REVIEW

Impairment of the autophagy−lysosomal pathway in Alzheimer's diseases: Pathogenic mechanisms and therapeutic potential

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Abstract   Alzheimer's disease (AD), the most common neurodegenerative disorder, is characterized by memory loss and cognitive dysfunction. The accumulation of misfolded protein aggregates including amyloid beta (Aβ) peptides and microtubule associated protein tau (MAPT/tau) in neuronal cells are hallmarks of AD. So far, the exact underlying mechanisms for the aetiologies of AD have not been fully understood and the effective treatment for AD is limited. Autophagy is an evolutionarily conserved cellular catabolic process by which damaged cellular organelles and protein aggregates are degraded via lysosomes. Recently, there is accumulating evidence linking the impairment of the autophagy−lysosomal pathway with AD pathogenesis. Interestingly, the enhancement of autophagy to remove protein aggregates has been proposed as a promising therapeutic strategy for AD. Here, we first summarize the recent genetic, pathological and experimental studies regarding the impairment of the autophagy−lysosomal pathway in AD. We then describe the interplay between the autophagy−lysosomal pathway

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and two pathological proteins, Aβ and MAPT/tau, in AD. Finally, we discuss potential therapeutic strategies and small molecules that target the autophagy−lysosomal pathway for AD treatment both in animal models and in clinical trials. Overall, this article highlights the pivotal functions of the autophagy−lysosomal pathway in AD pathogenesis and potential druggable targets in the autophagy−lysosomal pathway for AD treatment.

1. Introduction

Alzheimer's disease (AD), the most common neurodegenerative disorder, affects about 4%−8% of the elderly population worldwide[1,2]. Aging is the leading factor in the pathogenesis of AD. According to recent data from the World Alzheimer Report[3], there are about 10% of people above 65-year-old living with AD. In the United States, over 5.8 million people in 2020 suffered from AD and this number is expected to rise to 13.8 million in 2050[3]. China has become an aging society and there were about 164.5 million people aged 65 and above in 2019[4]. Recent studies showed that the overall prevalence of AD was estimated to be 0.04[5] and the pooled prevalence for aged people (55 years old) with mild cognitive impairment was as high as 12.2% in China[6]. The medicare cost for AD patients is enormous. For instance, the total cost of AD treatment is about $305 billion in 2020 in the USA[3]. Though the U.S. Food and Drug Administration (FDA) recently approved Aduhelm (aducanumab), a monoclonal antibody targeting Aβ (amyloid beta) for treating AD, there is also a controversial discussion regarding its effects, and the therapeutics for this disease are still unclear.

The main clinical features of AD are memory loss and cognitive dysfunction due to the loss of synapses in the brain[7]. Two hallmarks of AD pathology are the presence of extracellular senile plaques primarily composed of amyloid beta (Aβ), and the intraneuronal neurofibrillary tangles (NFTs), the main constituent of which is the aggregated microtubule associated protein Tau (MAPT/tau) protein[7,8]. While the aetiologies of AD are not fully understood, the elimination of Aβ and/or MAPT/tau aggregates is one of the most promising therapeutic strategies for this disease. The main route to remove Aβ and MAPT/tau aggregates is macroautophagy (hereafter referred to as autophagy)[9]. Autophagy is a highly conserved pathway for the degradation of intracellular long-lived proteins, protein aggregates and organelles (*e.g.*, mitochondrial) *via* lysosomes to maintain homeostasis under physiological conditions. The expansion of our knowledge on autophagy has revealed that impaired autophagy is linked to the pathogenesis of multiple chronic diseases including AD. Induction of autophagy by a variety of small molecules results in the clearance of Aβ and MAPT/tau aggregates, leading to beneficial effects in multiple preclinical AD models, suggesting that pharmacological activation of autophagy holds great promise for developing therapies for AD. Notably, other types of selective autophagy such as CMA (chaperone-mediated autophagy) and mitophagy have also been associated with AD, and the detailed discussion of these pathways goes beyond the scope of this review and can be found elsewhere[10,11]. Here, we summarize the characteristics of Aβ and MAPT/tau in AD, describe the regulatory mechanism of the autophagy−lysosomal pathway, review current evidence of dysregulated autophagy−lysosomal pathway in AD, discuss the interplay between autophagy and two pathological proteins, Aβ and MAPT/tau, illustrate autophagy enhancers in preclinical AD animals and clinical trials, and finally highlight potential pharmacological therapeutic strategies that target autophagy−lysosomal pathway for AD.

2. Aβ and MAPT/tau in AD

Key pathological features of AD include the senile plaques formation caused by the accumulation of Aβ and NFTs formation resulting from hyperphosphorylated MAPT/tau aggregates[7]. These toxic protein aggregates promote neuroinflammation and neuronal death. Aβ has been regarded as one of the central molecules leading to synaptic toxicity, memory and cognitive impairment in AD[12]. Aβ is generated by the cleavage of amyloid precursor protein (APP). APP is a type I transmembrane protein that can be cleaved by either α-secretase (non-amyloidogenic processing) or BACE1 (β-secretase; known as amyloidogenic processing) followed by γ-secretase cleavage (Fig. 1)[13−15]. In non-amyloidogenic APP processing pathway, APP is first proteolytically cleaved by α-secretase, producing sAPPα (secreted ectodomain APP alpha) and the membrane-associated APP-CTFα (APP C-terminal fragment alpha, C83)[7,16,92]. In amyloidogenic APP processing pathway, APP is firstly cleaved by BACE1/β-secretase to yield sAPPβ (soluble peptide APP beta) and the membrane-bound APP-CTFβ (APP C-terminal fragment beta, C99)[7]. Both APP-CTFα and APP-CTFβ can be proteolytically cleaved by γ-secretase into non-toxic P3 peptide and toxic Aβ (Aβ_{40} or Aβ_{42}), respectively (Fig. 1)[7,18]. The amyloidogenic pathway is predominant in AD[12,17], leading to the accumulation of Aβ. Aβ can aggregate and form Aβ oligomers, which will assemble into Aβ fibrils and finally form amyloid plaques, whose extracellular deposits have been observed in the brains of AD patients[2,12,18]. The amyloid hypothesis suggested that Aβ aggregation disrupted cell-to-cell communication, triggered neuroinflammation and finally destroyed brain cells (Fig. 1)[12]. Apart from Aβ aggregates, increasing evidence also indicated that Aβ oligomers also contribute to neurotoxicity and neurodegeneration in AD[19]. It seems that both Aβ oligomers and Aβ aggregates are involved in neurotoxicity in AD.

Apart from Aβ, NFTs that comprise MAPT/tau aggregates also play a critical role in AD pathogenesis[7,20]. MAPT is a microtubule-associated protein highly expressed in the axon of neurons, which is essential in stabilizing microtubules and promoting tubulin assembly into microtubules[20,21]. In AD and other MAPT-related neurodegeneration, MAPT becomes hyperphosphorylated and form insoluble aggregates, which are thus inability to maintain microtubule stability (Fig. 1)[21]. Finally,
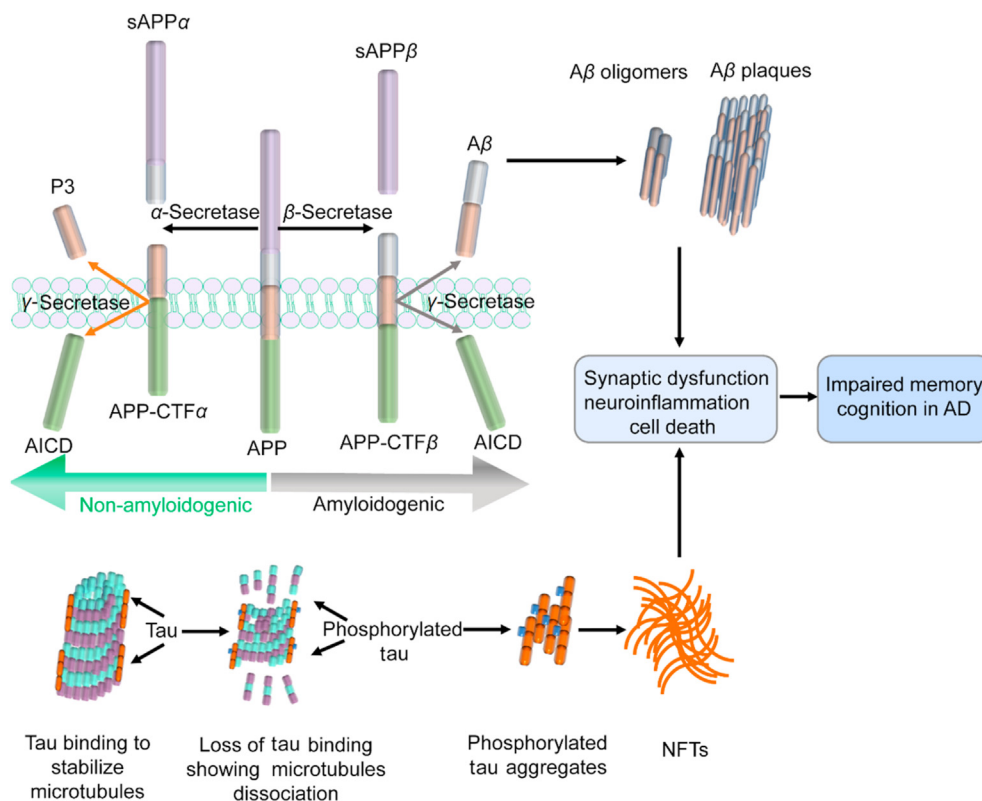
Figure 1 Aβ and MAPT/tau in AD. In non-amyloidogenic APP processing pathway, APP is first proteolytically cleaved by α-secretase to produce sAPPα and APP-CTFα. In amyloidogenic APP processing pathway, APP is firstly cleaved by β-secretase to yield soluble sAPPβ and the membrane-bound APP-CTFβ. Both APP-CTFα and APP-CTFβ can be proteolytically cleaved by γ-secretase into non-toxic P3 peptide and toxic Aβ. Aβ can be aggregated and form Aβ oligomers, which will assemble into Aβ fibrils and finally form amyloid plaques. MAPT is a microtubule-associated protein that is essential in stabilizing microtubules. In AD and other MAPT/tau-related neurodegeneration, MAPT/tau proteins become hyperphosphorylated and form insoluble aggregates, which are thus inability to maintain microtubule stability. Hyperphosphorylation MAPT/tau can aggregate and finally form NFTs. Amyloid plaques, Aβ oligomers and NFTs finally disrupt synaptic function, induce neuroinflammation, and cell death, which finally may induce memory dysfunction in AD. In addition, the synergistic effects of Aβ and Tau are also proposed to play important role in inducing neurodegeneration in AD.

hyperphosphorylated MAPT forms aggregates, and NFTs, which lead to the impairment of axonal transport, neurons death, and finally induce neurodegeneration (Fig. 1)[22]. Besides phosphorylation, acetylated MAPT has also been shown to affect its bind to microtubules, promote MAPT fibrillization, and play an important role in MAPT-mediated synaptic toxicity[23,24]. Interestingly, Aβ and MAPT have both independent and synergistic effects in inducing neurotoxicity, and the intimate interplay between soluble Aβ and MAPT/tau has been implicated in AD pathocascade[25,26]. As the accumulation of Aβ and MAPT, the synapses integrity and neural connectivity of the brain will be disrupted, which may finally induce neuronal death[25]. Importantly, Aβ fibrils and MAPT aggregates induce the hyperactivation of glia cells and subsequent neuroinflammation, which may also contribute to neurodegeneration in AD[25,27]. Considering the importance of Aβ and MAPT in AD, targeting Aβ and/or MAPT is probably the most promising strategy for anti-AD drug development.

3. The autophagy−lysosomal pathway

Autophagy is a highly conserved process for sequestering protein aggregates and damaged organelles into a double member structure termed autophagosomes, whose content will be subsequently delivered into lysosomes for degradation[9,28−31]. Autophagy process is generally divided into several steps, which include autophagy initiation, autophagosome formation, the fusion of autophagosomes with lysosomes, and lysosomal degradation (Fig. 2). Autophagy initiation is controlled by the Unc-51 like autophagy activating kinase 1 (ULK1) complex containing ULK1/2, ATG101, ATG13, and focal adhesion kinase family-interacting protein of 200 kDa (FIP200). Several key signaling pathways are known to regulate autophagy initiation, which includes adenosine monophosphate-activated protein kinase (AMPK) and mammalian target of rapamycin complex 1 (mTORC1). Nutrient deficiency and low energy status are well-defined autophagy inducers that inhibit mTORC1 and activate AMPK, respectively. AMPK activation and mTORC1 inhibition trigger autophagy initiation by phosphorylation and activation of ULK1 complex (Fig. 2)[32]. ULK1 complex activation recruits the VPS34/PIK3C3 PtdIns3K (phosphatidylinositol 3-kinase) complex comprising VPS34/PI3KC3, PIK3R4/VPS15, BECN1, ATG14L and nuclear receptor binding factor 2 (NRBF2) to a PAS (pre-autophagosomal structure) for the production of phosphatidylinositol 3-phosphate (PI3P)[33−35]. PI3P then recruits its effectors such as WD repeat domain phosphoinositide-interacting protein 2 (WIPI2) and downstream autophagy proteins (e.g.,
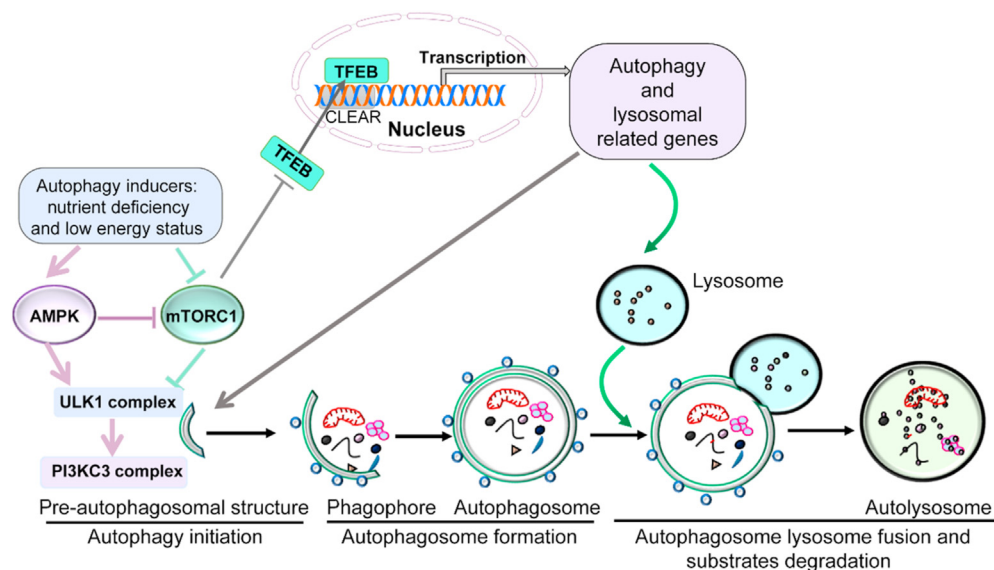
Figure 2 Autophagy process and its regulation. Autophagy is generally divided into several steps, which include autophagy initiation, the formation of autophagosomes, the fusion of autophagosomes with lysosomes and subsequent autophagic cargos degradation. MTORC1 and AMPK are upstream key kinases that control autophagy initiation. AMPK activation (in response to low energy status) and/or MTORC1 inhibition (in response to nutrient deficiency) promotes autophagy initiation by phosphorylation and activation of ULK1 complex, which further activates VPS34/PIK3C3 phosphatidylinositol 3-kinase (PtdIns3K) complex to produce PI3P. PI3P then recruits its effector proteins such as WIPI2 to form pre-autophagosome structure. The subsequent phagophores elongation and expansion will form autophagosomes, which are controlled by ATG5−ATG12−ATG16L complex and ATG8/MAP1LC3B, two conserved ubiquitin-like conjugation systems. The fusion of autophagosomes with lysosomes to form autolysosomes, within which autophagic cargos are degraded by lysosome hydrolases. Upon dephosphorylation (*e.g.*, upon starvation-induced MTORC1 inhibition), transcriptional factor EB (TFEB) is dissociated from 14-3-3 protein and subsequently moves from the cytosol into the nucleus, where it upregulates the expression of multiple genes responsible for autophagy and lysosome biogenesis. Thus, TFEB not only promotes the formation of autophagosomes, but also enhances lysosome functions.

ATG16L) to facilitate phagophores formation[9,28,29]. The subsequent elongation and expansion of phagophores are controlled by two ubiquitin-like conjugation systems, ATG12 and ATG8/LC3. By cooperation with ATG7 and ATG3, ATG5−ATG12−ATG16L facilitates the conjugation of MAP1LC3B-I/LC3B-I to lipid phosphatidylethanolamine (PE) to form lapidated MAP1LC3B-II/LC3B-II, a core component of autophagosome[28,29]. MAP1LC3B-II/LC3B-II promotes the sequestration of multiple autophagy substrates (*e.g.*, protein aggregates and mitochondria) into autophagosomes *via* a group of autophagy receptors such as SQSTM1/p62[28,29]. Finally, autophagosomes fuse with lysosomes to form autolysosomes, and autophagic cargos can be degraded by lysosome hydrolases. The fusion process is controlled by multiple factors including SNAREs (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors), small GTPase RAB7, EPG5 (ectopic P-granules autophagy protein 5 homolog), ATG14L, NRBF2 (Nuclear receptor-binding factor 2) and other factors[36,37].

In addition to its inhibitory effect on ULK1 complex-involved autophagy initiation, mTORC1 is known to negatively regulate the late stage of autophagy and lysosomal function[38,39,40]. One key mechanism is *via* targeting TFEB (transcription factor EB), a key transcription factor controlling autophagy and lysosome biogenesis[41,42]. Under normal physiological conditions, TFEB is phosphorylated by mTORC1 and predominantly retained in the cytoplasm by binding to YWHAZ/14-3-3 proteins[43]. Under starvation conditions and upon mTORC1 inhibition, TFEB is dephosphorylated and translocated from the cytoplasm into the nucleus, where TFEB upregulates the expression of various autophagy- and lysosome-related genes by directly binding to promoter regions containing the CLEAR (coordinated lysosomal expression and regulation) sequence[43−45] (Fig. 2). Though mTORC1 is the main kinase complex responsible for regulating TFEB phosphorylation, other kinases such as PRKCA/PKC and AKT also play a role in regulating TFEB activation[46]. Interestingly, we and others have shown that TEEB acetylation status is also critical for its transcriptional activities[47,48]. Furthermore, several other transcription factors including forkhead box O3 (FOXO3) and activating transcription factor 4 (ATF4) have also been implicated in controlling ATGs expression[49].

4. Impairment of the autophagy−lysosomal pathway in AD

4.1. Genetic evidence

Early-onset familial AD, accounting for 1%−5% of AD[50], is associated with mutations in APP, presenilin 1 (PSEN1) and presenilin 2 (PSEN2). Among them, PSEN1 and PSEN2 mutations are the most frequent known causes of early-onset familial AD[51]. PSEN1 and PSEN2 are key ingredients of the γ-secretase complex that regulates Aβ production, and these two gene mutations result in increased Aβ_{42}/Aβ_{40} ratio[51]. Apart from modulating Aβ production, PSEN1 also maintains lysosomal

acidification *via* regulating lysosome calcium channel mucolipin TRP cation channel 1 (MCOLN1)-mediated lysosome calcium homeostasis, and PSEN1 deficiency elevates lysosomal pH[52,53] (Table 1). Moreover, PSEN1 deficiency or mutation promotes mTORC1 activation, which subsequently inhibits TFEB-mediated autophagy and lysosome biogenesis[54]. PSEN2 mutation was also reported inhibiting autophagosome and lysosome fusion and consequently impaired autophagy flux[55] (Table 1). As such, PSEN1 and PSEN2 mutations induce impairment of the autophagy−lysosomal pathway, leading to the accumulation of protein aggregates and neurons death independent of γ-secretase.

In addition to familial AD-linked genes, gene-based tests and genome-wide association studies (GWAS) have found multiple loci in the genome associated with late-onset AD[56]. Among them, phosphatidylinositol binding clathrin assembly protein (*PICALM*), cathepsin D (*CSTD*), phospholipase D3 (*PLD3*), ubiquitin-like protein ubiquilin-1 (*UBQLN1*), *GRN* (granulin), sortilin-related receptor 1 (*SORL1*), and clusterin (*CLU*) have been implicated in regulating autophagy and/or lysosomal functions[57,98] (as summarized in Table 1 and Fig. 3A). *PICALM* is an AD risk gene[58], whose expression has been reported to be reduced in AD brains[59]. *PICALM* encodes a protein termed phosphatidylinositol binding clathrin assembly protein[60]. PICALM promotes the formation of clathrin-coated pits by interaction with and binding to clathrin and adaptor protein 2 (AP2), which are important for clathrin-mediated endocytosis[60]. Interestingly, PICALM/AP2 complex interacts with APP-CTFs and autophagic marker MAP1LC3B/LC3B and serves as an autophagic cargo receptor targeting autophagic degradation of APP-CTFβ, thereby modulating Aβ production[60]. Moreover, PICALM regulates both autophagosomes formation and the fusion of autophagosomes with lysosomes *via* modulating the endocytosis of soluble NSF attachment protein receptors (SNAREs) such as VAMP2 and VAMP8[61]. Consequently, *Picalm* deficiency promotes the formation of Aβ as well as results in the accumulation of MAPT protein, which exacerbates MAPT pathology in animal models *via* inhibiting autophagy[61,62]. Overall, these findings indicate that *PICALM* deficiency inhibits both autophagosome formation and the fusion of autophagosomes with lysosomes. *CTSD* gene encodes cathepsin D, a key lysosome enzyme that participated in the degradation of Aβ[63]. Genetic studies have suggested that *CTSD* variation was a key risk factor for AD[64,65], implicating that impairment of lysosomal function may link to AD pathogenesis. However, there was a report demonstrated that *CTSD* polymorphism was not a key risk factor for AD pathogenesis[66]. Though the underlying reasons for this discrepancy are unclear, APOEε4 carriers or non-carriers' status has been implicated to affect the association of *CTSD* with AD[65].

PLD3, a member of the phospholipase D protein family, is a 5′ exonuclease that specifically cleavages ssDNA to regulate inflammatory cytokine responses[67]. Whole-exome sequencing has identified that rare coding variants in the *PLD3* were associated with the increased risk to develop late-onset AD in European patients[68] and Chinese cohorts[69]. PLD3 expression is reduced in AD patients[68,70]. *Pld3* mutations reduced PLD3 activity and inhibited autophagy possibly *via* activation of mTOR in AD cell models[71], suggesting a possible link between the impairment of autophagy pathway in PLD3-mediated AD pathogenesis. However, whether and how PLD3-mediated autophagy impairment contributes to AD pathogenesis in animal models remains to be examined.

UBQLN1 encodes a ubiquitin-like protein ubiquilin-1, whose polymorphism has been suggested to be associated with AD[72] and the expression of ubiquilin-1 was reduced in the brains of AD patients[73]. *Ubqln1* deficiency led to increasing production of Aβ and neuronal cell death[73,74]. Apart from its role in delivering protein for degradation by the proteasome[74], it has also been shown that *Ubqln1* deficiency comprised the fusion of autophagosomes with lysosomes *via* interacting with LC3[75]. These results indicate that autophagy may also play a role in ubiquilin-1-mediated AD pathogenesis.

GRN encodes progranulin (PGRN) protein, a multiple functional glycoprotein. GRN mutations were identified as a risk factor for AD and frontotemporal dementia[76]. The decrease of PGRN levels can be detected in serum or cerebrospinal fluid of patients with GRN mutation since it is a secreted protein[77]. It has been shown that GRN mutation may be related to disrupting lysosomal functions[77] though underlying mechanisms are unclear.

SORL1 encodes sortilin-related receptor 1 protein that is critical for regulating the protein trafficking from Golgi to endosome[56,78]. *SORL1* rare coding variants are associated with the developing AD[79]. SORL1 regulates APP sorting and generation, and *Sorl1*-deficient mice have increased Aβ levels[80]. Importantly, homozygous mutations in SORL1 induced enlarged endosomes, lysosome dysfunction, and inhibited autophagosome flux[78]. Notably, APP, PSEN1 and SORL1 function within a common pathway for modulating endosome function.

CLU (also known as *APOJ*) encodes clusterin protein, which is a molecular chaperone that regulates protein folding[81]. GWAS results showed that *CLU* was also a late-onset AD risk gene[82,83]. Though how *CLU* mutation contributes to AD pathogenesis is largely unclear[84], it was found that CLU promoted LC3-lipidation and autophagosome biogenesis[85], suggesting a potential link between *CLU* mutation, autophagy lysosome dysfunction and AD. Several evidence showed that other late-onset AD risk genes including *CD2AP* (CD2 Associated Protein) and *BIN1* (bridging integrator 1) may be implicated in modulating autophagy−lysosomal pathway[86] though future mechanistic studies are required.

Overall, mutations in genes that affect autophagy and lysosome function are associated with an increased risk of both familial and late-onset AD.

4.2. Evidence from post-mortem analysis in AD patients

Post-mortem studies have provided mountains of evidence regarding the impairment of multiple steps of the autophagy−lysosomal pathway including autophagy imitation, autophagosomes formation, and autophagosome clearance in AD (Fig. 3A).

The dysregulated autophagy initiation machinery has been observed in the brains of AD patients and animal models. For instance, the expression of p-mTOR, p-RPS6KA1, p-RPTOR/Raptor, and mTORC1 upstream molecule RRAGC/Rag C were increased in the hippocampus of AD patients[87,88], suggesting the hyperactivation of mTORC1 signaling in AD, which may prevent autophagy initiation and autophagosome formation. The impairment of autophagosome biogenesis in AD was further confirmed by the finding that the expression of the key components that regulate autophagosome formation including *BECN1*, *NRBF2* and *ULK1/2* were reduced in the hippocampus of AD patients and in AD animal models (Fig. 3)[37,89−92].

Table 1 AD-related gene mutation links with the autophagy−lysosomal pathway.

| Gene name	Name of protein	Function	Association with AD	Relation with autophagy−lysosome pathway	Comments	Ref.
PSEN1	Presenilin 1	A key component of the gamma−secretase complex that regulates the Aβ generation	Familial AD associated gene	Maintains lysosomal acidification; PSEN1 deficiency inhibits TFEB-mediated autophagy and lysosome biogenesis	Mutations in PSEN1 and its homologous PSEN2 are the most frequent known causes of early-onset familial AD	52−54
PSEN2	Presenilin 2	Similar to PSEN1	Familial AD associated gene	PSEN1 deficiency inhibits autophagosome and lysosome fusion *via* deplete ER Ca^{2+} content	Mutations in PSEN1 and its homologous PSEN2 are the most frequent known causes of early-onset familial AD	55
PICALM	Phosphatidylinositol binding clathrin assembly protein	Promotes the formation of clathrin-coated pits	Its mutation is associated with an increased risk to develop late-onset AD	Affect both autophagosome formation and autophagosome−lysosome fusion	As a cargo for recruiting APP-CTFβ into autophagosomes for degradation	61,62
CSTD	Cathepsin D	A lysosomal aspartic protease	CTSD variation is a key risk factor for AD	An important lysosomal enzyme	Participated in degradation of Aβ	64,65
PLD3	Phospholipase D3	A 5′ exonuclease that cleavages ssDNA to regulate inflammatory cytokine responses	Mutation increases risk to develop late-onset AD	PLD3 mutations inhibited autophagy possibly *via* activation of mTOR	PLD3 is reduced in the brain of AD patients	68,71
UBQLN1	Ubiquitin-like protein ubiquilin-1	Physically interacts with proteasomes and ubiquitin ligases, and regulates proteasomal-mediated protein degradation	Its polymorphism is associated with AD	UBQLN1 deficiency inhibits autophagosome-lysosome fusion	Reduced in AD patients	75
GRN	Progranulin	A multifunctional glycoprotein	A risk factor for AD and frontotemporal dementia	Affect lysosomal functions	Reduced in the plasma and CSF of patients	76,77
SORL1	Sortilin-related receptor 1	Involved in regulating protein trafficking between the trans-Golgi network and endosomes	SORL1 variants increase risk for developing late-onset AD	Loss of SORL1 protein causes lysosomal dysfunction and inhibits autophagy flux	PSEN1, APP, and SORL1 act within a common pathway for regulating endosome functions	78
CLU	Clusterin	A chaperone that regulates protein folding	A late onset AD risk gene	MAP1LC3B-lipidation and autophagosome biogenesis	The third most significant genetic risk factor for late onset AD	84,85

Direct evidence linking autophagy to AD comes from a study by Nixon and colleagues[93], who found that the massive accumulation of autophagosomes was present in the dystrophic neuritis of AD brains as shown by immuno-electron microscopy analysis. This phenomenon was further verified in the APP/PS1 transgenic AD mice[94]. Importantly, the accumulation of autophagosome in the axon of the hippocampus of APP/PS1 mice was observed much earlier before the synaptic and neuronal loss[95], indicating the causative role of the impaired autophagy−lysosomal pathway in AD pathogenesis. The increased autophagy markers MAP1LC3B-II and SQSTM1/p62, and their colocalization with hyperphosphorylated MAPT in familial AD brains further highlighted the impairment of autophagosome clearance in AD[96,97]. The increased autophagosomes in AD may be due to the impaired lysosomal functions since it has been found that the changes of lysosome hydrolase CTSD/cathepsin D and lysosomal member protein lysosomal-associated membrane protein 1 (LAMP1) levels, and the mislocation of CTSD were found in AD brains[96,97].

In the APP/PS1 AD mouse model, the defective lysosomal proteolysis function was also observed[98], highlighting the impairment of lysosomal degradation capacity in AD.

However, pathological studies from different research groups also show variations and even inconsistent results regarding which steps that autophagy−lysosomal pathway is impaired in AD. This may be due to the dynamic process of autophagy. Dysfunctions of multiple pathological processes may vary at different stages of AD or in certain subpopulations of AD patients. Moreover, so far, the majority of studies mainly focused on neurons in AD, the roles of autophagy lysosome impairment in glia cells are largely unclear.

4.3. Evidence from AD animal models

Mounting evidence from autophagy deficiency animal models have also been established for the critical roles of autophagy in AD (summarized in Table 2 and Fig. 3A). For instance, Inoue et al.[99] found that forebrain specific *Atg7* deficiency mice exerted
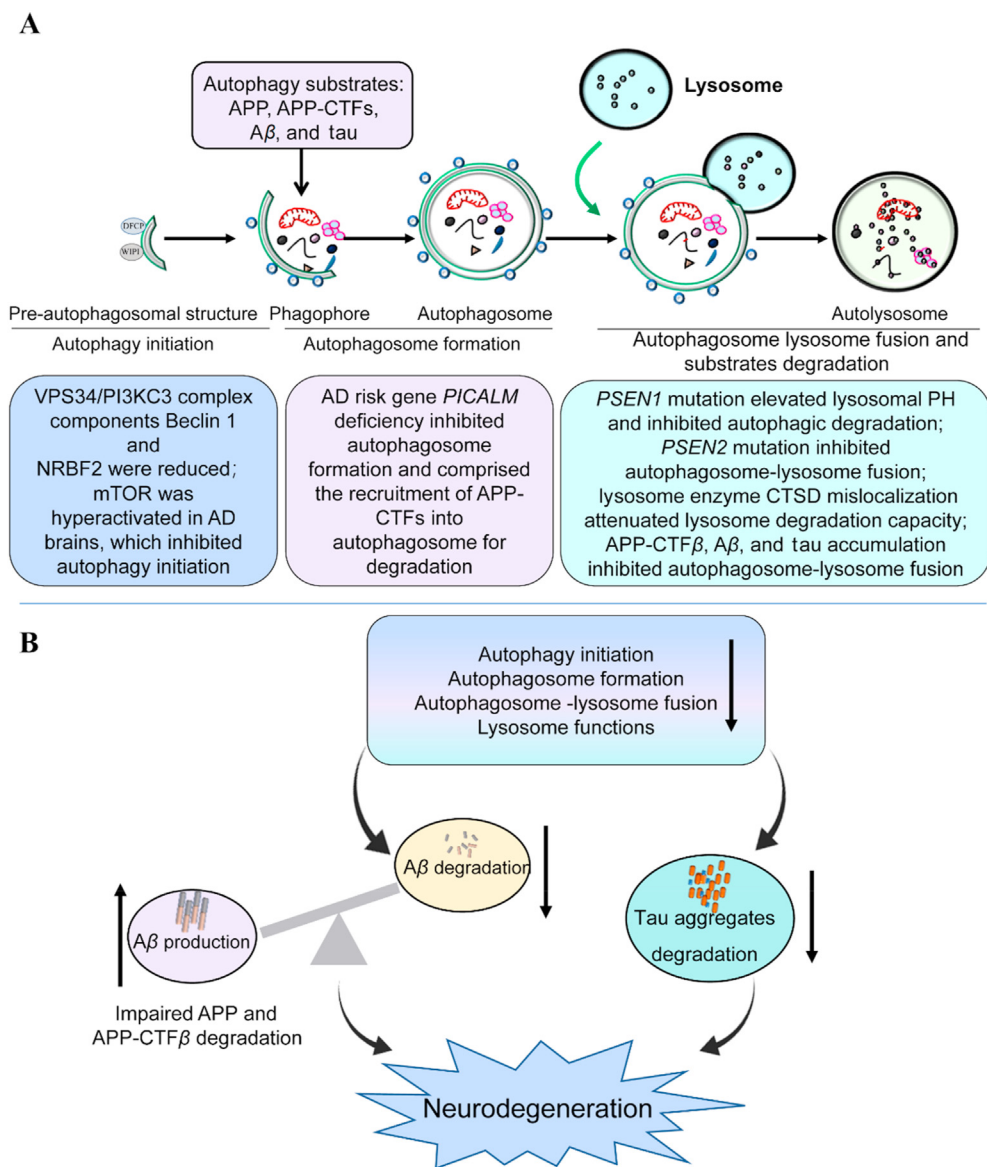
Figure 3 Impairment of autophagy−lysosomal pathway in AD. (A) Autophagy initiation, autophagosomes formation, and autophagosome−lysosome fusion and degradation are impaired in AD. First, the expression of two components of VPS34/PIK3C3 phosphatidylinositol 3-kinase (PtdIns3K) complex BECN1 and NRBF2 is reduced in AD, and hyperactivation of MTORC1 signaling in AD compromises autophagy initiation. Second, an AD risk gene *PICALM* deficiency not only inhibits autophagosomes formation but also comprises the recruitment of APP-CTFβ into autophagosomes for degradation. Third, familial AD associated *PSEN1* mutations enhanced lysosomal pH levels, and lysosome enzyme CTSD/cathepsin D mislocalization attenuates lysosome degradation capacity. *PSEN2* mutations, and autophagic substrates APP-CTFβ, Aβ, and MAPT/tau accumulation in AD have also been shown to inhibit the fusion of autophagosomes with lysosomes. (B) The impairment of autophagosome−lysosomal pathway compromised the degradation of APP and APP-CTFβ, thus promoting Aβ generation. Furthermore, the autophagosome−lysosome pathway dysfunction also inhibits MAPT/tau aggregates degradation. Accumulation of Aβ and MAPT/tau aggregates, two hallmarks of AD, may finally induce neurodegeneration.

an age-related neurodegeneration with the accumulation of autophagy substrates such as ubiquitin and SQSTM1/p62-positive protein aggregates, as well as the accumulation of phospho-MAPT. Importantly, inhibition of MAPT phosphorylation attenuated neurodegeneration in forebrain specific *Atg7* KO (knock out) mice[99]. These results highlighted the role of autophagy and phosphorylated MAPT in neurodegeneration. By using an APP transgenic mouse with excitatory neurons specific knock out of *Atg7*, it has been found that autophagy deficiency exacerbated neurodegeneration possibly *via* affecting Aβ secretion though the

underlying mechanisms are not fully understood[100,101]. In microglial cell, it has been shown that autophagy plays an important role in the degradation of Aβ fibrils *via* autophagy receptor OPTN/Optineurin, and microglial specific *Atg7* deficiency mice showed increased neuroinflammation upon exposure to exogenous Aβ fibrils[102]. Similarly, neural cells specific *Atg5* KO mice exerted neurodegeneration with motor dysfunction and accumulation of protein aggregates[103]. Apart from essential autophagy genes, beclin 1 and NRBF2, two components of VPS34/PI3C3 complex for autophagosome formation have also

Table 2 Animal models show links between autophagy deficiency and AD.

Gene name	Function in autophagy	Animal model	Phenotype associated with AD	Ref.
Atg7	An essential gene for MAP1LC3B lipidation	Forebrain-specific *Atg7* KO mice	These mice displayed accumulation of SQSTM1- and ubiquitin-positive inclusion, and phospho-MAPT protein; and showed aging-related neurodegeneration	99
Atg7	An essential gene for MAP1LC3B lipidation	APP transgenic mice with excitatory neuron-Specific *Atg7* deficiency	Exacerbated neurodegeneration; inhibited Aβ secretion and enhanced the intraneuronal accumulation of Aβ in Golgi	100,101
Atg7	An essential gene for MAP1LC3B lipidation	Microglia specific *Atg7* KO mice	Optineurin-mediated autophagic-degradation of Aβ fibrils in microglial cells and injection of extracellular Aβ fibrils increased neuroinflammation in microglial *Atg7* KO mice	102
Atg5	An essential gene for MAP1LC3B lipidation	Neural cells specific *Atg5* KO mice	Deficits in motor functions and accumulation of cytoplasmic inclusion bodies in neurons	103
Becn1	Regulates autophagosome formation	APP transgenic mice with heterozygous *BECN1* deficiency	Reduced neuron autophagy; increased Aβ deposition, and induction of neurodegeneration	89,92
Nrbf2	Regulates autophagosome formation	*Nrbf2* KO mice	*Nrbf2* deficiency resulted in the accumulation of Aβ in the hippocampus; memory and LTP deficits	90

been reported regulating Aβ and neurodegeneration[89,92]. For instance, aged *Nrbf2* KO mice with neuronal autophagy impairment, Aβ accumulation, and memory and LTP (long-term potentiation) deficits, further highlighting the importance of autophagy in AD. Notably, apart from modulating autophagy, autophagy-independent function of NRBF2 may also be involved in modulating learning and memory. Overall, these evidence from autophagy deficient mouse established critical links between autophagy impairment and AD pathogenesis.

Apart from canonical autophagy, defects in mitophagy have been implicated in the pathogenesis of multiple diseases including AD[11,104−106]. Mitophagy is a process for specifical degradation of damaged or superfluous mitochondria *via* lysosomes[107−111]. In mammals, a variety of proteins and pathways such as PINK1/parkin are identified to be necessary for mitophagy[112−116]. The accumulation of dysfunctional mitochondrial and impairment of mitophagy are commonly found in AD patients and AD animal models[11,104]. For instance, AD-associated APP mutation and Aβ accumulation caused mitophagy defective in AD animal models[117]. Defective mitophagy exaggerated Aβ and tau pathologies possibly *via* increasing oxidative stress and inducing energy deficits, and these events may finally contribute to synaptic dysfunction and cognitive deficits in AD[11,104,105]. In contrast, induction of mitophagy alleviated AD-associated-pathologies and improved memory deficits in multiple AD animal models[11,104,105]. Collectively, comprised mitophagy is critical for AD pathogenesis and induction of mitophagy may represent a promising therapeutic strategy.

5. Interplays between the autophagy−lysosomal pathway and Aβ and MAPT

5.1. The autophagy−lysosomal pathway participates in regulating Aβ homeostasis

Autophagy is crucial in degrading APP and its metabolites include APP-CTFβ and Aβ. It has been reported that overexpression of

TFEB to enhance autophagy and lysosomal biogenesis promotes APP degradation[118]. Similarly, APP-CTFβ was reported to be degraded in an autophagy-dependent manner[119]. Aβ is accumulated in the autophagosomes, which can be incorporated into autolysosomes for degradation *via* Aβ-specific degrading protease CTSD[93,97]. Interestingly, autophagic cargo protein OPTN/optineurin plays a critical role in degradation of extracellular Aβ fibrils by phagocytosis of microglia[102]. Though ubiquitin proteasome system (UPS) has also been involved in the degradation of Aβ, it was reported that proteasome only degrades monomeric Aβ$_{42}$ and low-molecular weight Aβ$_{42}$ oligomers. In contrast, autophagy degrades both the monomeric and high molecular weight Aβ aggregates[120]. These findings highlight the critical role of autophagy rather than UPS in promoting Aβ plaque degradation. Though several genes such as RRD1, SNF4, GCN4 and SSE1 were shown as critical regulators for autophagy-mediated Aβ$_{42}$ degradation in yeast[120], whether their homologous in mammalian cells also play a similar role is unclear. These results indicated that APP, APP-CTFβ and Aβ are autophagy substrates. BECN1 and NRBF2 are two main components of VPS34/PI3KC3 complex and they are crucial in regulating autophagy initiation[29,35]. It has been shown that *BECN1* overexpression triggered autophagy and reduced Aβ plaques and subsequently AD pathology in a transgenic mouse expressing human APP, and *vice versa* (Fig. 3)[91,106]. We recently found that NRBF2 not only regulates autophagosome biogenesis but also modulates autophagosome-lysosome fusion *via* modulating RAB7 activities. Interestingly, NRBF2 was reduced in the hippocampus of AD patients and 5XFAD transgenic mice[90,91], and NRBF2 interacted with APP and was essential for NRBF2-mediated APP-CTFβ degradation *via* autophagy[37,91]. Specifically, NRBF2 interacts with the CCZ1/MON1A complex to facilitate RAB7 activation, and NRBF2 also facilitated the interaction of APP with CCZ1/MON1A/RAB7 complex, which is important for the maturation of autophagosome containing APP-CTFβ for degradation, as such NRBF2-dependent autophagy is crucial for the maintenance of Aβ contents[37]. Furthermore, autophagy was also

reported modulating Aβ secretion[100]. Nilsson and colleagues[100] found that specific neuronal deletion of *ATG7*, an essential gene required for autophagosome formation, inhibited Aβ secretion and increased intraneuronal Aβ accumulation and exacerbated neurodegeneration in an AD mouse model Collectively, these results demonstrate that autophagy plays a major role in regulating both APP/APP metabolites degradation and Aβ secretion.

Importantly, the enhancement of autophagy has been demonstrated to reduce Aβ plaque formation and ameliorate memory deficits in multiple transgenic AD animal models. Activation of mTOR was found in AD mouse models[121,122], and genetic ablation of mTOR in Tg2576 mice not only induced autophagy but also Aβ deposits and rescued memory deficits[123,124]. Similarly, pharmacological inhibition of mTOR-mediated autophagy activation improved cognitive deficits and reduced Aβ levels in several AD transgenic mouse models that represent Aβ pathology[124,125]. Overall, these results indicate that autophagy is critical for maintaining Aβ homeostasis.

5.2. The autophagy−lysosomal pathway participates in regulating MAPT homeostasis

In addition to regulating APP/APP metabolites degradation, autophagy is also critical in modulating MAPT aggregation, phosphorylation and degradation. Several studies[126,127] have demonstrated that suppression of autophagy with 3-methyladenine (3-MA) or inhibition of lysosomal functions with lysosome inhibitor chloroquine (CQ) induced the formation of MAPT oligomers and aggregates formation, indicating that autophagy−lysosomal pathway regulates MAPT aggregates formation. In addition, brain specific deletion of *Atg7* resulted in autophagy deficiency, and subsequent accumulation of phosphorylated MAPT protein aggregates and neurodegeneration in mice, which may be due to the increased GSK3B/GSK3β, a main MAPT phosphorylation kinase in *Atg7* KO mice[99]. This finding indicates that autophagy may also affect MAPT phosphorylation status. Furthermore, the accumulation of MAPT in autophagosomes was found and MAPT can be degraded after the fusion of autophagosomes with lysosomes[128,129]. CTSD/cathepsin D is a critical enzyme for MAPT cleavage, and deletion of *Ctsd* resulted in MAPT accumulation and consequently neurodegeneration[130−132]. NDP52 (nuclear dot protein 52), an autophagy adaptor protein that is transcriptionally-induced by NRF2 (nuclear factor erythroid 2-related factor 2), was responsible for autophagy-dependent phosphorylated-MAPT degradation[133], further highlighting the critical role of autophagy−lysosomal pathway in promoting MAPT degradation. As aforementioned, the AD risk gene *PICALM* is critical for modulating autophagy, and its depletion increased the impairment of autophagy−lysosomal pathway and subsequent induction of MAPT aggregation and exaggeration of MAPT pathology in AD animal models[62], further indicating the role of defective *PICALM*-regulated autophagy−lysosome in MAPT-mediated AD pathogenesis. Furthermore, apart from phosphorylated MAPT, autophagy also plays a critical role in promoting acetylated MAPT degradation both *in vitro* and in animal brains and knockout *Atg7* in mouse brains increased acetylated MAPT[134].

Conversely, induction of autophagy facilitates the degradation of phosphorylated MAPT aggregates and acetylated tau and alleviates MAPT-induced pathology in multiple AD animal models[134,135]. Rapamycin treatment restored autophagy flux, reduced insoluble phosphorylated MAPT and MAPT tangle, and alleviated memory deficiency in MAPT transgenic AD mice[136,137], suggesting that pharmacological inhibition of mTOR to induce autophagy may effectively promote MAPT clearance in mice. Furthermore, genetic activation of autophagy and lysosome biogenesis by overexpression of *Tfeb*, a master regulator of autophagy and lysosome biogenesis, promoted the degradation of hyperphosphorylated and misfolded MAPT and rescued neurotoxicity in a tauopathy mouse model[138]. Overall, activation of autophagy−lysosomal pathway is critical for promoting MAPT aggregates degradation and attenuating MAPT-induced neurodegeneration.

Collectively, impairments of multiple stages in autophagy−lysosome pathway including autophagosomes formation, autophagy−lysosomes fusion, and lysosomal function lead to the accumulation of APP/APP-CTFβ, thereby promoting Aβ generation. Furthermore, autophagosome−lysosome dysfunction also results in the accumulation of MAPT aggregates. Accumulation of Aβ and MAPT aggregates, two hallmarks of AD, may finally induce neurodegeneration (Fig. 3B).

5.3. The effects of Aβ and MAPT on the autophagy−lysosome pathway

Though autophagy−lysosomal pathway plays a major role in modulating APP/APP metabolites and MAPT degradation, APP/APP metabolites (APP-CTFβ, Aβ) and MAPT also affect multiple steps of autophagy−lysosomal pathway. As aforementioned, APP-CTFβ is accumulated in AD patient brains and multiple AD animal models, which can be degraded by autophagy−lysosomal pathway. Paradoxically, APP-CTFβ overexpression could induce impairment of autophagy flux in mouse brains evidenced by accumulated MAP1LC3B-II and SQSTM1/p62, and this function is independent of Aβ since inhibition of γ-secretase to reduce the production of Aβ did not restore autophagy flux[139]. Furthermore, overexpression of mutant APP induced the accumulation of Aβ, which not only induced neurodegeneration but also induced autophagy inhibition with downregulated expression of autophagy markers including ATG5 in the hippocampus, suggesting that APP/Aβ may also inhibit autophagosome biogenesis[140]. Similarly, Aβ was reported to activate mTORC1, a negative regulator of autophagy, *via* promoting the phosphorylation of PRAS40 (proline-rich Akt substrate 40)[141], suggesting that Aβ may inhibit autophagy initiation. Additionally, $A\beta_{42}$ was reported to induce the accumulation of autophagosomes and contribute to neurodegeneration in fruit flies[142,143]. Apart from neurons, accumulation of Aβ also impairs autophagy in microglia cells though the underlying mechanisms are not fully addressed[144]. These results highlight that APP/APP metabolites are also critical for inducing the impairment of autophagy−lysosomal pathway. It would be interesting to understand the underlying molecular mechanisms of how APP/APP metabolites modulate autophagy−lysosomal pathway.

Apart from APP/APP metabolites, hyperphosphorylation of MAPT also causes autophagy and lysosome dysfunction. Microtubule has been well-established in promoting autophagosomes retrograde trafficking and subsequent autophagosome and lysosome fusion[145]. MAPT is essential in maintaining the stability of microtubules in axons, but phosphorylated MAPT in AD was unable to stabilize microtubules, and thus MAPT may inhibit autophagosomes movement and its subsequent fusion with lysosomes in neurons[146−148]. Furthermore, a recent study showed that MAPT accumulation inhibited the formation of endosomal sorting

complex transport-III (ESCRT-III), which is required for autophagosome−lysosome formation, by downregulating the expression of ESCRT-III associated factor *Ist1* (IST1 factor associated with ESCRT-III)[149]. MAPT overexpression also induced lysosomal aberrations in mice[150]. Overall, these results indicate that MAPT accumulation may result in the impairment of autophagy−lysosomal pathway. Together, Aβ and MAPT accumulation results in impairment of autophagy−lysosomal pathway. The impairment of autophagy−lysosomal in AD will lead to the accumulation of Aβ and MAPT aggregates, which further exacerbate the autophagy−lysosomal dysfunction. Thus, APP/APP metabolites and MAPT aggregates accumulation and impairment of autophagy−lysosomal form a vicious worse cycle, which may finally induce the formation of Aβ plaques and NFTs, and contribute to neurodegeneration in AD (Fig. 4).

6. Pre-clinical animal models for AD treatment with small molecule autophagy enhancers targeting the autophagy−lysosomal pathway

Given the importance of Aβ and MAPT in AD pathogenesis, and autophagy induction not only reduces the levels of both Aβ and MAPT but also alleviates AD pathology in multiple animal models, activation of autophagy represents a promising strategy for AD treatment. Multiple strategies targeting autophagy induction by small molecules have been shown to exert neuroprotective effects in AD animal models, including modulating upstream kinase mTOR and AMPK for autophagy induction, targeting autophagy components, activating TFEB, directly targeting lysosomes and other targets for enhancing autophagy. The following section highlights neuroprotective effects of autophagy enhancers in preclinical *in vivo* AD animal models, which are summarized in Table 3 and Fig. 5.

6.1. mTOR inhibitors

Rapamycin induced autophagy by inhibiting the mTORC1 pathway is one of the most thoroughly tested strategies for combating neurodegeneration including AD. Rapamycin is an immunosuppressant drug to prevent graft rejection and is also used for treating lymphangioleiomyomatosis[151]. Rapamycin promotes autophagy *via* binding the cytosolic protein FKBP1A/FKBP12 (FK-binding protein 12)[151]. It has been shown that rapamycin treatment lowered Aβ and MAPT levels, and rescued memory deficits in multiple AD animal models including 3XTg, P301S MAPT, and hAPP(J20) mice[125,136,152,153]. Rapamycin treatment also improved vascular and metabolic deficits in apolipoprotein E4 transgenic mice with pre-symptomatic AD though whether this effect was attributed to autophagy induction remains unclear[154]. However, chronic rapamycin treatment induces certain side effects such as glucose intolerance and hyperlipidemia, which may be due to its effects on inhibiting mTORC2. In addition, the anti-AD effect of rapamycin may also be involved in other pathways since rapamycin is a non-autophagy-specific compound. As such, more specific mTORC1 inhibitors, rapamycin paralogues, have been developed. Among them, everolimus and temsirolimus show improved effects, which have been approved by FDA for treating tuberous sclerosis and renal cell carcinoma, respectively[155]. Interestingly, everolimus is more stable than rapamycin in mice brains[156]. Everolimus inhibits mTOR in animal brains, reduces Aβ and tau levels, and improves cognitive deficiency in 3XTg transgenic AD mice[156]. Temsirolimus has also been shown

to induce autophagy by inhibiting mTOR in mice brains, which may contribute to its roles in reducing Aβ and MAPT, and improving motor deficit in multiple AD mice including APP/PS1[157], P301S[158], and Tg30 mice[159]. These findings demonstrate that everolimus and temsirolimus may be promising anti-AD candidates. In addition, an antihistamine drug latrepirdine (Dimebon) has also been demonstrated to activate autophagy *via* mTOR inhibition though it has multiple targets[160,161]. In TgCRND8 transgenic AD mice, latrepirdine was shown to improve cognitive impairment and Aβ neuropathology as well as restore autophagy impairment[160,161].

6.2. AMPK activators

Apart from mTOR inhibition, activating AMPK is another important way to induce autophagy. Multiple small molecules activation of AMPK such as metformin, resveratrol, and berberine[162] exert neuroprotective effects in AD animal models *via* autophagy induction. Metformin is the first-class anti-diabetic drug that can activate AMPK, and it has been demonstrated to exert neuroprotective effects in AD animal models including SAMP8 and APP/PS1 mice[163]. Metformin treatment also improved memory deficiency, reduced Aβ plaque loading and ptau levels in several AD animal models[164,165]. However, whether this neuroprotective effect depends on metformin-mediated autophagy induction *via* activating AMPK is unclear and its role in AD is controversial as a study also showed that metformin exaggerated AD pathology in P301S MAPT mice[166]. Future in-depth studies on exploring the effects and underlying mechanisms of metformin in AD are highly desired. Notably, metformin is a non-autophagy specific compound, and thus its beneficial effects in AD could also be partially attributed to other pathways. Resveratrol (3,5,4′-trihydroxystilbene), a natural polyphenol widely distributed in edible food including wide wine, peanut, blueberries, and grapes, is a caloric mimetic that has multiple biological activities[167]. It is an AMPK activator and directly binds to and activates SIRT1 to induce autophagy[167]. In the APP/PS1 transgenic AD mice, resveratrol has been shown to activate AMPK and reduce Aβ levels[168], suggesting a potential autophagy dependent neuroprotective effects of resveratrol. Notably, though resveratrol is an autophagy enhancer, autophagy-independent neuroprotective effects in AD animal models have also been reported, including anti-inflammation, anti-oxidant, and promoting non-amylogenic APP processing[169]. Berberine is widely distributed in botanical medical plants including *Coptis chinensis* and *Hydrastis canadensis*[170]. Berberine has multiple biological activities including metabolic anti-diabetes and anti-hypercholesterolemia, which may attribute to its effects on activation of AMPK[170,171]. In AD, berberine improved spatial learning capacity and memory retention, induced autophagy, and promoted the degradation of Aβ, APP, and aggregated MAPT levels in the 3XTg mice[172,173]. These findings demonstrate that multiple AMPK-dependent autophagy enhancers have neuroprotective effects in AD animal models.

6.3. TFEB activators

As aforementioned, upon activation, TFEB induces the expression of multiple autophagy- and lysosomal-related genes[41,174]. Genetic overexpression of TFEB alleviates AD disease progression *via* promoting both Aβ and MAPT degradation through the autophagy−lysosomal pathway in multiple AD animal models[118,175−177]. Additionally, the impairment of
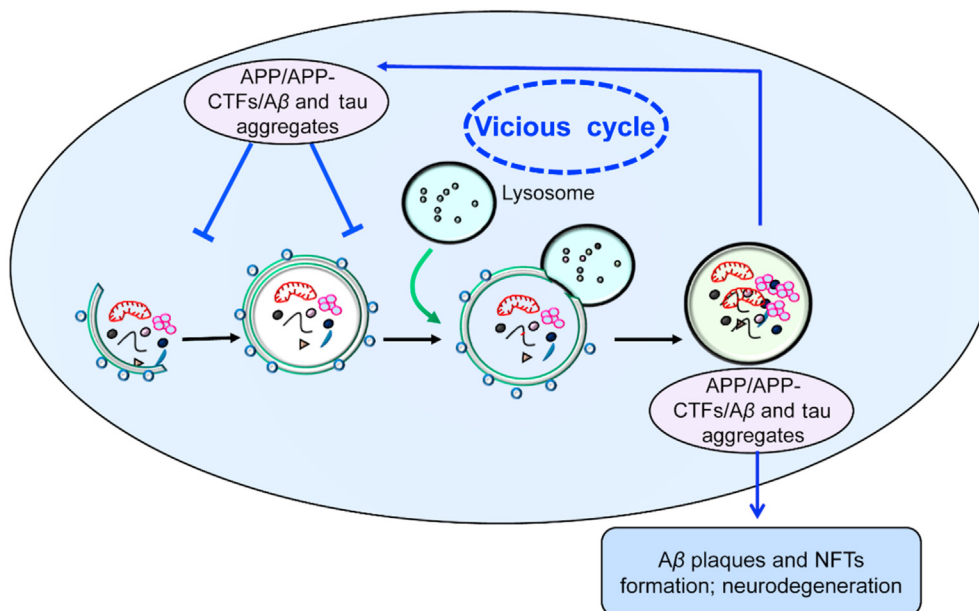
Figure 4 A vicious cycle between the autophagy−lysosomal dysfunction and accumulation of APP/APP metabolites and MAPT/tau aggregates in AD. The impairment of autophagy−lysosome pathway (such as genetic factor) in AD compromises the degradation and subsequent accumulation of APP, APP-CTFβ, Aβ, and MAPT/tau aggregates, which further induces the impairment of autophagy−lysosome pathway (*e.g.*, inhibit autophagosome formation, and autophagosome−lysosomes fusion). Thus, a vicious cycle of autophagy−lysosomal dysfunction and accumulation of APP/APP metabolites and MAPT/tau aggregates are formed, which may finally contribute to neurodegeneration.

autophagy−lysosomal pathway has been implicated in the pathogenesis of AD, further indicating that upregulation of TFEB to enhance autophagy and lysosomal biogenesis, thereby degradation of both Aβ and MAPT at the same time serves as a promising therapeutic strategy for AD treatment[175]. For instance, multiple TFEB activators have been identified to exert neuroprotective effects in AD animal models. Trehalose is a natural disaccharide that is commonly used as a preservative, humectant, and nutraceutical. Trehalose was reported to activate TFEB-mediated autophagy and lysosomal biogenesis[178,179]. It has been shown that trehalose alleviated AD pathology by reducing Aβ contents and attenuating the impaired cognitive and learning ability in multiple animal models[180,181]. Curcumin and its analogues have been reported to activates TFEB-mediated autophagy and lysosomal biogenesis[182−185]. Curcumin analogue C1 was reported to activate TFEB-mediated autophagy and lysosome biogenesis independent of mTORC1 inhibition[182,183]. C1 further attenuated both Aβ and MAPT pathology in 5XFAD, 3XTg and P301S MAPT mice by activation of TFEB[182,183]. HEP14 (5β-*O*-angelate-20-deoxyingenol) was demonstrated to bind to and activated PKCα and PKCδ, which inactivated GSK3B/GSK3β and facilitated TFEB dephosphorylation and activation[186]. Importantly, HEP14-mediated TFEB activation alleviates Aβ plaques in the brain of APP/PS1 mice[186]. TFEB can be transcriptionally upregulated by PPARA/PPARα activation[187]. Several small molecules such as aspirin, gemfibrozil, Wy14643, and cinnamic acid were reported to enhance TFEB-mediated autophagy and lysosome biogenesis by activation of PPARα[186,188−190]. These TFEB activators were also reported alleviating Aβ pathology in AD animal models as listed in Table 3[191,193−195]. Gypenoside XVII is a major saponin in ginseng, which was reported to activate TFEB-mediated autophagy and lysosome biogenesis[191] though the underlying mechanism is not fully understood. Interestingly,

gypenoside XVII not only improved spatial learning and memory deficits but also reduced Aβ plaques in APP/PS1 mice, which may be related to its role in promoting TFEB-mediated autophagy and lysosomal biogenesis[191]. Ouabain, a cardiac glycoside, was identified as a TFEB activator by high throughput screening. This compound was further demonstrated to enhance TFEB-mediated autophagy−lysosomal pathway by inhibiting mTOR, and reducing phosphorylated MAPT in transgenic AD flies and P301S transgenic mice[192]. Several other small molecule TFEB activators such as fisetin[193] and flubendazole[194] have also been shown to reduce phosphorylated MAPT in AD cell lines, future studies are required to test their effects in AD animal models. Collectively, these results suggest that pharmacological activation of TFEB by small molecules may represent a novel strategy to treat AD.

6.4. Restoration of lysosomal functions

Impairment of lysosome functions has been implicated in AD pathogenesis and thus directly restoring lysosomal functions has recently emerged as a promising strategy for AD treatment. Notably, PLGA-aNP (acidic nanoparticles of poly[D,L-lactide-*co*-glycolide]) lowered lysosomal pH, and restores lysosomal function in multiple cell models of neurodegenerative diseases associated with lysosomal dysfunction[195]. This nanoparticle also alleviated neuron loss in a Parkinson's disease animal model, highlighting its potential therapeutic effect *in vivo*[195]. Importantly, PLGA-aNP restores lysosomal functions and protects Aβ-induced toxicity in neurons[196], indicating the therapeutic potential of restoring lysosomal functions by PLGA-aNP in AD.

Recently, the impairment of ER (endoplasmic reticulum)-to-lysosome delivery of H^+/Cl^- exchange transporter chloride channel-7 (ClC-7) has been reported to play critical roles in PSEN1 deficiency and mutation cells with elevated lysosomal pH

Table 3 Autophagy enhancers tested in pre-clinical AD animal models.

Compound	Mechanism of action	Drug target	AD animal model	Main effects	Ref.
Rapamycin	mTORC1 inhibition	FKBP12	3XTg	Improved cognitive deficits and ameliorated Aβ and MAPT pathology Inhibited MAPT-induced neuronal loss, synaptotoxicity, and neuroinflammation Reduced cortical MAPT tangles, lowered hyperphosphorylation and insoluble MAPT levels	153,216
			P301L MAPT	Reduced Aβ$_{42}$ levels, improved learning and memory deficits	136,152
			PDAPP [hAPP(J20)]		125
Everolimus	mTORC1 inhibition	FKBP12	3XTg	Reduced Aβ and MAPT levels, attenuated cognitive deficit	156
Temsirolimus	mTORC1 inhibition	mTOR	APP/PS1	Reduced Aβ levels, induced autophagy, inhibited neuron apoptosis, and improved spatial cognitive functions Lowered hyperphosphorylation MAPT levels, rescued spatial learning and memory impairment	157
			P301S	Increased autophagy, reduced phosphorylated MAPT levels and neurofibrillary tangles	158
			Tg30		159
Latrepirdine	mTORC1 inhibition	Unknown	TgCRND8	Improved memory decline and reduced Aβ plaque	161
Metformin	AMPK activation	AMPK	SAMP8	Improved learning and memory, decreased Aβ and phosphorylated MAPT Decreased Aβ plaque load, inhibited inflammation	164
			APP/PS1		165
Resveratrol	AMPK activation	SIRT1	APP/PS1	Activated AMPK and reduced brain Aβ levels	168
Berberine	AMPK activation	Unknown?	3XTg	Improved spatial learning capacity and memory retention, induced autophagy and reduced Aβ, APP, and MAPT levels	172,173
Trehalose	TFEB activation	unknown	APP/PS1	Reduced Aβ deposit in hippocampus, and alleviated cognitive and learning ability Improved learning and memory	181
			Tg2576		180
C1 (Curcumin analogue)	TFEB activation	TFEB	5XFAD, 3XTg, P301S	Increased autophagy and lysosome biogenesis, improved learning and memory, decrease Aβ and phosphorylated MAPT	182,183
HEP14 (5β-O-angelate-20-deoxyingenol)	TFEB activation	PKC	APP/PS1	Activated TFEB, and ameliorated Aβ plaque formation	186
Aspirin	TFEB activation	PPARα	5XFAD	Decreased amyloid plaque pathology in a PPARα dependent manner	190
Gemfibrozil, Wy14643	TFEB activation	PPARα	APP-PSEN1ΔE9	Rescued cognitive and anxiety symptoms, reduced Aβ levels	188
Cinnamic acid	TFEB activation	PPARα	5XFAD	Reduced Aβ plaque burden, improved memory	189
Gypenoside XVII	TFEB activation	Unknown	APP/PS1	Improved spatial learning and memory deficits, reduced Aβ plaque formation	191
Ouabain	TFEB activation	Unknown	P301S transgenic AD flies and mice	Improved memory impairment and reduced phosphorylated MAPT	192

Table 3 (*continued*)

| Compound	Mechanism of action	Drug target	AD animal model	Main effects	Ref.
Lithium chloride (LiCl)	mTORC1-independent (inositol depletion)	IMPase	APP/PS1	Improved cognitive impairment and promoted the clearance of Aβ; Prevented MAPT hyperphosphorylation and NFT formation	202
			FTDP-17 MAPT mice		203
NAD⁺ precursor nicotinamide mononucleotide	Mitophagy inducer	NAD	*C. elegans*	Induced neuronal mitophagy and alleviated cognitive decline	104
UA	Mitophagy inducer	Unclear	APP/PS1; 3XTg	Induction of mitophagy, reduce Aβ in APP/PS1 mice and alleviated p-tau in 3XTg mice, improve memory deficiency in these two mouse models	104
Carbamazepine	mTORC1-independent (inositol depletion)	Unkown	APP/PS1	Improved spatial learning and memory deficits, and reduced Aβ plaque formation	206
PD146176	mTORC1-independent	12/15-Lipoxygenase inhibition	3XTg	Improved cognitive impairment, alleviated both Aβ and MAPT pathology	209

levels[197]. Interestingly, β_2-adrenergic agonists such as isoproterenol could restore lysosomal CLCN7/ClC-7 (chloride voltage-gated channel 7) levels and subsequent lysosome acidification in PSEN1 deficient cells[197]. Activation of β_2-adrenergic by clenbuterol could improve memory deficits in APP/PS1 mouse model of AD[198] though whether the underlying mechanism is related to the restoration of lysosomal PH is unclear. Overall, these studies underscore the potential of directly correcting lysosomal acidification deficits for therapy in AD and possibly in other autophagy-related neurodegenerative diseases.

6.5. Other autophagy enhancers

In addition to modulating canonical signaling pathways such as mTOR and AMPK for autophagy induction, a variety of other small molecules have also been implicated in inducing autophagy in an mTOR- and AMPK-independent manner. Among them, lithium chloride (LiCl), a drug for treating mental illnesses including bipolar disorder, has been shown to activate autophagy by inhibition of inositol monophosphatase (IMPase), which reduces free inositol and IP3 (myo-inositol-1,4,5-triphosphate) levels to induce autophagy[199]. In multiple animal models of AD, lithium chloride has been shown to exert neuroprotective effects[200,201]. For instance, lithium chloride treatment was reported to improve cognitive impairment and promote clearance of Aβ in APP/PS1 mice[202]. In mice overexpressing FTDP-17 (frontotemporal dementia and parkinsonism linked to chromosome 17) MAPT and GSK3B/GSK-3β, lithium chloride treatment inhibited MAPT hyperphosphorylation and NFTs formation[203], suggesting that lithium chloride could also reduce phosphorylated MAPT levels. However, apart from autophagy induction, we cannot exclude other mechanisms that contribute to its neuroprotective effects, such as lithium chloride-mediated inhibition of GSK3B/GSK-3β and Aβ aggregation formation[200,201]. Another mood-stabilizing drug carbamazepine has also been reported to induce autophagy *via* reducing inositol levels[204,205]. In the APP/PS1 mice, carbamazepine induced autophagy, improved spatial learning and memory deficits, reduced Aβ levels[206].

As mentioned above, defective mitophagy is linked to AD pathogenesis, and several small molecule mitophagy activators were shown to effectively improve memory deficits in AD mouse model[104]. Induction of mitophagy by NAD⁺ precursor (*e.g.*, nicotinamide mononucleotide) alleviated cognitive decline in *C. elegans* models of AD[104]. In the APP/PS1 mice, induction of mitophagy by urolithin A (UA) was shown to remove Aβ plaques, alleviate neuroinflammation and memory dysfunction[104]. In 3XTg mice, urolithin A was also shown to alleviate tau pathology and improve cognitive deficits[104]. These results indicated that mitophagy inducers such as NAD⁺ precursor and UA are promising anti-AD agents.

12/15-Lipoxygenase (12/15-LOX) is an endogenous enzyme that plays an important role in the oxidization of polyunsaturated fatty acids to produce many bioactive lipid metabolites such as 5-hydroperoxyeicosatetraenoic acid[207]. Recent studies revealed that genetic ablation or pharmacological inhibition of 12/15-lipoxygenase promoted autophagy[208], though the underlying mechanisms are not fully understood. Interestingly, a selective 12/15-lipoxygenase inhibitor PD146176 reduced the levels and deposition of Aβ, and MAPT neuropathology in 3XTg mice, and this neuroprotective effect may be mediated *via* activation of neuronal autophagy[209]. Notably, 3XTg mice over-expressing 12/15-lipoxygenase showed an exacerbation of AD pathology, which was accompanied by impairment of autophagy, further highlighting that induction of autophagy *via* inhibiting 12/15-lipoxygenase may represent a novel strategy for AD treatment[210]. Apart from the above compounds mentioned with *in vivo* validation for anti-AD effects, there are still multiple other agents that have been demonstrated to activate autophagy in cell lines but have not yet been investigated for their neuroprotective effects in AD animal models as reviewed elsewhere[9,211]. For instance, a natural compound corynoxine B has been reported to activate autophagy in a beclin 1-dependent manner and this small molecule promoted alpha-synuclein degradation and alleviated PD-pathology in fruit flies[212,213], but its effects in AD mouse models are awaiting further exploration. In addition, non-small molecules autophagy enhancers have also been developed. The
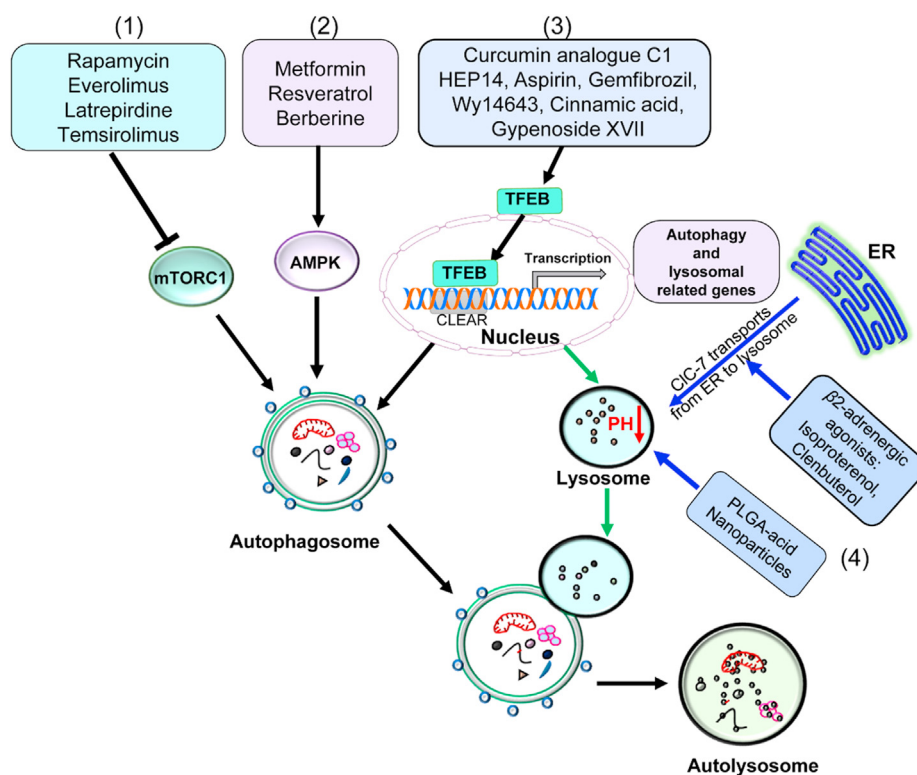
Figure 5 Strategies targeting the autophagy−lysosomal pathway for potential AD treatment. Targeting of upstream autophagy signaling such as (1) activation of AMPK (metformin, resveratrol, berberine) or (2) inhibition of MTORC1 (rapamycin, everolimus, temsirolimus, latrepirdine) can promote autophagosomes formation (3) Small molecules that activate TFEB (*e.g.*, curcumin analogue C1, HEP14, aspirin, gemfibrozil, Wy14643, cinnamic acid, and gypenoside XVII). Not only promote autophagy flux but also enhance lysosome functions, which may represent promising anti-AD agents. (4) Strategies through direct enhancing lysosomal functions including inhibition of ClC-7 transporter (*β*-adrenergic agonists: isoproterenol, clenbuterol) and acid nanoparticles. Importantly, above mentioned small molecule autophagy enhancers have been shown to reduce A*β* and/or MAPT/tau aggregates and alleviate memory deficiency in AD animal models, and some of them (*e.g.*, metformin) have shown promising results in clinical trials.

peptide Tat-beclin 1, was identified to activate autophagy both *in vitro* and *in vivo* and improve phenotypes of proximal and distal defects of the urea cycle in mice[214,215], though its effects in AD animal models are unclear. Overall, these results demonstrate that autophagy enhancers independent of AMPK and mTOR may also represent novel anti-AD candidates.

Collectively, multiple strategies can enhance autophagy by small molecules mediated regulation of various pathways including activation of AMPK, inhibition of mTOR, activation TFEB, or direct restore lysosomal functions to enhance autophagy, which has shown promising results in AD animal models (Fig. 5). However, it should be noted that the most above-mentioned autophagy activators have off-target effects. Therefore, whether autophagy indeed contributes to the neuroprotective effects still need further clarified in the future.

7. Clinical trials of autophagy enhancers for AD treatment

Notably, a variety of above-mentioned small molecule autophagy activators have been conducted or being tested for their efficacy in AD patients (Table 4). For instance, one of the most comprehensive investigated autophagy enhancers lithium was shown to exert potential beneficial effects on cognitive deficiency in patients with MCI (amnestic mild cognitive impairment, NCT01055392). In this study, patients with MCI received

lithium or placebo for 2 years, and a followed-up study for an additional 2-years. This study showed that the placebo control group showed a mild but significantly cognitive decline as reflected by total ADAS-Cog (The Alzheimer's Disease Assessment Scale−Cognitive Subscale) and CDR-SoB (the Clinical Dementia Rating scale) scores, but the lithium treatment group remain stably over 24 months[217]. Lithium treatment resulted in a significant increase in CSF A*β* contents[217]. These results indicate that lithium may be a potentially effective drug for MCI-AD patients. A phase IV clinical trial for investigating the effects of lithium in preventing cognition impairment in the elderly is being tested (NCT03185208).

Metformin is a classical anti-diabetic drug that activates AMPK. Pilot studies[218,219] have shown that the classical anti-diabetic agent metformin is a safe, well-tolerated agent, which attenuated certain cognitive decline (*e.g.*, reminding Test in the ADAS-Cog) in AD patients. However, its anti-AD effects are being tested on a relatively large-scale phase II/III trail (estimated to recruit 370 patients) (NCT04098666).

Although multiple studies in animal models showed that latrepirdine had anti-AD effect, and an initial 6-month phase II clinical study reported that latrepirdine improved cognitive dysfunction compared with placebo control[220], later studies conducted by Pfizer and Medivation found that this drug failed to improve cognitive deficit and further study was terminated

(NCT00838110, NCT00912288). The failure of this drug for treating AD may be due to latrepirdine having multiple functions unrelated to autophagy, which include blocking L-type calcium channels[160].

Rapamycin has been well-studied in inducing autophagy and it has neuroprotective effects in multiple AD animal models. However, the clinical trial aiming at investigating its safety, and feasibility for patients at an early stage of AD has just started

recently (NCT04629495). Notably, lithium, metformin, and rapamycin were reported to be safe in humans, and they are currently used for other diseases, further strengthening their feasibility for treating AD. Furthermore, other autophagy enhancers, such as diet-enriched natural small molecule resveratrol, were investigated for their anti-AD effects in humans.

It has been shown that 52-week resveratrol treatment (500 mg orally once daily) significantly reduced CSF MMP9 (matrix

Table 4 Clinical trials of autophagy enhancers in AD.

Agent	ClinicalTrial.gov NCT number	Trial title	Phase	Year	No. of subject	Results (if applicable)
Lithium	NCT01055392	Disease-modifying properties of lithium in the neurobiology of Alzheimer's disease	II	2007/2011	61	Lithiumimproved cognitive and functional decline after 24 months treatment, and increased CSF's $A\beta_{1-42}$ contents after 36 months treatment
	NCT02129348	Treatment of psychosis and agitation in Alzheimer's disease	II	2014/2020	77	N/A
	NCT03185208	Lithium as a treatment to prevent impairment of cognition in elders (LATTICE)	IV	2017/2023	80	N/A
Rapamycin	NCT04629495	Rapamycin − effects on Alzheimer's and cognitive health (REACH)	II	2021/2023	40 (estimated)	N/A
Latrepirdine	NCT00377715	Double-blind, placebo-controlled study of oral dimebon in subjects with mild to moderate Alzheimer's disease	II	2005/2006	183	Benefits in ADAS-cog compared with control
	NCT00838110	A Phase 3 study to evaluate the safety and tolerability of dimebon patients with mild to moderate Alzheimer's disease	III	2009/2010	742	Did not significantly improve ADAS-cog and CIBIC-plus
	NCT00912288	Phase 3 efficacy study of dimebon in patients with moderate to severe Alzheimer's disease	III	2009/2010	86	This study was terminated due to the lack of efficacy of NCT00838110
Metformin	NCT01965756	Effect of insulin sensitizer metformin on AD biomarkers	II	2013/2015	20	Metformin can penetrate into brain is safe, well-tolerated; metformin had a trend in the improvement of learning/memory and attention
	NCT00620191	Metformin in amnestic mild cognitive impairment (MCI)	II	2008/2012	80	Metformin improved the total recall of the selective reminding test in the ADAS-Cog, after adjusting for the baseline
	NCT04098666	Metformin in Alzheimer's dementia prevention (MAP)	II/III	2021/2015	370 (estimated)	N/A
Resveratrol	NCT01504854	Resveratrol for Alzheimer's disease	II	2012/2014	119	Reduced CSF MMP9 and $A\beta$ levels, but not MAPT levels; attenuated declines in (MMSE) mini-mental status examination scores
	NCT00678431	Randomized trial of a nutritional supplement in lzheimer's disease	III	2008/2010	39	Low-dose resveratrol is safe and well tolerated, its effect on AD remains uncertain
Trehalose	NCT04663854	Mycose administration for HealIng Alzheimer neuropathy (MASHIANE)	I	2020/2022	20 (estimated)	N/A

metallopeptidase 9) levels and modulated neuroinflammation[221]. Resveratrol attenuated the declines in CSF Aβ contents but did not affect CSF MAPT levels. Though, resveratrol also slowed the decline in MMSE (mini-mental status examination) scores[221], large scale studies are still required to further confirm its effects in improving cognitive and function in AD patients. A disaccharide trehalose was reported to activate TFEB-mediated autophagy and lysosome biogenesis and showed neuroprotective effects in multiple AD animal models, which are being tested for its anti-AD effects.

Overall, clinical trials have shown that autophagy enhancers hold great promise for developing novel anti-AD agents. However, since most of autophagy enhancers have multiple targets, further examination of the participant's expression levels of autophagic markers and their regulators will provide novel insight into the autophagy enhancers in AD therapy. For instance, resveratrol has multiple biological activities including anti-inflammation apart from its role in activating autophagy. As aforementioned, a clinical trial showed that it can induce adaptive immunity in AD patients[221]. Whether its anti-AD effects in human are related to autophagy remain elusive.

8. Conclusions and perspectives

Here, we have highlighted how pathogenic and genetic deficits contribute to the impairment of autophagy lysosome function and their link to AD pathogenesis, and illustrated the potential therapeutic potential of autophagy enhancers in AD animal models and humans. We argue that induction of autophagy may be an effective way to develop novel disease-modifying agents for AD. Specifically, impairment of autophagy−lysosomal pathway is mainly responsible for the accumulation of Aβ and NFTs, two hallmarks of AD. Thus, in terms of therapeutic intervention, the concept that induction of autophagy to remove Aβ and MAPT aggregates has been supposed to be an effective way to treat AD by targeting its roots causes. In addition, ageing is a major leading factor for the pathogenesis of multiple chronic diseases including AD[222,223]. Thus, understanding the underlying mechanisms of ageing will provide novel information on AD pathogenies and therapeutic targets. Accumulating evidence indicates that compromised autophagy is a hallmark of ageing, and autophagy plays critical role in modulating inflammation, ageing, and ageing-associated neurodegenerative diseases[222,223]. Induction of autophagy has shown promising beneficial effects in extending lifespan and alleviating ageing-associated disease including AD in lab animal models[162,223]. Future studies aiming at fully understanding the intricate relationships among autophagy, aging, and AD will eventually facilitate the development of novel agents (*e.g.*, autophagy enhancers) for treating AD. Importantly, autophagy is a pro-survival pathway that can reduce neuron death associated with neurodegeneration in AD. To this end, a variety of autophagy enhancers have been reported to exert beneficial effects in multiple AD animal models, and clinical trials.

However, the specific target of autophagy intervention in AD might be considered. Given that impairment of autophagosome maturation has been found in AD, and broad autophagy enhancers targeting upstream signaling pathways including mTOR and/or AMPK may lead to unexpected accumulation of autophagosomes, which might result in toxic effects, making it unsuitable for long-term use for AD treatment. In addition, the side effects of long-term mTOR inhibition should be considered since mTOR plays an important role in regulating synaptic plasticity, memory formation and retention in neurons[224]. The immunosuppressive effects of mTOR inhibitors such as rapamycin should also be considered. These issues can be avoided by using mTOR-independent autophagy enhancers targeting the whole autophagy process (*e.g.*, activating TFEB as aforementioned) or directly increasing lysosome functions. Similarly, the time point for intervening autophagy in AD might be a critical consideration. It has been shown that Aβ and tau are formed much earlier than the impairment of memory in AD patients[225], thus autophagy-based drugs before rather than after the onset of pathology may be more effective. Indeed, beneficial effects would be gained if autophagy is induced by rapamycin before, rather than after the formation of Aβ plaques and NFTs in 3XTg mice[153]. Therefore, future studies aiming at better understanding the dynamics of autophagy process and the exact mechanisms underlying the impairment of AD in different AD stages will also provide novel insight into the designing of specifically tailored autophagy enhancers for AD treatment. Finally, the vast majority of current studies mainly focus on investigating the roles of autophagy enhancers in neurons of AD. The function of autophagy in glia cells is so far unclear in AD. Given that autophagy also plays critical role in regulating inflammation and most autophagy enhancers also induce autophagy in glia cells, understanding the crosstalk between neurons and glia cells and their roles in AD upon autophagy induction is a critical issue for future studies. Further characterization of the roles of impairment of autophagy lysosomes in different AD stages and genetic and molecular subtypes of AD may provide new avenues for the discovery of novel therapeutic agents. Moreover, since most of the drugs may have off-target effects, developing innovative assays to detect dynamic autophagy flux in animals/humans to monitor the therapeutic efficacy of autophagy enhancers is highly desired both for animal models and clinical studies. Overall, we believe that targeting autophagy will yield novel therapeutic agents for AD though much work is still needed.

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Author contributions

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Conflicts of interest

The authors declare no conflicts of interest.

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