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Autophagy in Alzheimer's Disease

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Abstract

Autophagy is a vesicle and lysosome-mediated degradative pathway that is essential for protein homeostasis and cell health. In particular, compared to non-neuronal cells, neurons are dependent on high basal autophagy for survival.

There is emerging agreement that defects in autophagy are likely to contribute to the neurodegenerative processes in numerous diseases, including Alzheimer's disease (AD).

Autophagy-lysosome defects occur early in the pathogenesis of AD and have been proposed to be a significant contributor to the disease process. Given the fact that autophagy deficits are likely major contributors to the etiology of AD, the focus of this review will be on recent studies which support a role for autophagy deficits in AD.

Keywords

Alzheimer's disease; Autophagy; Degradative pathway; Lysosome defects

Introduction

Alzheimer's disease (AD) is the most common form of dementia which impacts more than 35 million people worldwide. The incidence of the disease doubles every 5 years after 65 years of age, with the diagnosis of 1,275 new cases per year per 100,000 persons older than 65 years of age [1]. This disease involves a progressive loss of synapses in the cerebral cortex and hippocampus leading to impaired memory and deterioration of cognitive functions [2, 3]. Although extracellular aggregates of the peptide A β (the amyloid plaques) and intraneuronal accumulations of abnormally processed tau (the neurofibrillary tangles)

define AD, it is clear that deficits in the autophagy-lysosomal pathway likely precede the formation of these pathological hallmarks [4]. Deficits in this pathway manifest as defective autophagy, the major degradative pathway of neurons [5], which likely contributes to the formation of the classic AD hallmarks of amyloid plaques and neurofibrillary tangles and the resulting cognitive decline. Given the fact that autophagy deficits are likely major contributors to the etiology of AD, the focus of this review will be on recent studies which support a role for autophagy deficits in AD and suggest possible sites of therapeutic intervention. We will begin with an overview of normal functions of autophagy, the molecular pathways involved in this process and factors related to reduction and induction of autophagy prior to discussing the role of defects in autophagy in AD pathology.

Normal functions of autophagy

The autophagy–lysosomal system is a complex chain of events leading to clearance of misfolded or damaged proteins and dysfunctional organelles, as well as contributing to membrane biogenesis and vesicular transport [6, 7]. This cellular machinery is induced by a variety of signals including nutrient starvation, oxidative stress, and neuronal excitotoxicity [6, 8, 9]. Via this process, cells can degrade damaged or unessential components and restore substrates for energy and cellular remodeling, which maintains cellular homeostasis [8]. This process is essential to clear long-lived proteins, aggregated protein, and damaged organelles [9].

There are three forms of autophagy:

- (a) Chaperone-mediated autophagy (CMA) involves cytoplasmic proteins being selectively delivered into the lysosome through recognition of specific motifs that are biochemically related to the pentapeptide KFERQ. First, CMA substrates are recognized in the cytoplasm by the heat shock cognate protein of 70 KD (hsc70). The chaperone–KFERQ-containing protein complex then binds LAMP (lysosome-associated membrane protein)-2A receptors on the lysosomal membrane, and translocates the target proteins into the lysosomes for degradation [10–12] (reviewed in [11–13]);
- (b) Microautophagy in which the cytoplasmic components are directly engulfed by the lysosome (reviewed in [14]);
- (c) Macroautophagy in which hierarchically ordered activity of autophagy-related (ATG) proteins leads to the cytoplasmic components being engulfed by autophagy vacuoles (AV) and degraded by proteases after fusion with lysosomes [8, 15, 16]. In addition to this bulk autophagy pathway, cargos can be selectively targeted to the autophagy machinery by specific autophagy receptor [17].

Macroautophagy, which we will refer to by the general term autophagy, is the focus of this review. It is considered the predominant contributor to the overall autophagy rate under most conditions. Its role as a causative or protective factor and even as a consequence of AD process is not clear yet.

Stages of autophagy

The initial step of autophagy is the appearance of a small membrane sac followed by its elongation. The elongated membrane, called the isolation membrane/phagophore, engulfs part of the cytoplasm and becomes a double membrane structure of multiple proteins, including microtubule-associated light chain 3-II (LC3-II), termed the autophagosome, through the fusion of the two ends of the membrane [4, 6, 8].

The maturation processes of the autophagosome includes fusion with lysosomes or other mature autophagic vacuoles which results in acidification and acquisition of proteolytic enzymes that degrade the inner membrane of the autophagosome, together with the contents. Additionally, endocytosed constituents can enter the autophagic pathway when endosomes fuse with autophagosomes to generate an amphisome [6, 8]. The stages are shown in Figure 1.

Most of the steps in this process require the cytoskeletal network proteins such as dynein and LC3-II (an autophagosomal membrane protein and a marker of autophagosomes [18]) which are associated with microtubule transport[8]. Although autophagic pathways appear to non-selectively engulf cytosolic molecules, they can also selectively trap certain cargoes by the association between LC3 and a cargo receptor such as p62, NRB1 (neighbor of BRCA1 (breast cancer early-onset 1)) or NDP52 (nuclear dot protein 52) [19-21].

P62 and NRB1 are both multifunctional proteins that are highly interact with ubiquitinated and misfolded proteins and the ATG8 family [19, 20, 22] which is critical for autophagosomal formation [18]. There is less information about NDP52. This molecule functions as an autophagic adaptor for the selective degradation of TRIF (Toll/IL-1 receptor homology domain-containing adaptor inducing IFN- β) and TRAF6 (TNF receptor-associated factor 6) in the periphery [23] and it was suggested that it plays a role in autophagosome maturation by docking with myosin VI together with T6BP and optineurin [24]. Recent studies indicated that NDP52 is a downstream facilitator of Nrf2 (nuclear factor erythroid 2-related factor 2)-mediated phosphorylated tau degradation by autophagy [21].

Regulation of autophagy

mTOR dependent signaling pathway

mTOR is kinase that is activated in the presence of growth factors and abundant cellular nutrients such as amino acids and inhibits the initiation step of autophagy by phosphorylation of ATG13, preventing its association with Unc-51-like kinase (ULK) and focal adhesion kinase family interacting protein of 200 kD (FIP200) recruitment. Starvation results in mTOR inhibition that activates phosphatases and partial dephosphorylation of ATG13 leading to autophagosomal formation [16, 25].

mTORC1 (mammalian target of rapamycin complex 1) is a complex that mediates the classic functions of mTOR [26, 27]. Recently, the Rag GTPases, which are also members of the Ras family of GTP-binding proteins, were shown to be amino acid-specific regulators of the mTORC1 pathway. Amino acids promote the translocation of mTORC1—in a Rag-

dependent fashion—to the lysosomal surface [16, 28]. MTORC1 regulate cell growth by coordination of upstream signals from growth factors. The tuberous sclerosis complex 1 (TSC1) and TSC2 proteins form a tumor suppressor complex that transmits growth factor and energy signals to mTORC1 by regulating the GTP-loading state of Rheb, a Ras-related GTP binding protein. When bound to GTP, Rheb interacts with and activates mTORC1 [28]. Upon growth factor depletion, the p110 β catalytic subunit of the class IA phosphoinositide 3-kinases (PI3Ks) dissociates from growth factor receptor complexes and increases its interaction with RAB5 (a small GTPase that is typically associated with early endosomes). RAB5 has been demonstrated to activate vacuolar protein sorting 34 (Vps34) and modulation of ATG5-ATG12 conjugation and autophagosome synthesis [29, 30].

MTORC1 blocks autophagy at the protein level. It directly phosphorylates and inhibits key components of ULK1/ULK2 complex. Also, by promoting the sequestration of TFEB (transcription factor EB) in the cytosol, modulates autophagy at the transcriptional level [31].

The activation of TFEB is regulated by mTORC1 and contributes to synchronize the two major cellular degradative systems, autophagy and lysosomes by upregulation of several proteins implicated in the formation of autophagosomes as well as proteins required for the fusion between autophagosomes and lysosomes [32]. Also, mTORC1 regulates TFE3 and ZKSCAN3 which are positive and negative regulators of lysosomal biogenesis and autophagy, respectively [33, 34].

Additional molecules involved in macroautophagy regulation include 5' AMP-activated protein kinase (AMPK) [35] and death-associated protein kinase (DAPK) [36]. Activation of AMPK decreases mTOR signaling activity to facilitate autophagy and promotes lysosomal degradation of A β . Additionally, increase in cytosolic Ca²⁺ can induce autophagy by inhibition of mTOR through the CaMKK β (Ca(2+)/CaM-dependent protein kinase kinase β)-mediated activation of AMPK [35, 37]. DAPK is a positive mediator of cell death and a bona fide tumor suppressor. Oxidative stress induces DAPK activation and under these stress conditions DAPK activates PKD (protein kinase D), which induces the formation of both autophagosomes and autolysosomes- through phosphorylation [36, 38].

mTOR independent signaling pathway

Although the canonical mTor dependent autophagy pathway is the most well studied, autophagy can be initiated by mTor independent pathways and be mediated through non-canonical signaling events as well. Here we will highlight a few examples of these alternative pathways.

Ca²⁺ signaling has been linked to canonical and non-canonical, mTOR-independent autophagy [39, 40]. Ca²⁺ signals that mediate by IP3 receptor were reported to inhibit autophagy [41, 42]. In contrast, an increase in cytosolic Ca²⁺ enhanced autophagy [43, 44].

Lithium (Li⁺) has also been reported to stimulate autophagy in an mTOR-independent manner by inhibiting inositol monophosphatase (IMPase). Li⁺ activation of autophagy could be reversed by manipulations that raised cytoplasmic IP3 levels, implicating

phosphatidylinositol signaling in the regulation of autophagy. Importantly, Li^+ was without effect on mTORC1 activity, suggesting that phosphatidylinositol regulation of autophagy was mediated by a non-canonical pathway that operated additively and independently of mTOR [39].

ATG5 and ATG7 are believed to be essential genes for autophagy, however Nishida et al. [45] showed that mouse cells lacking ATG5 or ATG7 were still able to form autophagosomes/autolysosomes resulting in autophagy-mediated protein degradation when subjected to certain stressors. Interestingly, formation of LC3-II by LC3 lipidation did not occur during this process. This alternative process of autophagy was regulated by several autophagic proteins, including Ulk-1 and Beclin1. Additionally, unlike conventional autophagy, autophagosomes seemed to be generated in a RAB9-dependent manner by the fusion of isolation membranes with vesicles derived from the trans-Golgi and late endosomes [45, 46].

Trehalose, a disaccharide present in many non-mammalian species, has been shown to activate autophagy in an mTOR independent manner [47]. In vivo studies have provided evidence that trehalose reduced pathology in a tauopathy mouse model [48] and in an amyotrophic lateral sclerosis mouse model where mutant SOD1 is expressed [49]. Interestingly in the ALS mouse model where trehalose had beneficial effects, rapamycin, a classical activator of mTOR-dependent autophagy, actually accelerated motor neuron degeneration and shortened the lifespan of the mice [50]. These studies suggest that in addition to increasing autophagy rapamycin likely affects other signaling pathways that may negatively impact neuronal survival, a caveat that needs to be considered when pursuing therapeutic strategies which are directed towards increasing autophagy [50].

Presenilins as autophagy modulators

Presenilin 1 (PS1) and PS2 are multi-pass membrane proteins that serve as the catalytic center of γ -secretase which mediates intra-membrane cleavage of over 20 known substrates, including amyloid precursor protein (APP). These ubiquitous trans-membrane proteins have diverse biological roles in wnt signaling pathway, calcium channel regulation, cell adhesion, apoptosis, neurite outgrowth and synaptic plasticity [51, 52] (reviewed in [53]). There is now evidence that PSs may also play a role in mediating autophagy. Knockdown of PSs show an inefficient clearance of long-lived proteins. It was also shown that γ -secretase inhibitors do not adversely impact autophagy, suggesting that the role of PS in autophagy is independent of γ -secretase activity [54]. Wilson et al. found that autophagic-lysosomes increase and selectively accumulate α - and β -synuclein in PS1 $-/-$ neurons, implicating PS1 deficiencies in mechanisms of α - and β -synuclein trafficking and degradation which could play a role in α -synuclein lesions in neurodegenerative diseases such as familial AD [55] and Parkinson's disease [56].

Lee et al. [57] reported that loss of PS1 activity lead to impairments in lysosomal function as a consequence of not properly acidified lysosomes, caused by failed maturation of the proton trans-locating V0a1 subunit of the vacuolar (H^+)-ATPase and targeting to the lysosome. Their finding of impaired autolysosomes in PS1-deficient cells was consistent with other

studies [54, 55, 58, 59]. Dobrowolski et al. confirmed LysoTracker findings of their study [59]. However, not all studies are in agreement, for example Neely et al. did not observe a deficit in lysosomal acidification in PS double knockout (DKO) MEF cells and PS1 KO primary neurons using LysoTracker red [54]. Recent studies emphasize that LysoTracker is not a substitute for direct measurement of lysosomal acidification [60]. Above all, lysosome acidification deficits detected by direct measurement of lysosome pH [60, 61] confirmed the earlier Lee et al. [57] findings. Zhang et al. reexamined these issues in mammalian cells and in brains of mice lacking both PS1 and 2, as well as just PS1 and have been unable to find evidence that the turnover of autophagic substrates, vesicle pH, V0a1 maturation or lysosome function is altered compared to wild-type counterparts. According to their transcriptome studies, using PS conditional knockout mice, they concluded PS has role in regulating lysosomal biogenesis but not in cellular autophagosomal regulation [62]. They could not find changes reported by others because conditional partial deletion of PS, specifically in neurons resulted in measurement of elements that are present in all cells including the more abundant glia which may not be a sensitive approach to detecting changes in a subpopulation of affected neurons. Several potential technical issues that may be responsible for discrepancies between these recent studies and experiments of Lee et al. [57, 63] are using different methods to measure pH and performing biochemical analysis of the V0a1 N-glycosylation state and subcellular localization using different cellular sources. Nonetheless, as a whole, the evidence indicates that PSs likely play a role in mediating degradative processes, although further studies are required to clearly define the mechanisms by which PSs affects these processes in these different cellular contexts.

Tung et al. found that cells deficient in PS1 exhibit reduced levels of p62 protein which is a cargo-receptor shuttling tau for degradation. The down regulation of PS1 mediated through an Akt/AP-1 pathway led to a significant decrease in both the protein and mRNA transcript of p62, concomitant with attenuated p62 promoter activity. Their study suggests a novel function for PS1 in modulating p62 expression to control the proteostasis of tau [64].

APP processing and autophagic dysfunction

As a result of aging and metabolic or oxidative stress, indigestible or incompletely degraded materials accumulate in a family of mature lysosomal compartments which includes dense bodies, multivesicular bodies, and autophagic vacuoles (AVs) [65, 66]. AVs contain the components for A β generation, APP, and its processing enzymes, BACE and PS [67, 68].

Deficiency of Beclin1 in cultured neurons and transgenic mice results in increases in APP, A β and the C-terminal fragment (CTF), whereas its over expression reduces the accumulation of A β [69-71]. Beclin1 levels are reduced in affected gray matter of patients with early stages of AD [71]. In transgenic mice that express human APP, genetic reduction of Beclin1 expression increased intraneuronal A β accumulation, extracellular A β deposition, and neurodegeneration. It also caused microglial changes and profound neuronal ultrastructural abnormalities. Administration of a lentiviral vector expressing Beclin1 reduced both intracellular and extracellular amyloid pathology in APP transgenic mice. From these results it was concluded that Beclin1 deficiency disrupts neuronal autophagy,

impairs A β clearance by lysosomes, modulates APP metabolism, and promotes neurodegeneration in the mice [71].

Additionally, A β could be a regulator of autophagy. Intracellular A β could modulate this process by an Akt-dependent pathway, RAGE-calcium-CaMKK β -AMPK pathway or induction of mitochondrial reactive oxygen species (ROS) generation, thus act as an intrinsic checkpoint of its own homeostasis [72-74].

Several studies determined autophagy as a potential therapeutic target to ameliorate neuronal pathogenesis in AD:

Rapamycin, an mTOR inhibitor and an autophagy inducer, is able to reduce A β and Tau pathology and improve cognition [75, 76], only when treatment begin prior to the onset of widespread plaque and tangle accumulation [77]. Also, autophagy induction is necessary for rapamycin's effects on A β [78].

Lysosomal proteases play crucial roles in regulating and executing several steps in the autophagic pathway. The cathepsin lysosomal acidic proteases participate directly in lysosomal substrate clearance by degrading vesicular content [79]. Genetic ablation of cystatin B or treatment with lysosomal protease inhibitors enhanced lysosomal activities and ameliorated amyloid pathologies and memory deficits in an AD mouse model [80, 81]. These data is in consistent with Lee et al. [57] findings that indicted impaired fusion between autophagosomes and lysosomes during the autophagic process and acidification in AD.

Steele and coworkers provided evidence that in cultured cells, the antihistamine latrepirdine (Dimebon) stimulated mTOR- and ATG5-dependent autophagy. Chronic administration of latrepirdine to TgCRND8 (APP K670M, N671L, V717F) attenuated the behavioral deficits, and reduced A β neuropathology [66]. Their work in the current article support the notion of Oddo et al. through evidence that autophagic failure occurs as a delayed pathology following the deposition of A β in a mouse model with intact murine PS1. Nonetheless, latrepirdine failed in Phase III clinical trials as a treatment for AD [82].

Zhu et al. reported that arctigenin (a natural product from *Arctium lappa* (L.)) can both inhibit A β production by suppressing BACE1 expression and promote A β clearance by enhancing autophagy through mTOR s inhibition and AMPK/Raptor pathway activation in cells and APP/PS1 transgenic AD model mice. In this study, arctigenin treatment of the APP/PS1 transgenic mice significantly decreased senile plaques and efficiently reduced the memory impairment [83]. This might be the first reported natural product able to both inhibit A β production and promote its clearance.

Recently, Shin et al. [84] investigated whether mesenchymal stem cells (MSCs) could enhance autophagy and therefore exert a neuroprotective effect through modulation of A β clearance in A β -treated neuronal cells. Administration of MSC to A β -treated animals upregulated BECN1/Beclin 1 expression and significantly increased autophagosome induction, maturation of late AVs, and fusion with lysosomes in addition to significantly reducing A β levels in the hippocampus in the A β -treated mice, concomitant with increased

survival of hippocampal neurons. Two other studies showed that transplantation of Human umbilical cord-derived MSCs activate M2-like microglia which associate with improved cognitive function and A β deposition reduction in A β PP/PS1 transgenic AD mouse model [85, 86]. Figure1 addresses some potential therapies and their site of action.

The aforementioned information suggest that we need therapeutic approaches that can enhance lysosomal activity and/or autophagosome/lysosome fusion, besides to targeting the autophagic pathway before the onset of autophagic or lysosomal failure [57, 77]. Additionally, agents with dual-functional activities in both A β production inhibition and upgrading its clearance might demonstrate more potent anti-AD properties [83].

Tau and autophagic dysfunction

The two major pathways involved in tau metabolism under physiological and pathological conditions are autophagy-lysosome and ubiquitin–proteasome systems (UPS) [87, 88]. There is a long time that UPS considered as a critical route for degradation of short lived nuclear and cytosolic proteins such as tau, recently it has been well-established that tau can be cleared by autophagy (for a review see: [89]) [88]. Autophagy but not the UPS can degrade protein aggregates [88]. Impairment of both pathways have been described in AD [4, 90].

There are reports that have demonstrated UPS as a principal tau degradation system [91-94]. Even so, they all investigated over expressed tau in cell lines instead of endogenous tau in primary neurons. There is evidence that endogenous tau and its over expressed form undergo different degradation pathways [95].

In an effort to elucidate the importance of autophagic degradation of tau, several groups conducted studies in different cell systems including neuronal cell lines [96-100]. Different tau fragments might be degraded through distinct autophagic pathways; the full-length molecule is preferentially degraded through macroautophagy, whereas tauRD K280 (a truncated tau containing pro-aggregated repeat domains that functions as a seed for initiating tau aggregate formation) is translocated to lysosomes through CMA machinery [97]. There are two CMA targeting motifs in the 4th repeat domain (336QVEVK340 and 347KDRVQ351) of tau which are recognized by Hsc70 and delivered to the lysosomal LAMP-2A receptor [101]. Another caspase-cleaved tau, tau C, is present in AD brains and more tending to aggregate and induce neurotoxicity than natural one. This molecule is appeared to be preferentially degraded through autophagy in a faster turnover rate than the full length tau [96]. Clearance of soluble tau species is likely to involve selective autophagy adaptor proteins to target tau to the autophagic machinery. Recent studies have provided evidence that NDP52 acts as an autophagy adaptor for tau in neurons facilitating tau degradation by autophagy [21]. Treatment of primary neurons with trehalose resulted in a reduction in tau levels via autophagy [98]. In this report, the UPS inhibited by application of proteasome inhibitor MG132 and epoxomicin. The results suggested that UPS plays no major role in tau degradation in primary neurons.

Studies from animal models also provide evidence that autophagy contributes to the clearance of tau [48, 102, 103]. The TSC1 and 2 are negative regulators of mTOR and in

TSC2+/- mice endogenous mouse tau levels and phosphorylation are increased. Further in P301LS mice treatment with rapamycin ameliorated tau pathology and the associated behavioral deficits indicating a role for autophagy in modulating tau levels [102]. Treatment of mice that over express a mutant form of tau (P301S) with trehalose resulted in increased indicators of autophagy in the brain concurrent with a decrease in the number of neurons containing tau inclusions as well as decrease in the amount of insoluble tau [48].

Methylene blue (MB), a phenothiazine derivate, was distinguished as an inhibitor of tau aggregation, in vitro [104]. It reduced the levels of 12kD tau, A β oligomer levels and amyloid deposits in mouse models [105-107]. MB was recently tested in a phase II clinical trial for AD and has shown encouraging results as significant improvement in cognitive functions was reported for patients on MB compared to patients on placebo [108]. Its mechanisms of action are still unclear and possibly depend on the active concentration. At micromolar doses, it reduces tau aggregates by direct prevention of tau-tau binding [104]. High concentration modulates Hsp70 activity which correlates with reduction of tau in cells and animal models [109, 110]. Administration of MB in a wide range of doses, resulted in increased autophagy via mTOR signaling pathway and attenuated tau pathology, in primary neurons and JNPL3 mice, which express P301L-tau [103]. These and other studies indicate that the deficits in autophagy which occur in AD, likely contribute to the accumulation of tau [89] and therefore approaches that increase autophagy should attenuate tau accumulation.

Concluding remarks

Deregulation of autophagy impairs neuronal homeostasis and the balance of energy and nutrient homeostasis which likely contribute to AD pathogenic processes. In the past decade there has been a surge in autophagy research which has significantly increased our understanding of the pathways and mechanisms involved in mediating autophagy Figure1. However our understanding is far from complete and there is still much to learn about autophagy and its role in AD. Nonetheless it appears evident that therapeutic strategies that enhance autophagy have the potential to be beneficial in AD.

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Figure 1.

AMPK: 5' AMP-activated protein kinase, mTORC1: mammalian target of rapamycin complex 1, RHEB: Ras homolog enriched in brain, TSC: tuberous sclerosis, AKT: protein kinase B, ERK: Extracellular signal-regulated kinase, PI3K: phosphoinositide 3-kinase, ULK: UNC-51-like kinase, Atg: Autophagy protein, RAB5: GTP binding protein, LC3: microtubule-associated light chain 3, MSC: mesenchymal stem cell, MB: Methylene blue.