degrade large protein aggregates or impaired organelles which are too large to go into the narrow entrance of proteasome chamber.

Brains are the most vulnerable organ in most lysosome disorders, indicating that neurons might rely on autophagy even more heavily than other cells to maintain protein homeostasis. Unusual

structures such as large dendritic and axonal cytoplasm in neurons cause difficulty for them to remove impaired organelles and other waste in time (5). Two key components of autophagy locate on distinct places that autophagic vacuoles generated in axons should travel long distances to lysosomes mainly locating near the cell body. In addition, unlike other mitotic cells, neurons are not able to divide to disperse harmful substances (62). Aging will worsen the situation that neurons are gradually losing the ability to efficiently clear wastes, eventually resulting in abnormal accumulated autophagic substrates. Altogether, neurons are prone to suffer from autophagic proteolytic damage.

Notably, numerous stress responses promote misfolded protein aggregation at ER. ER stress is associated with unfolded protein response (UPR) (38), which is initiated by Inositol-requiring enzyme 1 α (IRE1α) (19). IRE1 is an ER transmembrane sensor that activates the UPR to maintain the ER and cellular function and downstream target c-Jun N-terminal kinase (JNK) (50, 90, 93, 130). ER stress can regulate Beclin1 in autophagosome formation (14). Protein misfolded diseases are usually accompanied with ER stress. UPR stands for a cell survival pathway to modulate autophagy to reduce protein aggregation and remain proteostasis (38, 93). In brief, various regulation mechanisms emerge to maintain proteostasis within cells making the modulation of autophagy as a promising strategy for therapeutic purposes.

AUTOPHAGY AND ALZHEIMER'S DISEASE

AD is the most common neurodegenerative disease that is characterized by extracellular amyloid-β (Aβ) plaques which are cleaved products of amyloid precursor proteins (APPs) and intracellular neurofibrillary tangles which are composed of aggregated hyperphosphorylated tau protein (135).

Under normal circumstance, autophagosome vesicles are rare in brains. Strikingly, detailed ultrastructural analyses have shown that dystrophic neurites in AD brains contain autophagosome vesicles (35, 87). Further study demonstrates that increased autophagy vacuoles are found in Presenilin 1 (PS1)-rich locations (35). Accumulation of autophagy vacuoles is likely arising from impaired clearance rather than the induction of autophagy itself, suggesting the modulation of late steps of autophagy as a possible therapeutic strategy for AD. Accordingly, treatment with autophagy enhancer rapamycin significantly increases autophagosome fusion with lysosome *in vitro* (35).

PS1 is a ubiquitous transmembrane protein, whose cleaved form is the catalytic subunit of γ-secretase complex, which induces the intra-membranous cleavage of APP (16). Generally, APP is firstly cleaved by β-secretase to produce β-C-terminal fragment (βCTF), which is cleaved by Presenilin 1 (PS1) to produce Aβ. Mutant PS1 is considered to contribute to AD pathogenesis by interfering cleavage of APP. Recent investigations have shown that PS1 can also decrease Aβ levels by directing βCTF degradation through autophagy (8). Moreover, PS1 is involved in the fusion of autophagsome and lysosome. Lack of phosphorylation on PS1 1 Ser367 impedes the fusion of autophagosome and lysosome in mouse brain. And then, this inhibition of autophagy reduced βCTF degradation leading to the accumulation of Aβ in the brain (7). These observations imply that PS1 could be a promising target for the treatment of AD through autophagy.

However, PS1 is a vital mediator in lysosomal turnover of autophagic substrates. PS1 is an ER chaperone to facilitate maturation and targeting of the v-ATPase V0a1 subunit to lysosomes, which is a key component in acidification and substrate degradation (60). Further investigation demonstrates that PS1 also maintains Ca^{2+} homeostasis by regulating acidification of lysosome (59). Loss of acidification leads to dysfunction of lysosome that impedes fusion of autophagosome to lysosome, thereby accumulation of autophagosomes. In addition, lysosome dysfunction causes cargo-specific deficits of axonal transport leading to AD-like neuritic dystrophy (61). Based on these observations, it is reasonable to suspect that restoring the proteolytic function of lysosome may enhance the removal of protein aggregations. In line with this notion, deletion of cystatin B, an inhibitor of lysosome cysteine protease in AD mouse models promotes the clearance of abnormal protein aggregations in lysosomal compartments (133).

Genome-wide association studies (GWAS) have identified additional proteins involved in autophagy that are also closely linked with AD, such as the phosphatidylinositol binding clathrin assembly protein (PICALM/CALM). CALM is involved in endocytic trafficking to regulate endocytosis of SNAREs that enhance autophagy to clear tau aggregations (81).

Beclin1, a key factor in autophagosome formation has been shown to be transcriptionally suppressed in AD brains (96). Under pathogenic conditions, Caspase 3, a key component in apoptosis pathway, may cleave Beclin1 protein and lead to autophagy disruption. The cleaved form of Beclin1 is therefore regarded as a common *in vitro* marker for apoptosis in AD pathogenesis (104). Another potential marker for the pathology of AD is nuclear factor erythroid derived 2 like 2 (Nrf2). In response to oxidative stress, Nrf2 could induce autophagy receptor NDP52 (51) to stimulate autophagy and remove aggregated tau proteins (43). Meanwhile, Nrf2 as a vital transcription factor can also regulate the transcription of autophagy related proteins (92).

AUTOPHAGY AND PARKINSON'S DISEASE

PD is the second most common neurodegenerative disease that is characterized by selective loss of dopamine neurons in substantia nigra pars compacta, and intracellular inclusions of Lewy body and Lewy neurites composed of α-synuclein and polyubiquitinated proteins (20). In the post-mortem brain samples of PD patients, dysfunctional lysosomes and accumulation of autophagosomes were observed in neurons (22), indicating a pathogenic role of autophagy in PD. The main component of Lewy bodies is misfolded and aggregated α-synuclein (20, 45, 123). When lysosome is inhibited, the level of α-synuclein is increased, suggesting a close link between α-synuclein degradation and autophagy. Previous studies have shown that basically all forms of α-synuclein can be degraded by autophagy (22, 37, 58), while monomeric α-synuclein is also degraded by the proteasome (126). Transcription factor EB (TFEB), a key modulator for autophagy (113), has been widely demonstrated to relieve pathology of neurodegenerative diseases. Over-expression of TFEB could decrease the damage of lysosome by inducing its biogenesis, thus ameliorating the α-synuclein

pathology (21, 49). Taken together, these results suggest an essential role of autophagy in the prevention and treatment of synucleinopathy in PD.

Mutations in leucine rich repeat kinase 2 (LRRK2) represent the most common cause of autosomal dominant form of PD (123). Over-expression of LRRK2 G2019S mutation in differentiated SH-SY5Y cells results in shortening dentric and autophagosomes aggregation (98). *In vivo* experiments have demonstrated that the up-regulation of LRRK2 G2019S impairs autophagic flux with aging (107). The VPS35 D620N mutation that causes autosomal-dominant PD destabilizes WASH complex leading to defect of autophagosome formation and compromises trafficking of autophagy protein ATG9 (137).

Besides, mutations in parkin RBR E3 ubiquitin protein ligase (PARKIN) and PTEN induced putative kinase 1 (PINK1) are the main causing factors for autosomal recessive forms of PD, accounting for 50% of familial cases in Europe (48). These two proteins coordinate mitophagy to selectively degrade mitochondria by autophagy. Damaged mitochondria are delivered and sequestered within double membrane autophagosome, ultimately cleared by autolysosome. In this process, the proteasome-mediated degradation of PINK1 is stalled in depolarized mitochondria leading to accumulated PINK1 on the mitochondrial outer membrane where it phosphorylates ubiquitin and recruits parkin. In turn, the activated parkin can ubiquitinate outer membrane proteins, which are subsequently phosphorylated by PINK1. The outcome of these linkages greatly actives parkin and elicits a positive feedback involving more ubiquitinated proteins of mitochondria (46, 56, 70, 85, 86).

GWAS has identified a few lysosome related genes associated with PD. The protein ATP13A2 involved with lysosomal ATPase, is found mutated in autosomal recessive forms of early-onset Parkinsonism (24, 100). Down-regulation of ATP13A2 results in decreased lysosomal degradation in dopaminergic neurons and accumulation of α-synuclein protein (120). Subsequently, depletion of ATP13A2 leads to ubiquitination and degradation of SYT11 that induces lysosome dysfunction and increases accumulation of mutant α-synuclein (4).

Autosomal recessive mutations in the gene *GBA* which encodes lysosomal hydrolase cause defects in autophagosome-lysosome pathway and aggregation of α-synuclein (1). Depletion of ATP6AP2 which is essential for lysosomal acidification and function, has been associated with Parkinsonism (1). Moreover, loss of VPS13C function causes mitochondrial dysfunction and lysosome dysfunction and is associated with autosomal recessive Parkinsonism (1, 63).

AUTOPHAGY AND HUNTINGTON'S DISEASE

HD, the most common polyglutamine disease, is a devastating autosomal dominant neurodegenerative disease. HD is characterized by CAG repeat tri nucleotide in the first exon of the huntingtin (*HTT*) gene which leads to polyglutamine (polyQ) expansions and pathogenic aggregation (39, 42).

Aggregated autophagosomes could be observed in HD models (68), although autophagosome formation is not affected by HD pathology. Huntingtin plays a key role in autophagosome transport. In HD models, depletion of huntingtin results in abnormal accumulation of autophagosomes with engulfed mitochondria which is indicative of impaired cargo degradation (140).

In addition, there are aberrant interactions between autophagy and onset of HD. One polymorphism in the Atg7 is associated with an earlier onset form of HD (75). Beclin1 could reduce *HTT* mRNA level with aging (115). Dysfunction of loading into autophagosomes has been observed in cellular and animal models of HD, causing an impaired autophagic protein degradation despite of increased autophagic vesicles contents. The autophagy selective substrate p62/SQSTM1 is commonly treated as a crucial marker for autophagic flux especially in the cargo-recognition machinery which transports substrates to autophagosomes. Deficiency in such machinery is prevalent in HD models (68). Moreover, up-regulation of casein kinase 2 (CK2) which phosphorylates p62/SQSRM1 reduces large inclusion formation of mutant huntingtin (71).

Compared with mutant HTT, non-mutant HTT seems to coordinate with autophagy in a different manner. For example, wild-type HTT can bind p62 to enhance its role in autophagy and interact with ULK1 to evoke autophagy. In addition, Atg11 shares resemble structure with HTT to play a role in autophagosome formation. On the contrary, knock-out of dynein reveals increased levels of autophagosomes and impaired autolysosomes, accompanied with increased aggregation of mutant huntingtin (101).

AUTOPHAGY AND ALS

Amyotrophic lateral sclerosis (ALS) is a paralytic and fatal disease characterized by selective loss of motor neurons in brain and spinal cord giving rise to muscle weakness and atrophy. Most cases in ALS are sporadic, while the familial form accounts for approximately 10%. Mutations in chromosome 9 open reading frame 72 (C9ORF72), superoxide dismutase 1 (SOD1), TDP-43 and fused in sarcoma/translated in lip sarcoma (FUS/TLS) are common causes for familial type of ALS (94).

Several reports have demonstrated that autophagy is associated with ALS. Immunostaining experiments in transgenic mice with mutant *SOD1* G93A have shown that autophagy is activated (82). The aggregated autophagosomes in cytoplasm indicate that autophagy is activated in degenerated motor neurons in ALS cases (82). Notably, excess autophagosomes and autolysosomes are closely associated with p62/SQSTM1 positive inclusions, suggesting an impaired cargo digestion in lysosome (112). Other studies have shown that increased autophagosomes are tightly related with the decreased phosphorylation of mTOR in numerous genetic ALS models (82).

Growing evidence has shown that mutations in autophagy-related proteins are closely associated with the onset of ALS. Earlier studies have indicated that depletion in subunits of endosomal sorting complexes required for transport (ESCRT) causes abnormal multivesicular bodies (MVBs) with autophagosomes and is considered to be associated with ALS (29). In addition, mutations in ESCRT subunit charged multi vesicular body protein-2B (CHMP2B) are found in patients with ALS which impair ESCRT function leading to accumulation of ubiquitinated proteins and p62 (29). Autophagy receptor p62/SQSTM1 which binds both LC3 and ubiquitin to target ubiquitinated substrates to autophagosomes has been involved in ALS cases. Clearance of mutant SOD1 via ubiquitin proteasome system or autophagy is coordinated by p62/SQSTM1. Similarly, over-

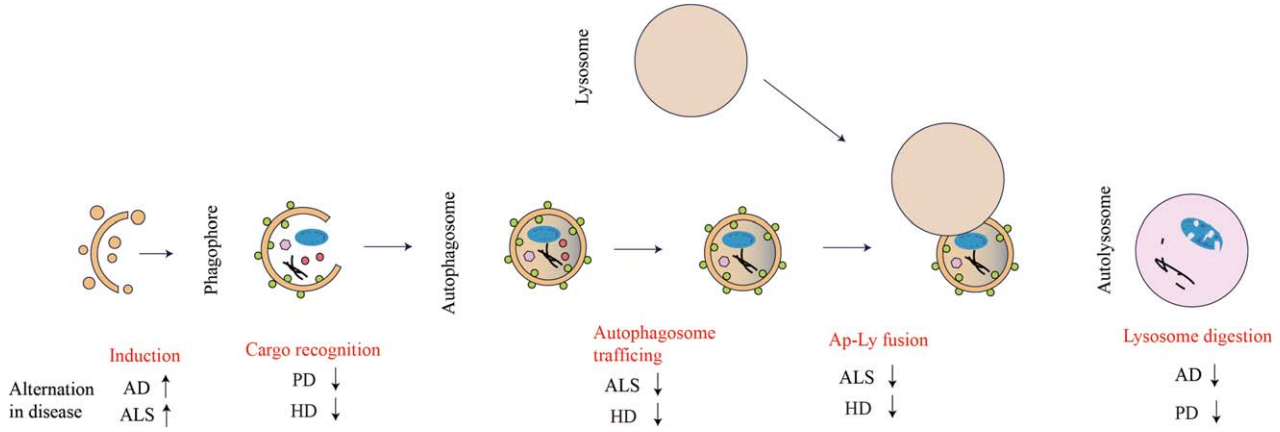

Figure 2. Overview of autophagic flux and impaired states in neurodegenerative diseases. This schematic diagram shows the procedures through the autophagic flux from formation of the autophagosome to fusion with the lysosome. Red text highlights refer to the dysfunctional steps in autophagy, along with related neurodegenerative diseases. Arrows' directions stand for activation or inhibition.

expression of p62/SQSTM1 could reduce TDP-43 aggregation via autophagy or proteasome *in vitro* (6).

Moreover, multi groups have identified a link between serine/threonine kinase TANK-binding kinase 1(TBK1) and ALS (52). One recent study has shown that TBK1 is the upstream regulator of autophagy receptor optineurin (OPTN) (80). Both TBK1 and OPTN play key roles in mitophagy (129). Since mitochondria are the place for not only generating energy but also executing cellular apoptosis, clearance of damaged mitochondria is essential for cellular homeostasis. These investigations suggest mitophagy as a new etiology of ALS. *Ubiquilin2 (UBQLN2)*, a proteasome shuttle factor, plays a key role in formation of autophagosome. Mutations in *UBQLN2* lead to cognitive deficits, shortened lifespan and neuron loss in mouse models (23, 57). A detailed illustration of alternations in neurodegenerative diseases in autophagic flux is shown in Figure 2.

AUTOPHAGY AS A THERAPEUTIC TARGET FOR NEURODEGENERATIVE DISEASES

Links between autophagy and neurodegenerative diseases promote an intriguing question: whether the modulation of autophagy could slow down disease progression. Emerging evidence has shown that autophagy enhancement could efficiently ameliorate neuropathology and neurodegeneration via either an mTOR-dependent or -independent pathway. Thus various reagents targeting for autophagy have been investigated (102, 116, 127).

Autophagy is activated by diverse signaling classified as mammalian target of rapamycin (mTOR) dependent and mTOR independent pathway (Figure 3).

mTOR dependent pathway

The serine/threonine protein kinase mTOR is a core component of two distinct complexes, mTOR complex 1 (mTORC1) and mTORC2. mTORC1 negatively regulates autophagy, while mTORC2 does it in an opposite way (32). Under normal conditions, autophagy is suppressed by mTOR. Since mTORC1 phosphorylates and inhibits core autophagy complex composed of ULK1, Atg13 and FIP200. Rapamycin interacts with immunophilin FK506-binding protein (FKBP12) to form a complex which inhibits the kinase activity of mTORC1, thus inducing autophagy (11, 66). Additionally, some drugs indirectly target mTOR such as Nilotinib, which could stimulate AMPK pathway in mTOR dependent manner to induce autophagy (136).

mTOR independent pathway

Besides autophagy, mTOR-dependent pathway plays a wide role among several other biology reactions. To avoid potential adverse effects induced by mTOR multiple function, studies on mTOR-independent pathway are increasingly emphasized. For example, more and more pharmacological drugs have been screened for regulating autophagy and have been shown to influence diverse signaling pathways including calcium flux, inositol phosphates and epigenetics.

Lithium decreases the level of Inositol 3-phosphates (IP3), which is a second messenger binding to its receptor on the ER leading to Ca^{2+} release into cytoplasm. Rilmenidine reduces level of cAMP (110) which could bind IP_3 to regulate intracytosolic Ca^{2+} levels (10). Resveratrol could active autophagy via epigenetic mechanisms (124). Nilotinib can upregulate AMPK pathway to active autophagy (37, 65). Although great achievements have been made in the discovering of novel mTOR-independent autophagy modulators, till now, the detailed mechanisms underlying autophagy regulating effects of these molecules remains exclusive and efforts are still needed for their clinical application.

Alzheimer's disease

Previous studies have reported that the application of rapamycin can reduce fibrillary tangles and amyloid plaques in brains and rescue cognitive deficits (5, 9, 67). Rapamycin analogue temsirolimus also shows similar effects in AD mouse models (41). Arctigenin, a natural product from Arctium lappa, can inhibit Aβ production and promote Aβ clearance by activating autophagy through inhibiting AKT/mTOR signaling (141). Latrepirdine is a pro-neurogenic compound that reduces accumulation of Aβ42 by stimulating autophagy (117). GTM-1, a novel small molecule, can attenuate Aβ

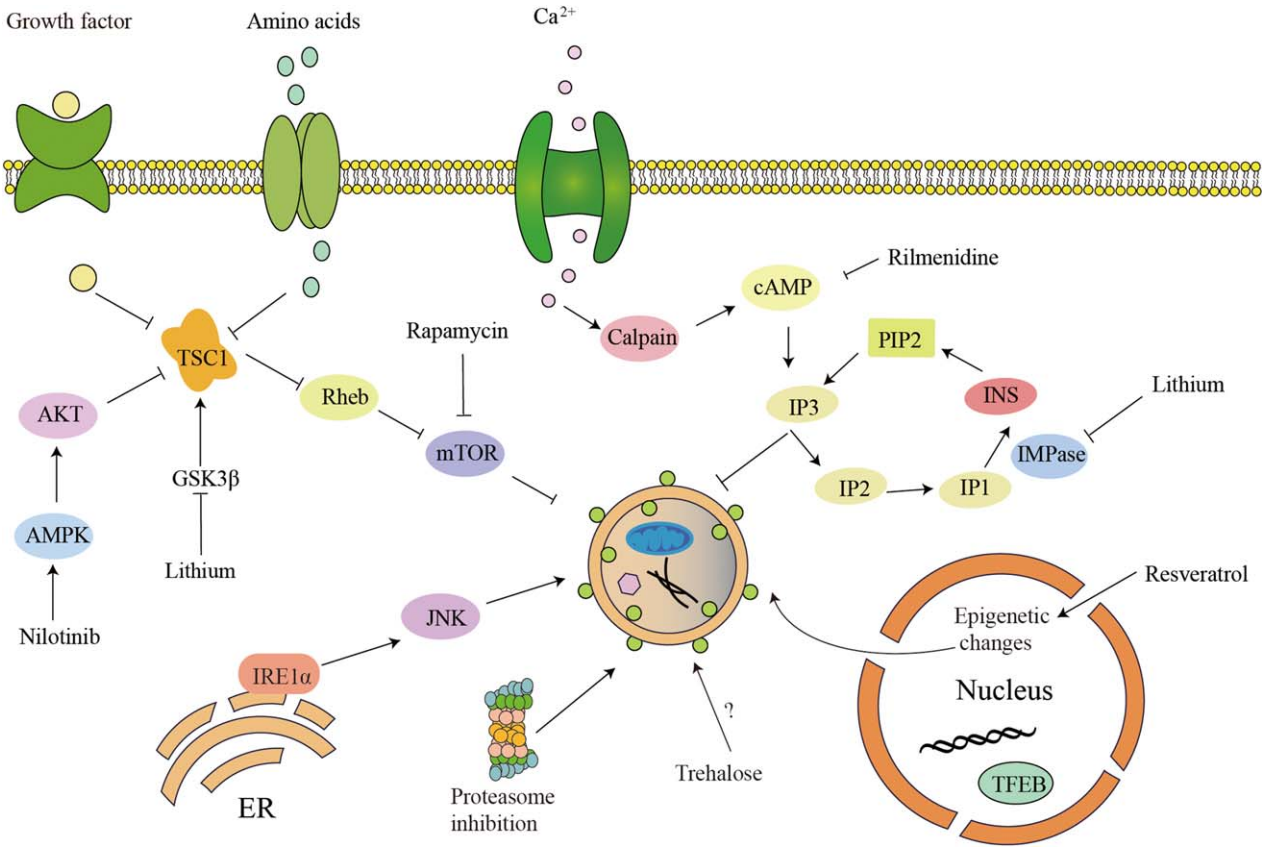

Figure 3. Molecular targets of autophagy up-regulating agents. This schematic diagram shows representative molecular agents involved in autophagy activation through regulating autophagy-related pathways. Either the mTOR-dependent or mTOR-independent pathway could play a negative role in autophagy. In addition, suppression of these pathways will result in activation of autophagy.

oligomer-induced neurotoxicity via inducing autophagy in an mTOR-independent manner (18). Nilotinib, a tyrosine kinase inhibitor, can enhance interaction of parkin and Beclin1 that lead to clearance of Aβ (65). Notably, it also plays a clearance role in PD-related parkin mutant models (37). Trehalose, a natural disaccharide, is beneficial for removing abnormal proteins. It has been demonstrated to reduce accumulation of Aβ (103). Trehalose rescues the learning impairment by reducing Aβ deposits in APP/PS1 mice (25). Since trehalose is free of toxic effects at high concentrations suggesting a promising prospect for clinical applications in human tauopathies.

Parkinson's disease

Resveratrol induces autophagy via AMPK/SIRT1 pathway to protect neurons form rotenone induced toxicity *in vitro* (83). Administration of Nilotinib contributes to clearing α-synuclein aggregation via autophagy, and rescues dopaminergic neuron loss (37). Notably, in proteasome inhibition-induced mouse models, proteasome dysfunction leads to activation of autophagy that serves a compensatory mechanism to clear protein aggregation and decrease cell death (34). Further enhancement of autophagy by pharmacological drugs or molecular inhibitors can attain similar effects. Trehalose contributes to reducing α-synuclein mutants *in vitro* (109). In addition, trehalose increases the number of dopamine neurons and the dopaminergic activity in the midbrain in PD mouse models (103). Lithium facilitates clearance of mutant α-synuclein *in vitro* (28).

Huntington's disease

Rapamycin reduces huntingtin accumulation and cell death in cell models of HD (102, 111). Lithium could partially rescue cell death (12, 110). Trehalose could bind expanded polyglutamine to delay pathology in HD mouse models (109, 119). Rilmenidine could enhance autophagy to remove mutant huntingtin fragments in cell models via mTOR independent pathway (105). Lithium could reduce mutant huntingtin protein aggregates and cell death (30).

Amyotrophic lateral sclerosis

Interestingly, rapamycin plays two opposite roles in ALS animal models. For example, rapamycin treatment in SOD1G93A mouse models further augments motor neuronal degeneration and lead to more death of ALS mice (139). However, in mutant TDP-43 models, rapamycin treatment decreases pathology of ALS (125). These contradictory findings may be because of different pathogenic proteins overexpressed and their different impact on autophagy in the two animal models of ALS. Further studies have demonstrated that rapamycin administration impairs autophagic flux, although it significantly increases the number of autophagosomes in mutant

SOD1 models. Trehalose could induce autophagy via mTOR independent pathway and significantly decrease SOD1 aggregation, reduce ubiquitinated protein accumulation in the motor neurons of SOD1 mice (13, 138).

In addition, developing novel chemicals to modulate autophagy reveals a promising prospect. For example, single-walled carbon nanotubes (SWNT) restore normal autophagy by reversing abnormal activation of mTOR signaling and deficits in lysosomal proteolysis, thereby facilitates elimination of autophagic substrates. These findings suggest that SWNT could serve as a novel neuroprotective approach to AD therapy (131).

AUTOPHAGY IN CLINICAL DIAGNOSIS OF NEURODEGENERATIVE DISEASES

Recently, emerging evidence in clinics implies that autophagy is in close association with neurodegenerative diseases. Biochemical analyses show the dramatic increase of the autophagosome marker LC3 in postmortem brains of AD patients and confirm its co-localization with hyperphosphorylated tau (97). Besides, some proteins known to regulate autophagy have been newly implicated with the pathogenesis of AD. In postmortem brains of AD patients, increase of tetraspanin impedes the fusion of autophagosome with lysosome, which leads to the abnormal accumulation of APP (33). Another study shows that decrease of immunophilin FKBP52 (FK506-Binding Protein of MW $\sim$ 52 kDa), a protein co-localized with lysosome, is accompanied with accumulation of neurofibrillary tangles (72). In postmortem brains of PD patients, expression of toll-like receptor 2 (TLR2) is elevated in neurons and spatially correlated with the pathological α-synuclein aggregation and increase of autophagy receptor SQSTM1 (26). Aberrant alternation of LAMP2, which is a significant marker in lysosome, is closely related with the early pathology of PD (84). These findings suggest that autophagy is commonly aberrant in neurodegenerative diseases. However, further studies are still required to explore specific autophagic pathways or signaling involved in different types or sub-types of neurodegenerative diseases.

CONCLUDING REMARKS

Overall, increasing evidence indicates dysregulated autophagy plays a key role in the pathogenesis of neurodegenerative diseases, and implies potential therapeutic strategies to ameliorate neurodegenerative diseases through regulating autophagy. However, mechanisms of autophagy regulation on proteostasis and general metabolism remain to be further investigated, especially through linking the interplay between specific proteins involved in autophagy and progression of diseases. Furthermore, other resident cells in the brain such as microglia might also be involved in the process of autophagy. It remains largely unknown whether and how microglia cooperates with neurons and non-neuronal cells to regulate autophagy. As to autophagy-inducing agents, treatment dose and duration should be carefully chosen and examined, as overactivation of autophagy could result in detrimental effects in accelerating the progression of neurodegenerative diseases.

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