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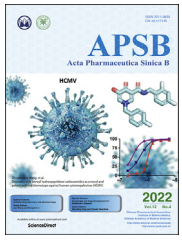

REVIEW

Pharmacological modulation of autophagy for Alzheimer's disease therapy: Opportunities and obstacles

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Abstract Alzheimer's disease (AD) is a prevalent and deleterious neurodegenerative disorder characterized by an irreversible and progressive impairment of cognitive abilities as well as the formation of amyloid β (Aβ) plaques and neurofibrillary tangles (NFTs) in the brain. By far, the precise mechanisms of AD are not fully understood and no interventions are available to effectively slow down progression of the disease. Autophagy is a conserved degradation pathway that is crucial to maintain cellular homeostasis by targeting damaged organelles, pathogens, and disease-prone protein aggregates to lysosome for degradation. Emerging evidence suggests dysfunctional autophagy clearance pathway as a potential cellular mechanism underlying the pathogenesis of AD in affected neurons. Here we summarize the current evidence for autophagy dysfunction in the pathophysiology of AD and discuss the role of autophagy in the regulation of AD-related protein degradation and neuroinflammation in neurons and glial cells. Finally, we review the autophagy modulators reported in the treatment of AD models and discuss the obstacles and opportunities for potential clinical application of the novel autophagy activators for AD therapy.

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1. Introduction

Alzheimer's disease (AD) is the most prevalent neurodegenerative disorder, which is characterized by an irreversible and progressive impairment in cognitive function and deficiency in memory abilities. The pathological hallmarks of AD are extracellular amyloid β (Aβ) plaques and intracellular neurofibrillary tangles (NFTs)[1]. The extracellular Aβ plaques are composed of Aβ peptides, which are generated through the proteolytic cleavage of the amyloid precursor protein (APP, a causative protein of familial AD) at C-termini by integral α-, β- and γ-secretases[2,3]. The number of amino acids in Aβ peptides ranges from 37 to 49[2]. However, Aβ_{42} is the most predominant toxic species and the main component of Aβ plaques in AD brain[4]. Once Aβ peptides are generated by enzymatic processing of amyloid precursor protein (APP), the species can be released into the extracellular space to form insoluble plaques as disease progresses. The intracellular NFTs are consisted mainly of the hyperphosphorylated tau which is a microtubule-associated protein and connects with tubulin to regulate the stabilization of microtubules[5]. In pathological conditions, tau protein is abnormally hyperphosphorylated by tau-related kinase and becomes misfolded and aggregated to form the intracellular NFTs[6].

Autophagy is an evolutionarily conserved catabolic process whereby organelles and proteins are degraded through lysosomes. Three major autophagic pathways have been identified: chaperone-mediated autophagy (CMA), microautophagy, and macroautophagy, which employ different cellular signaling molecules and proteins to carry out lysosomal degradation. We will focus the review on macroautophagy (henceforth referred to as autophagy) in AD.

Autophagy is controlled by mechanistic target of rapamycin complex 1 (mTORC1), which is a nutrient sensor and master regulator of metabolic cellular signaling pathways[7,8]. Under nutrient rich conditions, mTORC1 is activated, which suppresses the initiation of autophagy by phosphorylating proteins in Unc51-like kinase 1 (ULK1) complex and transcription factor EB (TFEB). Conversely, under nutrient poor conditions, the activity of mTORC1 is inhibited, causing induction of autophagy through the dephosphorylation of ULK1 complex and TFEB. Dephosphorylated TFEB will translocate into the nucleus to upregulate autophagy and lysosome associated genes in the nucleus thus promoting autophagic degradation[9]. The dephosphorylation of ULK1 activates ULK1 complex to phosphorylate Beclin1[10] and ATG14L[11], two protein subunits in the class III phosphoinositol-3-phosphate kinase VPS34 complex and thus activates VPS34 kinase activity—a critical process for the nucleation of the phagophore membrane. The phagophore then is expanded to form the isolation membrane and prompt the engulfment of cytoplasmic contents. Two ubiquitin-like conjugation systems are involved in the elongation of the isolation membrane. The first one is the covalent conjugation of ATG12 to ATG5, a process carried out by ATG7 (E1-like) and ATG10 (E2-like), to form ATG5−ATG12 complex which then binds ATG16L. The second one is the conjugation of phosphoethanolamine to LC3[12], which requires ATG7 and ATG3 (E2-like) as well as ATG5−ATG12−ATG16 complex. The lipidation of LC3 is required for the association of LC3 with the autophagosome, which is a double membrane structure and aids in cargo sequestration through various autophagy receptors[13]. Eventually autophagosomes deliver sequestrated cargoes along microtubules tracks and then fuse with lysosomes for cargo degradation[14]. Studies have identified endosomal or lysosomal proteins (*i.e.*, LAMP1, RAB7, and HOPS complex proteins) and various SNARE proteins (*i.e.*, Syntaxin17, VAMP7/8, and SNAP29) as key mediators of autophagosome-lysosome fusion[15]. Growing evidence shows that autophagy can selectively degrade cargos such as aggregated proteins and damaged mitochondria to maintain intracellular homeostasis through a family of proteins called autophagy adaptors or receptors[16–19]. Although autophagic dysfunction was implicated in the development of AD, the precise role of autophagy in the pathogenesis of the disease remains elusive.

2. Autophagy dysregulation in AD

2.1. Evidence for autophagy dysregulation in AD

In the post-mortem brain of AD patients, accumulation of autophagy vacuoles (AVs) including autophagosomes and autolysosomes has been observed, in contrast to control subjects[20–22]. AVs accumulation indicates either excessive autophagy biosynthesis or dysfunctional autophagy clearance[23]. Evidence shows that increase of autophagy activity with accumulated AVs could be a response to Aβ accumulation and promotion to the degradation of Aβ in AD brain[24]. A recent study has reported that the protein level of ATG5, a key protein in autophagosome formation, is elevated in primary rat cortical neurons with Aβ treatment and in the plasma of AD patients with dementia[25]. However, AVs accumulation could also reflect autophagy dysfunction in AD. For instance, it's reported that in the post-mortem brain of patients, accumulation of AVs is due to the impairment of endosomal-lysosomal trafficking[22]. In addition, the failure of proteolysis in lysosome, which results in the intraneuronal accumulation of AVs, has also been observed in the brain of AD mouse model[26,27]. CCT (chaperonin containing TCP-1) is required for autophagy-lysosomal activity in primary neurons[28]. Recent study identified a reduced expression of CCT in the brain of AD patients[29], implying the reduction of autophagy−lysosomal activity in AD. Apart from AV accumulation, impaired autophagosome synthesis has also been implicated in the pathogenesis of AD. A recent study has reported a decreased protein level of ATG7 in the hippocampus and cerebral of an AD mouse model[30]. These findings indicate the correlation of the impaired autophagy and AD (Fig. 1).

2.2. Mechanism for autophagy dysregulation in AD

2.2.1. Roles of AD-related genes in autophagy regulation

Presenilin-1 (*PS1*) is a causative gene to familial AD[31]. Apart from functioning in Aβ cleavage as a part of γ-secretase complex, PS1 is an ER chaperone for V0A1 which is a subunit of V-ATPase and required for lysosomal acidification[27]. Available evidence indicates that AD-associated mutations in *PS1* disrupt autophagosome degradation as a result of lysosomal V-ATPase dysfunction in AD patient-derived cells[27]. Presenilin-2 is another causative gene to familial AD[32]. Recent study has shown that mutations in presenilin-2 impair autophagy through disturbing Ca^{2+} homeostasis[33,34]. APOE4 encoded by ε4 allele of *APOE* gene is a major genetic risk factor for sporadic AD by increasing the occurrence and lowering the age of onset of AD[35,36]. Disease-

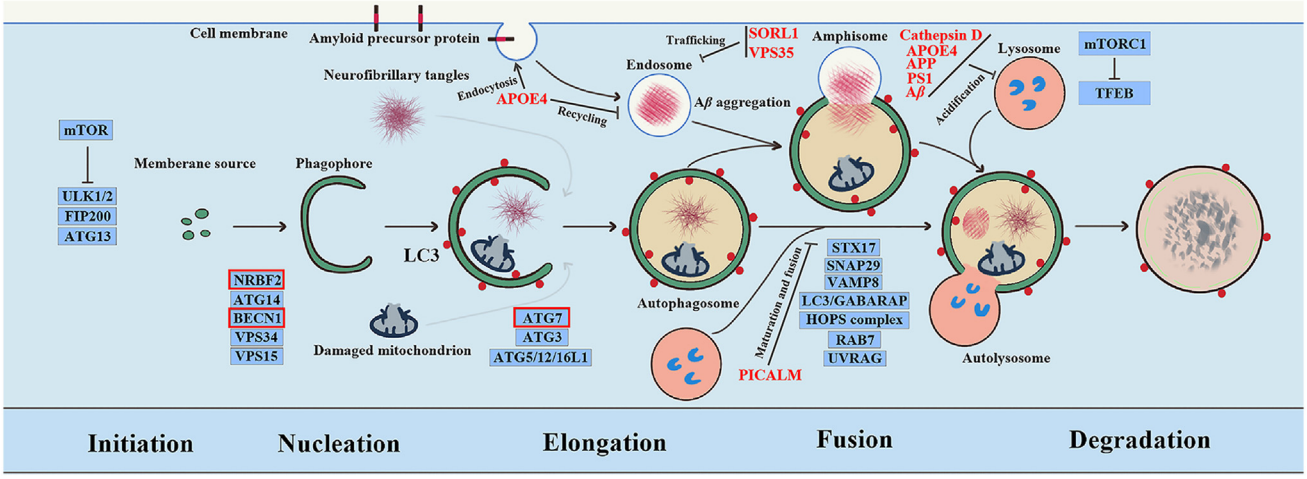

Figure 1 Autophagy process and dysfunction in AD. The autophagy process starts with the formation of an isolated membrane to sequester the protein aggregates and intracellular organelles to form a double membrane structure called phagophore. During elongation, phagophore expands to form autophagosome. Upon the autophagosome formation, it directly fuses with lysosome and endosome to generate autolysosome and endolysosome, respectively, to digest and recycle cargo. Pathogenic proteins including Aβ aggregates and hyperphosphorylated tau, as well as damaged organelles including mitochondria can be efficiently cleared by autophagy to maintain neuronal homeostasis. The autophagy proteins highlighted with red rectangular lines are those downregulated in AD models. The proteins with red color are AD-associated proteins impairing autophagy−lysosome/later endosome pathway in different steps.

related APOE4 variants and the pathogenic mutations in APP have been reported to cause the upregulation of RAB5 and endocytosis[37−40], which may result in accumulation of overloaded and dysfunctional lysosomes and consequently reduce the clearance of autophagic cargos. Indeed, accumulation of autophagy substrates have been observed in AD mouse models due to lysosomal dysfunction[22,41]. PICALM is a clathrin adaptor protein and plays crucial roles in clathrin-mediated endocytosis. The variants in *PICALM* gene are reported to be linked with AD and confer loss of function in the brain of AD patients[42−44]. Depletion of PICALM disrupts the endocytosis of VAMP proteins, such as VAMP2, VAMP3 and VAMP8, which are involved in autophagosome maturation and autophagosome−lysosome/late endosome fusion, respectively[44]. Thus, disruption of PICALM is expected to cause the impairment of both autophagosome formation and fusion with lysosomes[44].

2.2.2. *Beclin1−PI3KC3 complex dysfunction in AD*

As an autophagy regulatory protein, Beclin1 is one of the key components of PI3KC3 complex which plays an important role in the biogenesis of autophagosome[45,46]. In AD brain tissues, Beclin1 is down-regulated at both protein and messenger RNA levels, which causes the reduced autophagosome formation[47]. Moreover, in a transgenic mouse model of AD, genetic reduction of Beclin1 protein level causes intraneuronal and extracellular accumulation of Aβ species, and neurodegeneration[47]. Our recent study has found significant downregulation of multiple components of two autophagy kinase complexes Beclin1−PIK3C3 and ULK1/2-FIP200 specifically in the parahippocampal gyrus (BA36)[48]. We showed that NRBF2 is a novel component in PI3KC3 complex and the protein level of NRBF2 is reduced in both human AD parahippocampal gyrus and hippocampus of 5XFAD AD mouse model[48−50]. In addition, we demonstrated that overexpression of NRBF2 reduces Aβ species in human cells with overexpression of APP protein; conversely knockout of NRBF2 increases Aβ species in N2a cells[50]. And overexpression of NRBF2 by AAV transduction causes the reduction of Aβ species and consequently improves the memory abilities in 5XFAD AD mouse model[48]. These findings indicate the dysfunction of Beclin1−NRBF2−PI3KC3 complex in human AD brains and mouse models of AD, and importantly they suggest NRBF2 as a potential drug target in AD.

2.2.3. *Vesicle trafficking defect in AD*

Intracellular protein trafficking through endosomes plays an important role in the regulation of normal neuronal function[51,52]. Retromer is a multimodular assembly which is critical for sorting and trafficking cargos out of endosomes[53,54]. Proteins cargos from the endosomes are mainly delivered to three possible destinations: recycling to the plasma membranes, transporting to the trans-Golgi network, and sorting to the lysosomes for degradation[53]. Recent study has shown that retromer is required for the translocation of mTORC1 to lysosomal membrane upon amino acids stimulation to control mTORC1 activity and autophagy activation[55]. Previous study has indicated the retrograde transport of autophagosomes through fusion of late endosomes in neurons[56]. AD related gene mutations are associated with the trafficking dysfunction of retromer or endosome[57], which implicates the trafficking defect in AD. SORLA, encoded by *SORL1* gene, is a neuronal sorting receptor and has multiple roles in retromer-dependent trafficking, endocytic sorting and APP processing regulation[58−61]. Decreased expression of SORLA and rare truncated mutations with loss of function have been detected in AD patients[62−65]. A more recent study has validated the defects in neuronal endosomal traffic by depletion of SORLA in hiPSC-derived neurons[66]. In addition, as a core protein in retromer,

VPS35 is a master conductor of endosomal traffic. Mutations of VPS35 have been identified in AD, and VPS35 protein is deficient in regionally vulnerable AD brains[67–69]. Genetic reduction of VPS35 protein level causes an increase of Aβ levels and cognitive impairments in an AD-like amyloidosis mouse model[70,71]. Conversely, overexpression of VPS35 significantly reduces the levels of Aβ species and phosphorylated tau and ameliorates spatial learning and working memory in the triple transgenic (3×Tg) mouse model of AD[72]. A most recent study by examining cerebrospinal fluid (CSF) in AD patients with dementia and mouse with neuronal-specific knockout of VPS35 identifies the correlation of retremer-dependent endosomal trafficking defect and AD pathology[73]. Consistent with this notion, recent studies have shown that depletion of VPS35 causes an aberrant lysosome function and autophagy impairment, which subsequently leads to accumulation of cytoplasmic tau aggregates[74,75]. These studies suggest a defect in retromer-dependent trafficking in AD pathogenesis.

2.2.4. *Lysosomal dysfunction in AD*

Lysosome is the organelle that contains various proteolytic enzymes to degrade protein aggregates and other damaged organelles with high-capacity[76–78]. The routes that cargos are delivered to lysosomes include autophagy, phagocytosis and endocytosis. In mammals, maintenance of adequate lysosomal function is crucial for post-mitotic neurons[79,80]. Multiple lines of evidence have demonstrated lysosomal dysfunction in AD[22,81,82]. Aging is one of the common risk factors for the major neurodegenerative diseases including AD. It's reported that aging is accompanied by the decline of lysosomal proteolytic activity, which subsequently causes the accumulation of protein aggregates[77,83]. Aβ is produced from APP by the sequential cleavage of β-secretase-1 and γ-secretase. Aβ itself can induce the alkalinization and dysfunction of lysosome by inhibiting the nuclear translocation of TFEB and subsequently blocking the expression of osteoporosis-associated transmembrane protein 1 OSTM1, a vital protein involved in lysosome acidification in primary microglia cells[84]. Accumulation of Aβ species is one of the key pathological hallmarks of AD. While normal product of Aβ by APP metabolism serves various physiological functions, APP mutations will cause increase of Aβ production and thus induce the lysosomal dysfunction, which, in turn, results in further accumulation of Aβ species due to impaired degradation[85,86]. Recent study has shown an intraneuronal lysosomal accumulation of APP-CTFs (APP carboxyl-terminal fragments) and oligomeric Aβ species in a transgenic mouse with APP mutation (E693Q), which implicates the lysosomal dysfunction in AD mouse model[87]. Apart from APP, other AD causative proteins have been reported to associate with lysosomal dysfunction, such as APOE, Cathepsin D, CLN5, and PS1. APOE4 enhances the endocytosis of APP and BACE1, and promotes their colocalization in early endosomes while impairing endosome recycling, which subsequently causes the accumulation of APP-CTFs in endosome[88,89]. Expression of human APOE4 in the brain of mice that was subjected to neprilysin inhibitor, an Aβ-degrading enzyme, increases Aβ localization to lysosome and elevates the levels of enlarged lysosomes, which is accompanied by the loss of hippocampal CA1 neurons and pronounced cognitive deficits[90]. Previous study showed that in response to Aβ species APOE4 traffics to lysosome and causes lysosome leakage in N2a cells by forming a reactive intermediate, which potentially insert into the lysosomal membrane[91]. Recent study has shown that APOE4 disrupts lysosomal integrity and causes a leakage of Cathepsin D to cytosol[92]. Cathepsin D is a peptidase and involved in the protein degradation in lysosome. It's reported that genetic variants of Cathepsin D, which increase the risk of AD, impact the degradation capacity of lysosome[93,94]. Recent study by a whole-exome sequencing identifies a missense variant in *CLN5* in AD patients and validates a loss of function of this variant by impairing Cathepsin D maturation[95], consistent with the dysfunction of lysosome in protein degradation. It has been suggested that AD-linked *PS1* mutant increased Aβ production and caused lysosomal dysfunction by impairing the function of lysosomal V-ATPase, which is required for lysosomal acidification[27,96]. More recently, by performing an integrative gene co-expression network analyses of multi-omics profiling, Wang et al.[97] identified ATP6V1A as a key regulator in top-ranked neuronal modules which are most affected by late-onset AD. They confirmed the effects of ATP6V1A reduction in neuronal activity and motor function in *in vitro* and *in vivo* which are further compromised by adding Aβ_{42} species[97]. Previous studies have shown that knockdown of ATP6V1A inhibits drug-induced acidification of lysosome in HeLa cells and *de novo* heterozygous mutations of *ATP6V1A* cause abnormal lysosomal acidification leading to abnormalities in neuronal morphology[98,99]. The evidence highlights the involvement of lysosomal dysfunction in the development of AD.

3. Autophagy regulates AD pathology

3.1. *Neuronal autophagy regulates degradation of AD-related proteins and protects neuron survival*

Autophagy is critical for the maintenance of cellular homeostasis and functionality in neurons[100–102]. The axon degeneration and neuron death have been observed in mice with specific depletion of autophagy essential genes in neurons[103,104]. By tracking the spatiotemporal dynamic of autophagosome in each compartment of neuron with GFP-labeled autophagy protein LC3[13,105], the pathway for neuronal autophagy has been presented. The neuronal autophagosomes are predominantly produced in the distal axon where the core autophagy proteins, such as ATG5 and ATG13, are recruited to the specific sites of endoplasmic reticulum[106–109]. After formation, distal autophagosomes will fuse with later endosomes likely to facilitate the tracking to the soma[56,107,110]. As travel to the proximal axon and soma where lysosomes are enriched, autophagosomes will fuse with lysosome to form the degradative autolysosomes[107,110]. This retrograde pathway in neuronal autophagy provides a mechanism for the degradation of cytosolic and organelle cargos delivered from distal axon to the soma. One study has shown that the selective degradation of ubiquitinated substrates by neuronal autophagy plays a crucial role in axon guidance during nervous system development[111]. In addition, disruption of autophagy has been reported to be associated with the defects in axonal outgrowth[112]. These findings suggest that neuronal autophagy is critical for the maintenance of the proper connectivity of the brain.

The dysfunction of proteins involved in autophagosome formation in neurons is associated with AD pathology. In AD models, genetic reduction of Beclin1 protein level disrupts neuronal autophagy and causes intraneuronal and extracellular accumulation of Aβ species, and consequent neurodegeneration as

well as neuronal ultrastructural abnormalities[47]. Conversely, increase of Beclin1 protein level ameliorates the amyloid pathology in an APP transgenic mice[47]. In addition, our recent study demonstrates that depletion of NRBF2 causes a significant reduction of autophagy flux in hippocampus and overexpression of NRBF2 in the hippocampus *via* AAV-mediated transduction reduces β-amyloid levels in AD mouse model[48]. The role of neuronal autophagosome trafficking in AD pathology is poorly defined; however, growing evidence has demonstrated the dysfunction of retromer or endosome trafficking in neurons in AD[113,114]. A recent study has shown that neuronal-specific knockout of VPS35, a core protein of the retromer complex, in mice disrupts retremer-dependent endosomal trafficking and causes accumulation of AD-related proteins such as tau and APP[73]. Conversely, neuronal-specific overexpression of VPS35 significantly reduces the levels of phosphorylated tau and Aβ species, and ameliorates spatial learning and working memory in the triple transgenic (3×Tg) mouse model of AD[72]. In addition, a recent study has shown that pharmacological and genetic inhibition of NHE6, a primary protein involved in proton leak channel in early endosome, completely restores the APOE4-induced recycling block of APOE receptor and counteracts Aβ-induced LTP suppression in *APOE*-knockin mice[115]. These findings suggest that upregulation of autophagosome formation and retremer-dependent endosomal trafficking can ameliorate AD pathology.

A recent study has reported that pharmacological activation of autophagy facilitates the clearance of Aβ species in primary neuronal cells and thus provides neuroprotection[116]. Apart from the degradation of Aβ species, neuronal autophagy is also required for the reduction of phosphorylated tau to improve neuronal survival and function[21,117]. To support this notion, a previous study has shown that postnatal forebrain-specific knockout of *ATG7*, a core autophagy gene, causes an accumulation of phosphorylated tau in cerebral cortex and hippocampus, as well as neuron loss in hippocampus[118]. Recent study has reported that induction of autophagy in neurons by repeated ultrasound treatments promotes the clearance of neuronal tau and improves motor function in a tau transgenic mice[119]. Overexpression of BAG3, a facilitator of autophagy, attenuates the accumulation of pathological tau in primary neurons, whereas depletion of BAG3 exacerbates it[120,121]. Recently, autophagy receptors in selective autophagy have been linked to the clearance of phosphorylated tau in neurons and their overexpression can ameliorate tau pathology *in vivo*[122,123]. These findings suggest that neuronal autophagy plays a critical role in ameliorating AD pathology through promoting the degradation of AD related proteins.

3.2. *Glia autophagy regulates neuroinflammation in AD*

Neuroinflammation, an inflammatory process in brain, can be triggered in response to various stress: pathogen infection, brain injury, or toxic metabolites[124,125]. The inflammatory cytopathology is sponsored by the reactions in glia, especially microglia and astrocytes[125,126]. Microglia are the resident immune cells in the brains and comprise around 10%−15% of the overall cells in CNS. Microglia have a capacity of phagocytosis with two main states, resting and activated states[127]. In most cases, microglia keep in the resting states; however, upon pathological insult by endogenous or exogenous stimulations, microglia will modify their shapes and transform into the activated states to enable the phagocytic function and trigger inflammatory response by release of multiple cytokines and mediators[127]. It's reported that the receptors in the cell-surface including CD36, CD47 and Toll-like receptors, such as TLR4 and TLR6, are essential for the initiation of the immune response to the insulted stimulations[128,129]. The actions of microglia will control the fate of neurons around them[130].

As a detrimental factor in CNS, neuroinflammation plays a critical role in pathogenesis of various neurodegenerative diseases including AD[131]. While acute microglia activation is beneficial by reducing environmental stresses, chronic expose of microglia to the inflammatory mediators such as Aβ and cytokines will cause over-activation of microglia, leading to neuroinflammation and neurotoxicity[132−135].

Growing studies have determined the role of autophagy in the regulation of neuroinflammation in neurodegenerative diseases including AD[136,137]. NLRP3 (nucleotide-binding oligomerization domain-, leucine-rich repeat- and pyrin domain-containing 3) is a well-studied inflammasome, an important component in the innate immune system, and abundantly expressed in microglia[138]. Previous study indicates that knockdown of autophagy gene *ATG7* or *LC3* causes the activation of NLRP3 inflammasome and increased secretion of pro-inflammatory cytokine IL-1β in cultured microglia treated with Aβ species[139]. Mice with depletion of microglial *ATG7* display increased inflammation in the hippocampus with fibrillar Aβ injection, resulting in neuronal damage[139]. More recent study has shown that reduction of the protein level of Beclin1 in microglia increases the protein level of NLRP3 and the production of inflammatory cytokines IL-1β and IL-18[140]. These results indicate that activation of microglial autophagy may inhibit the NLRP3 inflammasome-mediated inflammation through regulating the release of inflammatory cytokines, enhance the degradation of AD-related species, and promote neuron survival.

As the professional phagocytes in brain, microglia are capable of engulfing different types of cargos including neuronal debris and protein deposits such as Aβ species through phagocytosis which mainly endures in immune cells. In AD, abnormal engulfment of Aβ and synaptic terminals may occur due to dysfunctional phagocytosis in microglia. A recent study has shown that knockdown of Beclin1 reduces Aβ uptake in cultured microglia and *ex vivo* brain slice from aged, plaque-depositing APP transgenic mice[69]. It has also found that reduced Beclin1 diminishes the recycling of phagocytic receptor CD36[69]. These results suggest that microglial autophagy maintains normal function of phagocytosis to mediate the internalization of Aβ in microglia. Another study has recently discovered the LC3-associated phagocytosis (LAP) in macrophages[141], which promotes the functional cross-talk of autophagy and phagocytosis during the immune response. While it is evident that phagocytosis can evokes a pro-inflammatory response in macrophages, the regulation of LAP is associated with a net elevation of anti-inflammatory cytokines[142,143]. The involvement of autophagy machinery in phagocytosis may promote degradation efficiency of extracellular cargos by microglial phagocytosis[144]. More recently, Heckmann et al.[145] have identified LC3-associated endocytosis (LANDO) pathway in microglia. They found that mice with depletion of LANDO in microglia display increased production of pro-inflammatory cytokine in hippocampus and accumulation of neurotoxic Aβ potentially due to impaired recycling of Aβ receptors[145]. They also showed that 5XFAD mice lacking LANDO in microglia show impaired neuronal signaling, accelerated neurodegeneration, and memory impairment[145]. These results implicate the critical role of LAP/LANDO in microglia in the regulation of neuroinflammation in AD.

Apart from microglia, autophagy in astrocyte, another type of glia in CNS, also play a key role in the regulation of neuroinflammation in AD. Recent studies have shown that activation of autophagy in astrocytes effectively inhibits the Aβ-induced NLRP3−caspase-1 activation[146,147], which implicates the neuroprotective role of astrocyte autophagy to counter the neuroinflammation in AD.

3.3. *Genetic modulations of autophagy in AD*

Several studies have tested the idea that genetic activation of autophagy ameliorates AD pathology in animal models. Beclin1 and NRBF2 play an important role in the biogenesis of autophagosome through maintaining the function of PI3KC3 complex[45,46,49,148]. Overexpression of Beclin1 or NRBF2 to stimulate autophagy can reduce the amyloid pathology and consequently improve the memory abilities in mouse models of AD[47,48]. Recent studies demonstrate that constitutive activation of autophagy by a knock-in point mutation of Beclin1-F121A, which blocks its interaction with BCL2 (an Beclin1 inhibitor), significantly reduces Aβ species levels and restores the survival in 5XFAD mice[149,150]. Induction of autophagy by overexpressing autophagy gene *ATG5* ameliorated the morphological defects in a zebrafish model with tau mutant A152T[151]. Previous studies have shown that overexpression of TFEB, a master regulator of lysosomal pathways, reduces neurofibrillary tangles and restores behavior defects in a transgenic mouse model of tau with rTg4510 mutation[152,153]. Recent studies have reported that activation of TFEB in astrocytes by exogenous expression effectively counters the pathogenesis of amyloid plaque in APP/PS1 transgenic mice and reduces the tau pathology in the hippocampus of PS19 mice[154,155]. In contrast to the exogenous expression of TFEB, a most recent study has reported that a loss of function of TFEB exacerbates tau pathology in a transgenic mouse model of tau with P301S mutation[156]. These studies indicate that genetic activation of autophagy can ameliorate AD pathology (Fig. 2).

4. Autophagy modulators for AD therapy

4.1. *Chemical autophagy modulators in AD models*

Increasing evidence indicates that autophagy modulation is a promising strategy for the treatment of neurodegenerative diseases. Since 2008 an array of studies has investigated the potential of chemical autophagy modulators in the alleviation of AD-related pathologies, raising enthusiasm for the development of therapeutics targeting autophagy for the disease intervention. Through a comprehensive literature search, we identified 92 articles that reported beneficial effects of chemical autophagy modulators in AD models (Supporting Information Table S1). A total of 73 compounds have been studied in these publications for their autophagy modulation and anti-AD properties. Among these compounds, the vast majority are reported as autophagy inducers (including 4 mitophagy inducers), while only 4 are autophagy inhibitors (Fig. 3A). Among 69 autophagy inducers, 37 compounds have been reported as the mTOR-dependent regulator; whereas the rest induce autophagy *via* mTOR-independent mechanisms including AMPK/Raptor pathway activation, Beclin1 induction, SIRT1-coupled LKB1−AMPKα, TyrRS−PARP1−SIRT1, and Wnt−GSK3β−β-catenin signaling pathways. While mTOR-dependent autophagy inducers are the major type of autophagy regulators showing anti-AD activity, selective autophagy modulators, such as mitophagy inducers, have been recently shown as potential candidates for preventing AD.

4.2. *Analysis of research quality: Experimental design and autophagy-related assays*

To evaluate the research quality in the 92 publications, we analyzed the autophagy-monitoring methods, pharmacological activities, disease models, and drug dosages of the compounds used in these publications (Table S1). We found that these studies generally consist of two parts: 1) determine the type of chemical autophagy modulation, 2) evaluate the beneficial effects of autophagy modulators in various AD models. In order to obtain an objective and quantitative view of the 92 studies, we applied the autophagy modulator scoring system (AMSS)[157]. In the AMSS-A section that measures the methodological competency of autophagy assays, we found that the quantification of the autophagosomes, the biochemical changes associated with autophagosome formation, and the degradation of the autophagic substrates are frequently used; however, nearly half of the studies (41/92) lack autophagic flux assay (Fig. 3B). In summary, only one third of the studies (30/92) examined all 4 aspects of autophagy characterization in AMSS-A, indicating that many studies lack rigorous analysis and therefore the property of the compound as autophagy inducer or inhibitor is inconclusive (Fig. 3B). In the AMSS-B section, only one quarter of the studies (7/30) score 4 or above, indicating that most of the studies fail to provide sufficient analysis of the pharmacological and functional properties of chemical modulators (Fig. 3C). We also analyzed the "citations" and "reproducibility" of these publications. Three studies have been cited at least 500 times (data from Web of Science), and four compounds have been reported at least four times (Fig. 3D). Finally, we summarized the well-characterized chemical autophagy modulators for their potential in the treatment of AD based on the citation, reporting time, and methodological integrity (Table 1).

4.3. *Chemical autophagy modulators associated with anti-AD activity*

Various types of small-molecule compounds known as autophagy modulators have been reported to alter AD related pathologies including the deposition of amyloid plaques and neurofibrillary tangles, the presence of neuroinflammation and oxidative damage, and the loss of neurons and synapses[203,204]. Here we review some well-characterized autophagy modulators and discuss their anti-AD activities.

4.3.1. *Rapamycin*

Rapamycin is a macrocyclic natural product isolated from the soil bacteria *Streptomyces hygroscopicus*. It was approved by FDA in US as an immunosuppressive drug, which can effectively prevent acute rejection and improve renal function in kidney transplant recipients[205]. Rapamycin is a well-known autophagy inducer through prevention of mTOR dimerization and activation[206]. By far many studies have demonstrated the beneficial effect of rapamycin-induced autophagy activation on AD models. These studies showed that in various AD models, including cells or mice overexpressing mutant APP, tau, or PS-1, rapamycin can reduce the levels of Aβ, APP, and hyperphosphorylated tau and

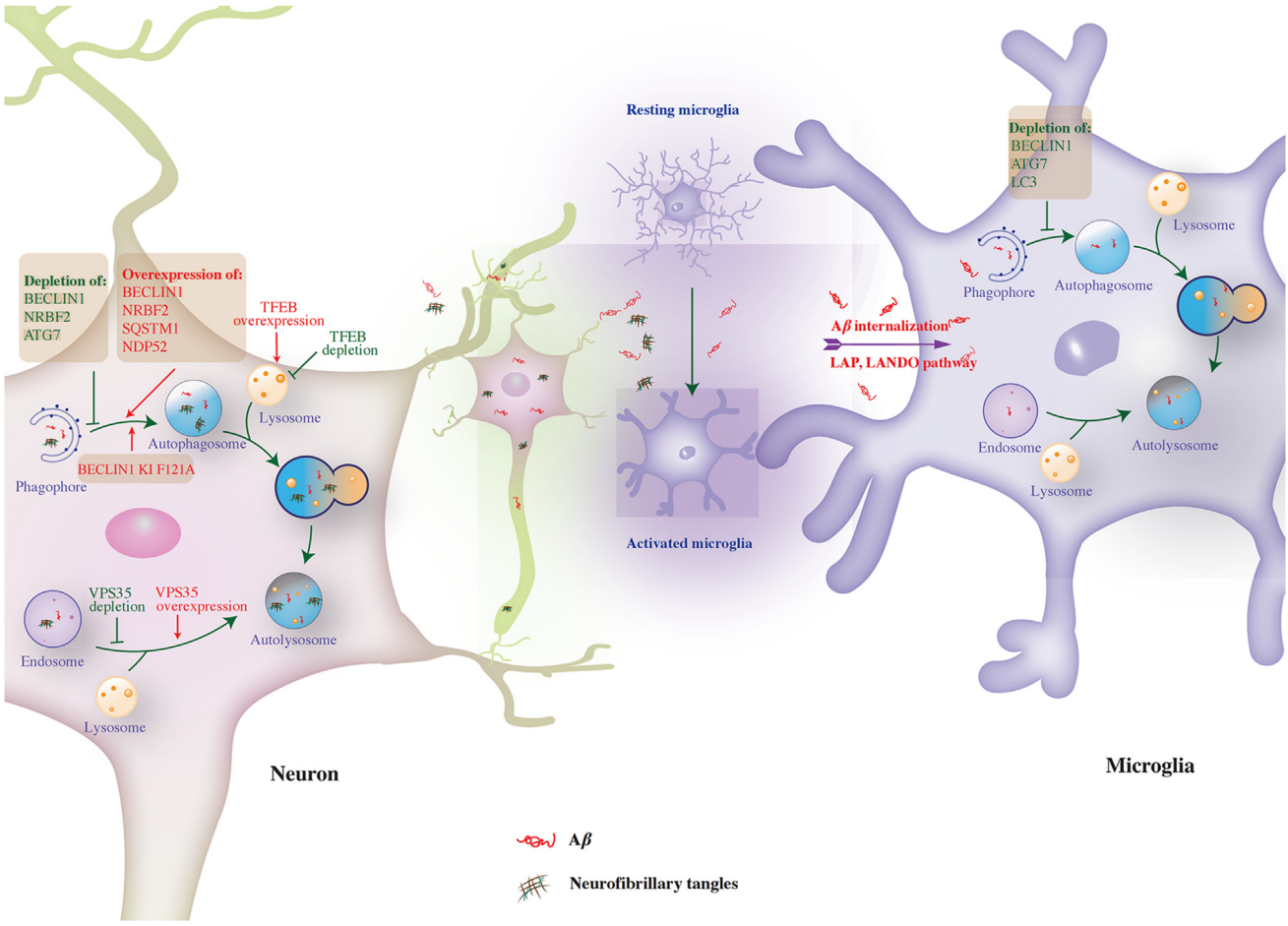

Figure 2 Autophagy in neurons and microglia regulates AD pathology. In neurons, depletion of autophagy key proteins impairs neuronal autophagy and causes accumulation of Aβ species and neurofibrillary tangles, and eventual neurodegeneration in AD models, whereas overexpression of autophagy proteins reduces AD pathology by promoting the clearance of Aβ species and neurofibrillary tangles in AD models. In microglia, depletion of autophagy key proteins impairs neuronal autophagy of Aβ species and causes activation of NLRP3 inflammasome, release of pro-inflammasome cytokins (*e.g.*, IL-1β), and eventual neuroinflammation. In addition, disruption of LAP/LANDO impairs the phagocytosis of Aβ species and causes neuroinflammation in AD models.

improve learning and memory impairments through autophagy induction[158−164].

4.3.2. *Trehalose*

Trehalose is a natural, non-reducing sugar composed of two molecules of glucose. It is abundant in some bacteria, fungi, yeast, plant, and invertebrates, where it acts as a beneficial substance to help cells survive under severe environmental stress condition[207]. It's reported that trehalose plays a neuroprotective role by inhibiting Aβ aggregation and reducing cell death[208]. In addition, trehalose improves the exercise behavior and anxiety of Parkin-deficient/tau overexpression mice and tau transgenic mice with P301S mutation through reducing the levels of Aβ, tau, and hyperphosphorylated tau[165,167,168]. Studies have shown that the neuroprotective role of trehalose in the cell model is blocked by the autophagy inhibitor 3-MA[166]. Recent studies have investigated autophagy modulation and the molecular mechanisms of trehalose in neuroprotection. Interestingly, trehalose triggers the autophagic flux in an AMPK-dependent manner, but not mTOR-dependent manner through inhibition of solute carrier 2A (SLC2A) transporters and prevention of cellular glucose import[209,210]. Considering the safety and tolerance of trehalose administration, it is a promising autophagy inducer and can be further developed into a complementary medicine for the treatment of AD[211].

4.3.3. *Resveratrol*

Resveratrol, a polyphenol derived from grape skins and seeds, plays a vital role in the prevention and treatment of neurodegenerative diseases. Increasing evidence shows that resveratrol has various beneficial pharmacological activities, including anti-oxidative stress, anti-inflammation, anti-apoptosis, and neuroprotective property[212]. We collected and analyzed the publications about the anti-AD effects of resveratrol as an autophagy modulator. In primary neurons with two-point mutations in human APP, resveratrol significantly reduces Aβ level in an autophagy-dependent manner. Also, AMPK activation and AMPK-target mTOR inhibition have been confirmed as molecular mechanisms by which resveratrol enhances autophagy[169]. In various cellular and animal AD models, resveratrol can attenuate neuroinflammation and promote the release of neurotrophic factors, which can be reversed by chemical autophagy inhibitors[169−171,213,214]. In addition, resveratrol can induce Parkin-mediated mitophagy, attenuate Aβ-induced oxidative stress and apoptosis, thereby maintaining mitochondrial function[172]. At present, many clinical trials have shown that oral

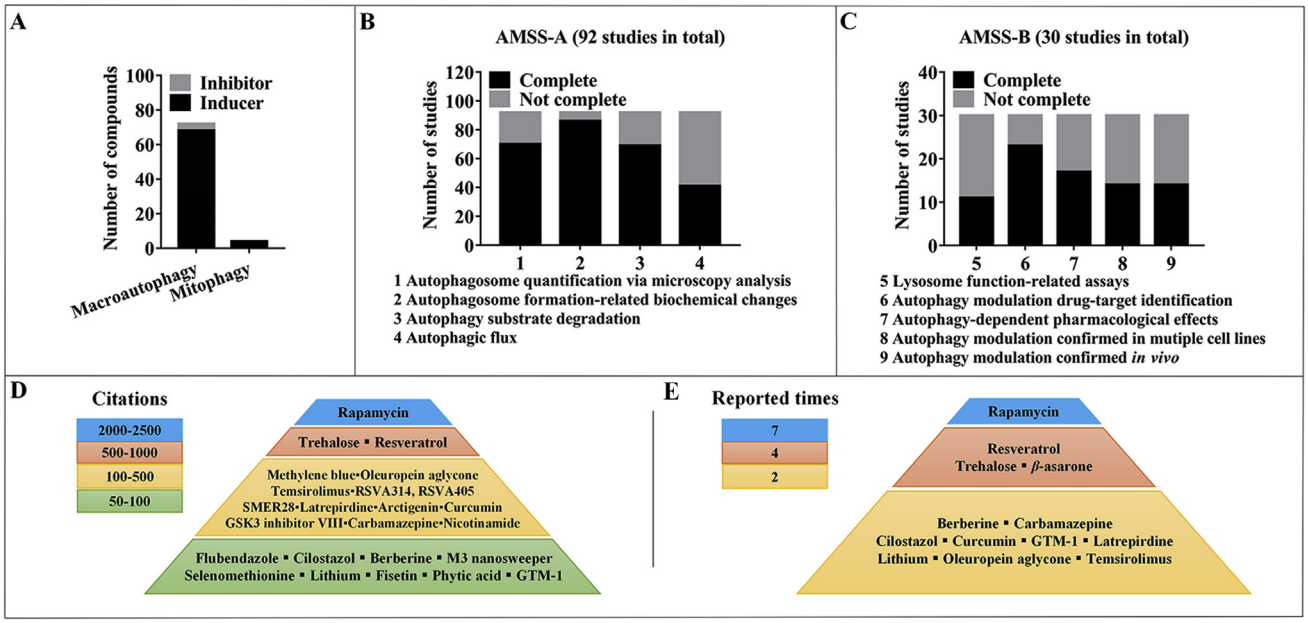

Figure 3 Analysis of research quality of chemical autophagy modulators. (A) The number of compounds belonging to autophagy inducers or inhibitors. (B) and (C) The number of studies completed in each category in Autophagy Modulator Scoring System (AMSS) -A and -B. (D) The list of autophagy modulators studies most cited. (E) The list of autophagy modulators reported at least twice.

resveratrol can improve cognitive deficits and innate immune functions, and reduce Aβ levels in plasma and cerebrospinal fluid[215–217]. Notably, a relative high dose of resveratrol is safe and well-tolerated[215]. The results also showed that two analogs of resveratrol, RSVA314 and RSVA405, can promote CaMKKβ-dependent AMPK activation and mTOR inhibition, and enhance the autophagic clearance of Aβ in several mutant APP overexpressing cell lines[177].

4.3.4. *Berberine*

Berberine is a natural isoquinoline alkaloid isolated from Coptidis Rhizoma. It has been widely used in Chinese herbal medicine for more than two thousand years. In recent years, due to its roles in anti-oxidative stress, anti-inflammation, and anti-apoptosis, berberine is considered a promising compound against AD[218]. Increasing evidence demonstrates that the neuroprotective activity of berberine is through autophagy modulation. In primary hippocampal neurons, berberine can reduce the intracellular levels of Aβ, APP, and BACE-1. In 3×Tg-AD transgenic mice, berberine shows similar effects and improves spatial learning and memory. Both *in vitro* and *in vivo* studies have confirmed that berberine induces autophagic flux by targeting the PtdIns3K/Beclin1 pathway[186]. Another study shows that berberine attenuates hyperphosphorylated tau level by activating Akt and inhibiting GSK3β and promotes the autophagic clearance of tau through the PtdIns3K/Beclin1 pathway. *In vivo* study indicates that berberine can also improve spatial learning ability and memory retentions[187]. Over the past 30 years, berberine hydrochloride has been widely used in China to treat intestinal infections. An extensive database of pharmacokinetics, toxicology, and adverse drug reactions may provide strong evidence to support the future clinical trial of berberine in the treatment of AD.

4.3.5. *Curcumin*

Curcumin is the main polyphenol extracted from Curcumae Longae Rhizoma. In the past decade, extensive research has shown that curcumin has many beneficial pharmacological effects, including anti-cancer, anti-virus, anti-arthritis, anti-oxidative stress, anti-inflammation, and neuroprotective property[219]. Increasing evidence indicates that autophagy induction is an important mechanism of curcumin's neuroprotective role. *In vitro* studies have shown that curcumin can inhibit Aβ aggregation, reduce the level of full-length APP, and attenuate the oxidative stress induced by H_2O_2. *In vivo* studies have shown that oral curcumin can inhibit Aβ production and improve memory impairment. The exploration of the molecular mechanisms shows that curcumin induces the autophagic flux in a manner dependent on GSK-3β inhibition, mTOR inhibition, and TFEB nuclear translocation[191,192]. The clinical trials of curcumin show that curcumin is well tolerated by oral administration and has good clinical effects. However, low water solubility and poor bioavailability limit the further extensive clinical trials of curcumin. To this end, the researchers synthesized curcumin derivatives and examined their anti-AD activity. Curcumin analog C1, as a TFEB nuclear translocation activator and autophagy inducer, reduces the levels of Aβ, APP, CTF-β/α, and tau, and improves learning and memory impairments in cell models and tau P301S, 3×Tg-AD, and 5×FAD transgenic mice[220,221].

4.3.6. *Lithium*

Lithium is a first-line drug approved for the treatment of bipolar depression. It has shown promising neuroprotective effects through various biological mechanisms, including anti-oxidative stress, anti-inflammation, promotion of neurotrophic factor synthesis, and autophagy regulation. Among these beneficial pharmacological activities, lithium-induced autophagy plays an important role in the treatment of AD[222]. In an *in vitro* study, researchers used human tauopathy brain extracts as a seed to induce similar tau aggregation in the cell model. The results show that lithium reduces insoluble tau and p62 levels and increases autophagic vacuoles and the LC3-II protein level[195]. In transgenic mice overexpressing human mutant tau, oral lithium for 4 months

Table 1 List of autophagy modulators and their therapeutic effects in AD models.

Compd.	Effect on autophagy	Autophagy modulation mechanisms	Disease model	Phenotypic effect	Dosage		Ref.
					In vitro	*In vivo*	
Listed by citations							
Rapamycin	Induction	■ mTOR inhibition ■ Wnt/GSK3β/β-catenin signaling pathway	■ SwAPP-SH-SY5Y ■ APPSwe/PSEN1dE9 transgenic mouse ■ PDAPP transgenic mouse ■ Tau P301S transgenic mouse ■ 3×Tg-AD transgenic mouse ■ AAV-hTauP301L-injected mouse	■ Reduce Aβ, APP, tau, and p-tau levels ■ Prevent neurodegeneration, axonal and synapse loss, and neuroinflammatory reactive gliosis ■ Increase cell viability ■ Improve learning and memory impairments	50−100 nmol/L	1−15 mg/kg (Oral administration and i.p.)	158−164
Trehalose	Induction		■ Tau-N2a ■ Epoxomicin-treated NB69 ■ Parkin deleted/tau overexpressing mouse ■ Tau P301S transgenic mouse	■ Reduce Aβ, tau, and p-tau levels ■ Increase cell viability ■ Improve the motor behavior and anxiety	50−150 mmol/L	1%−2% in drinking water	165−168
Resveratrol	Induction	■ AMPK activation and AMPK target mTOR inhibition ■ TyrRS−PARP1−SIRT1 signaling pathway	■ APP-N2a ■ APP-HEK293 ■ J20 (PDGF-APPSw, Ind) transgenic mouse primary neurons ■ Aβ-incubated PC12 ■ APPSwe/PSEN1dE9 transgenic mouse ■ 3×Tg-AD transgenic mouse	■ Reduce Aβ level ■ Promote the release of neurotrophins and synaptic biomarkers ■ Attenuate inflammation ■ Increase cell viability	1−40 μmol/L	300−557 mg/kg (Oral administration)	169−171
	Mitophagy induction		Aβ-incubated PC12	■ Attenuate oxidative stress and apoptosis ■ Alleviate mitochondrial damage	3 μmol/L		172
Oleuropein aglycone	Induction	■ mTOR inhibition	■ TgCRND8 transgenic mouse	■ Reduce Aβ level ■ Prevent cognitive deficits	90 μmol/L	50 mg/kg (Oral administration)	173,174
Temsirolimus	Induction	■ mTOR inhibition	■ SwAPP-HEK293 ■ Okadaic acid-incubated SH-SY5Y ■ APPSwe/PSEN1dE9 transgenic mouse ■ Tau P301S transgenic mouse	■ Reduce Aβ and p-tau levels ■ Attenuate cellular apoptosis ■ Improve learning and memory impairments	100 nmol/L	20 mg/kg (i.p.)	175,176

■ RSVA314 ■ RSVA405	Induction	■ CaMKKβ-dependent activation of AMPK and mTOR inhibition	■ SwAPP-N2a ■ APP-SH-SY5Y ■ APP-HEK293	■ Reduce Aβ level	1−3 μmol/L	177
SMER28	Induction		■ APP-N2a	■ Reduce Aβ and APP-CTFs levels	10−50 μmol/L	178
Latrepirdine	Induction	■ mTOR inhibition	■ GFP-Aβ42-yeast ■ TgCRND8 transgenic mouse	■ Reduce Aβ and CTF-β/α levels ■ Prevent cognitive deficits	0.25−50 μmol/L	3.5 mg/mL (Oral administration) 179,180
Arctigenin	Induction	■ mTOR inhibition ■ AMPK/Raptor pathway activation	■ SwAPP-HEK293 ■ APPSwe/PSEN1dE9 transgenic mouse	■ Reduce Aβ level ■ Improve memory impairment	1−40 μmol/L	3 mg/kg (i.p.) 181
Listed by reported times						
β-Asarone	Induction		■ Aβ-incubated PC12	■ Reduce Aβ and APP levels ■ Increase cell viability	12−144 μmol/L	182
	Mitophagy induction	■ PINK1/Parkin-mediated pathway	■ Aβ-injectedrat	■ Reduce Aβ level ■ Improve learning and memory impairments		15−30 mg/kg (Oral administration) 183
	Inhibition	■ PI3K/Akt/mTOR pathway	■ APPSwe/PSEN1dE9 transgenic mouse	■ Reduce AChE, Aβ, and APP levels ■ Improve learning and memory impairments		10−40 mg/kg (Oral administration) 184,185
Berberine	Induction	■ PtdIns3K/BECN-1 pathway	■ Mouse primary hippocampal neurons ■ 3×Tg-AD transgenic mouse	■ Reduce Aβ, APP, tau, and p-tau levels ■ Improve learning and memory impairments	1 μmol/L	50−100 mg/kg (Oral administration) 186,187
Carbamazepine	Induction	■ mTOR-independent	■ APPswe/PSEN1dE9 transgenic mouse ■ 3×Tg-AD transgenic mouse	■ Reduce Aβ level ■ Improve learning and memory impairments		100 mg/kg (Oral administration) 163,188
Cilostazol	Induction	■ mTOR inhibition ■ SIRT1-coupled LKB1/AMPKα signaling pathway	■ SwAPP-N2a ■ Retinoic acid-incubated N2a	■ Reduce Aβ and APP-CTF-β levels	10−30 μmol/L	189,190
Curcumin	Induction	■ mTOR inhibition and GSK-3β inhibition ■ Promote TFEB nuclear translocation	■ SwAPP-SH-SY5Y ■ APPSwe/PSEN1dE9 transgenic mouse	■ Reduce Aβ and APP levels ■ Reduce ROS level ■ Improve memory impairment	2.5−20 μmol/L	160, 1000 ppm (Oral administration) 191,192

(*continued on next page*)

Table 1 (*continued*)

Compd.	Effect on autophagy	Autophagy modulation mechanisms	Disease model	Phenotypic effect	Dosage		Ref.
					In vitro	*In vivo*	
GTM-1	Induction	■ mTOR-independent pathway	■ MC65, SH-SY5Y ■ 3×Tg-AD transgenic mouse	■ Reduce Aβ level ■ Improve learning and memory impairments	8−20 μmol/L	2.3−6 mg/kg (Oral administration)	163,193
Latrepirdine	Induction	■ mTOR inhibition	■ GFP-Aβ_{42}-yeast ■ TgCRND8 transgenic mouse	■ Reduce Aβ and CTF-β/α levels ■ Prevent cognitive deficits	0.25−5 μmol/L	3.5 mg/mL (Oral administration)	179,180
Lithium	Induction		■ Tau-SH-SY5Y ■ JNPL3 (P301L) transgenic mouse	■ Reduce tau and p-tau levels ■ Prevent motor disturbance	10 μmol/L	1−2 g/kg (Oral administration)	194,195
Listed by methodological integrity (AMSS = 9 or 8 points)							
Methylene blue	Induction	■ mTOR inhibition	■ Tau-CHO ■ JNPL3 (P301L) transgenic mouse	■ Reduce tau and p-tau levels	200 nmol/L	0.02 mg/kg (Oral administration)	196
GSK3 inhibitor VIII	Induction	■ Promote TFEB nuclear translocation	■ APP-CHO ■ N2asw	■ Reduce Aβ, APP, and APP-CTFs levels	10−30 μmol/L		197
Fisetin	Induction	■ mTOR inhibition ■ Promote TFEB/Nrf2 nuclear translocation	■ Tau-T4	■ Reduce p-tau level	2.5−10 μmol/L		198
LX2343	Induction	■ mTOR inhibition	■ APP-CHO ■ Streptozotocin-incubated SwAPP-HEK293	■ Reduce Aβ level ■ Improve learning and memory impairments	5−20 μmol/L	10 mg/kg (i.p.)	199
Selenomethionine	Induction	■ mTOR inhibition	■ Aβ-incubated PC12 ■ Aβ injected-mouse	■ Increase cell viability ■ Prevent cognitive deficits	100 μmol/L	6 μg/mL in drinking water	200
Dihydroartemisinin	Induction	■ mTOR inhibition	■ SwAPP-N2a ■ SwAPP-SH-SY5Y ■ APPSwe/PSEN1dE9 transgenic mouse	■ Reduce Aβ and APP levels ■ Improve memory impairment	1 μmol/L	20 mg/kg (Oral administration)	201
Thioperamide	Induction	■ CREB-1-mediated autophagy	■ Aβ-incubated mouse primary cortical neurons ■ APPSwe/PSEN1dE9 transgenic mouse	■ Reduce Aβ level ■ Ameliorate neuronal loss ■ Prevent cognitive deficits	1 μmol/L	1−5 mg/kg (i.p.)	202

can reduce the levels of hyperphosphorylated and aggregated tau, decrease the number of neurofibrillary tangles, and attenuate movement disorders. Moreover, lithium increases LC3-positive puncta and decreases p62 protein level in mice brain tissue, indicating that lithium-induced autophagic flux plays an important role in exerting neuroprotective effects[194]. Mechanistically lithium induces mTOR-independent autophagy through inhibition of inositol monophosphatase and consumption of free inositol[223]. Many clinical trials have been conducted to test lithium as a treatment for patients with AD and mild cognitive impairment. The results indicate that lithium administration shows safety and tolerability[224], and a meta-analysis suggests that lithium therapy may improve cognitive impairment in patients with AD[225]. Up today, a clinical trial has entered phase 4 (www.ClinicalTrials.gov, NCT code: 03185208).

4.3.7. *Methylene blue*

Methylene blue (methylthioninium chloride) is a cationic dye that belongs to the phenothiazine class. It is the first synthetic compound and has been widely used in clinical treatment for more than 100 years. At present, methylene blue has been approved by the FDA to treat methemoglobinemia, neurotoxicity caused by ifosfamide, and to prevent urinary tract infections in elderly patients[226]. Recently, the protective role of methylene blue in neurodegenerative diseases has attracted increasing attention from the scientific community[227]. Increasing evidence suggests that methylene blue can attenuate the formation of amyloid plaques and neurofibrillary tangles and slow down the cognitive decline of various AD models. A study that explored the relationship between methylene blue-modulated autophagic flux and AD[196] indicate that nanomolar concentrations of methylene blue can significantly reduce the levels of tau and hyperphosphorylated tau in cells, primary neurons, and organotypic slice cultures. Knockdown of Beclin1 eliminated the beneficial effects and confirmed that methylene blue promotes tau clearance through the autophagy-lysosomal degradation pathway. In transgenic mice overexpressing human mutant tau, a low dose of methylene blue (20 μg/kg) can reduce tau levels. In addition, both *in vitro* and *in vivo* studies have confirmed that methylene blue induces mTOR-dependent autophagy. Some clinical trials have been completed; however, the results indicate that the use of methylene blue only in the early stages of AD may improve learning and memory impairments. Considering that methylene blue has many desirable properties, including good solubility in aqueous media, low toxicity, ability to cross the blood−brain barrier, and the apparent effect of inhibiting the aggregation of toxic proteins, it should be investigated further as a lead compound for the treatment of AD.

4.3.8. *Others*

Many studies have explored the autophagy modulation and anti-AD activity of FDA-approved drugs. Carbamazepine is an FDA-approved drug for the treatment of epilepsy, trigeminal neuralgia, manic and mixed episodes of bipolar I disorder[228,229]. Carbamazepine significantly reduced cerebral amyloid plaque burden and Aβ level through the autophagic clearance pathway, and rescued the spatial learning and memory deficits in transgenic mice overexpressing human mutant APP, PSEN1, and MAPT[163,188]. Cilostazol is approved for medical use to help relieve the symptoms of intermittent claudication in peripheral vascular disease[230]. In transgenic and chemicals-induced AD cellular models, studies confirmed that cilostazol significantly induced autophagy and reduced Aβ and APP-CTF-β levels[189,190]. Latrepirdine was first used as an antihistamine drug and its neuroprotective effect in AD and Huntington's disease has been widely explored in recent years[231]. Although latrepirdine failed to improve AD symptoms in the phase III trial, it significantly reduced Aβ and CTF-β/α levels and prevented cognitive deficits through an mTOR-dependent autophagic flux[179,180]. Dihydroartemisinin is a semi-synthetic derivative of artemisinin and is used to treat malaria. It has various beneficial pharmacological activities, including anti-cancer, anti-arthritis, anti-oxidative stress, anti-inflammation, and neuroprotective property. In recent years, dihydroartemisinin has been shown to improve learning and memory in AD models, as well as to promote the clearance of Aβ and APP through autophagy induction[201]. By now, these chemicals have been in clinical use for decades, and their pharmacological activity, toxicology, and pharmacokinetics have been documented in detail. They might be the promising lead compounds to develop novel autophagy inducers as anti-AD drugs.

4.3.9. *Summary on the chemical autophagy modulators*

From the above discussion, it is obvious that mTOR-dependent autophagy modulators are still the most studied type and more than half of the known autophagy inducers (37/69) tested on the AD models are mTOR-dependent. The anti-AD potentials of mTOR inhibition have been repeatedly reported[232], and the lifespan extension effect of mTOR inhibition[233] further support the therapeutic value of mTOR inhibitors in AD treatment. Though the side effects including mouth sores, impaired wound healing, gastrointestinal discomfort, and the increased risk of infection have been reported to occur during mTOR inhibitor rapamycin treatment, a recent clinical trial revealed that rapamycin administration up to 8 weeks is well tolerant in older individuals[234]. Except rapamycin, natural compounds resveratrol and trehalose are the most frequently reported autophagy inducers displaying neuroprotective effects on AD models (Fig. 3). These two compounds have been used as food supplements or additives for decades with good safety. Collectively, rapamycin, resveratrol and trehalose are probably the most promising autophagy inducers to be tested in clinical trials for AD therapy.

5. Obstacles

Genetic and pharmacological enhancements of autophagy have displayed therapeutic potentials in multiple neurodegenerative disease models including AD, promoting the identification of chemical autophagy modulators as novel anti-AD drug. Despite the increase of the list for autophagy modulators, none has been successfully developed for clinical use due to numerous obstacles. The primary obstacle is the lack of specificity of described autophagy modulators in autophagy modulation. Multiple chemical molecules that are used to inhibit or activate autophagy show a low pharmacological specificity for their targets in autophagy process. For instance, acute administration of rapamycin inhibits mTORC1 with a relatively high specificity, however chronic rapamycin administration will cause mTORC2 disassembly[235,236]. Rapamycin not only activates autophagy, but also inhibits mTORC1-mediated translation and cellular proliferation[236,237]. The similar case is that 3-MA, a non-selective phosphoinositide 3-kinase (PI3K) inhibitor, blocks the catalytic activities of multiple PI3K complexes. Many components of autophagy machinery display autophagy-independent functions, thus activation or

inhibition of autophagy *via* targeting these proteins will affect their functions beyond autophagy. Additional obstacle is the lack of knowledge for current autophagy modulators in targeting autophagy in specific cell types in the nervous system. Autophagy is a highly conserved pathway and all cells share the same core autophagy machinery. Due to poor cell type specificity, when triggering neuronal autophagy, autophagy modulators may also induce undesired autophagy response in tissues and may even cause potential adverse response. Further obstacle lies in the difficulties in monitoring autophagy flux *in vivo* (*e.g.*, animal models and human tissues). Given the complexity of autophagy, there is no single reliable marker for autophagy activation and combination of multiple assays is always needed to determine autophagy flux. Though a series of assays have been developed to monitor autophagy in mammals, most are only applicable for *in vitro* models. Although transgenic mice models carrying autophagy flux marker tandem fluorescence-tagged LC3 were developed and may provide a tool to monitor autophagy flux *in vivo*, they do not allow live imaging of autophagy flux in intact brain in a dynamic manner. Without appropriate assays and biomarkers to monitor autophagy flux in human tissues, it is challenging to evaluate autophagy flux at different stages of AD. It also poses a challenge to determine the right time point to induce autophagy as well as to optimize the dosage of autophagy modulators in patients. Finally, obstacle exists in the lack of substrate-selective autophagy modulators for precise and specific degradation of toxic/aggregated proteins and damaged organelles associated with diseases. Non-selective autophagy is a low economical process that are strongly induced under extremely stressed conditions like starvation, oxidative stress or cytotoxicity, at the cost of bulk degradation of cellular content. A cost-effective way for autophagy to maintain cellular homeostasis is to selectively degrade substrates like aggregated proteins and damaged organelles. Indeed, the attempt to search for selective autophagy modulators have led to the discovery of several selective autophagy modulators that enhance aggrephagy or mitophagy[183,238]. However, most of the selective autophagy modulators are still not so "selective" and can also trigger global autophagy.

6. Perspective

Autophagy regulates degradation of AD related proteins in neurons and neuroinflammation process, thus protecting neurons. In the brain, activation of autophagy is expected to reduce protein aggregates in neurons; however, available autophagy activators have poor specificities to autophagy. It is also unclear how they mediate activation of autophagy in neuron, which have distinct mechanism of regulation. In addition, lysosomal dysfunction is implicated in the AD pathogenesis[81], and simply inducing autophagy initiation may lead to abnormal autophagosome accumulation and overproduction of Aβ[82,239]. Thus, identification of autophagy activators that selectively targets lysosomal activity is desirable to ameliorate AD pathology. Indeed, stimulating lysosomal proteolytic activity in an AD mouse model (TgCRND8) by genetically deleting Cystatin B, an inhibitor of lysosomal cysteine proteases, rescues autophagic-lysosomal pathology, reduces intraneuronal Aβ species, and ameliorates the deficits of learning and memory[240]. Similar therapeutic effects are observed in 5×FAD and 3×Tg-AD mice by pharmaceutically activating TFEB activity[221]. Furthermore, development of novel autophagy modulators for AD therapeutics should explore those targeting selective autophagy including aggrephagy and mitophagy. Various autophagy receptors were shown to mediate the clearance of AD-related proteins[122,123]. Searching small molecules boosting neuron's ability to specifically remove the disease-related proteins via the autophagy receptors should be performed in the near future[241,242].

Another promising therapeutic approach is to target microglial autophagy which plays a critical role in the regulation of neuroinflammation in AD[131,136,137]. Phagocytosis and cytokine release are the primary functions of microglia. Autophagy is known to play a key role in promoting microglia phagocytosis[243] and suppressing inflammatory responses[140,244]. However, in acute brain damage conditions like stroke, autophagy also mediates pro-inflammatory cytokine release[245]. Microglial Beclin1 not only modulates NLRP3 inflammasome-mediated inflammation, but also regulates phagocytosis through controlling the recycling of phagocytic receptor[69,140]. Microglia isolated from human AD patients display severe reduced protein levels of Beclin1[69]. Thus, it is possible that specific activation of microglial Beclin1 would ameliorate AD pathology due to its function in anti-inflammation and LAP/LANDO of Aβ species. PRKAA1 is a known inducer of autophagy[246]. Previous study has shown that the activators of PRKAA1 such as AICAR and metformin can induce microglia autophagy and subsequently enhance the degradation of Aβ species in cultured microglia[139]. A recent study has shown that activation of autophagy by a potent autophagy inducer alborixin substantially clear Aβ species in microglial N9 cells[116]. It's reported that genetic activation of Beclin1 by a knock-in point mutation of Beclin1-F121A significantly reduces Aβ species levels, ameliorates memory deficits, and improves the survival in 5XFAD mice[149]. Thus, identification of novel microglial autophagy modulators that target autophagy proteins such as Beclin1 and Rubicon should be helpful in therapeutic development for AD.

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Author contributions

Zhenyu Yue, Jia-Hong Lu and Zhiqiang Deng conceived the project. Zhiqiang Deng and Yu Dong searched the literatures and drafted the manuscript. Zhenyu Yue, Jia-Hong Lu, Zhiqiang Deng, Yu Dong and Xiaoting Zhou edited and revised the manuscript. All authors approved the final manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

Appendix A. Supporting information

Supporting data to this article can be found online at https://doi.org/10.1016/j.apsb.2021.12.009.

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