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REVIEW

Preserving Lysosomal Function in the Aging Brain: Insights from Neurodegeneration

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Abstract
Lysosomes are acidic, membrane-bound organelles that serve as the primary catabolic compartment of the cell. They are crucial to a variety of cellular processes from nutrient storage to autophagy. Given the diversity of lysosomal functions, it is unsurprising that lysosomes are also emerging as important players in aging. Lysosomal dysfunction is implicated in several aging-related neurodegenerative diseases including Alzheimer's, Parkinson's, amyotrophic lateral sclerosis/frontotemporal dementia, and Huntington's. Although the precise role of lysosomes in the aging brain is not well-elucidated, some insight into their function has been gained from our understanding of the pathophysiology of age-dependent neurodegenerative diseases. Therapeutic strategies targeting lysosomes and autophagic machinery have already been tested in several of these diseases with promising results, suggesting that improving lysosomal function could be similarly beneficial in preserving function in the aging brain.

Key Words Lysosome · aging · neurodegeneration · autophagy · therapeutic targets

Introduction

Lysosomes are dynamic organelles that function as a major catabolic center in the cell. They participate in cellular homeostasis by serving as the terminal degradation compartment in autophagy, modulating nutrient status and interacting with other organelles [1]. There is growing evidence for the role of lysosomes in aging. Some studies suggest that processes such as lysosomal acidification and autophagy decrease with age, potentially altering nutrient storage and clearance of damaged proteins and organelles [2]. The importance of lysosomes in the aging brain is also supported by the many neurodegenerative diseases presenting with lysosomal dysfunction [3]. Thus, improved knowledge of the pathophysiology of these diseases as well as identification of therapeutic targets will strengthen our understanding of and ability to preserve lysosomal function in the aging brain.

Lysosomal Functions Related to Aging

Lysosomes participate in several cellular functions including nutrient processing, autophagy, and inter-organelle contacts. There is emerging evidence that these functions may be perturbed in age-related disease processes, including those affecting the central nervous system, as well as in physiological aging.

Nutrient Storage and Sensing

Lysosomes play a critical role in nutrient homeostasis, serving as both centers for nutrient processing via a host of degradative enzymes and sites for storage of metabolites including amino acids, lipids, and ions. The transport of metabolites into the lysosome depends on transmembrane transporters and channels, whose activity is coupled to a proton gradient established by the highly conserved H^+-ATPase or V-ATPase [4, 5]. V-ATPase pumps protons into the vacuolar lumen, and the subsequent extrusion of these protons drives ions and metabolites inward [6, 7].

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Amino Acids

Lysosomes sense nutrient availability through the activities of V-ATPase and the conserved serine–threonine kinase, mTORC1, and both are sensitive to changes in amino acid levels [2, 8]. The activity of the V-ATPase is regulated through association–dissociation of its many subcomplexes [9]. In states of low amino acids, the V-ATPase becomes fully associated, generating a proton gradient to promote the influx of substrates into the lysosome [10]. The nutrient sensing function of the V-ATPase is additionally linked to the mTORC1 pathway via the V-ATPase, Rag-GTPases, and Ragulator complex. During states of nutrient abundance, mTORC1 associates with lysosomes to promote cellular growth and limit autophagy [11, 12]. The relationship between mTORC1 and lysosomal amino acid sensing is further evidenced by the lysosomal arginine transporter, SLC38A9, which communicates amino acid status to mTORC1 [13, 14]. This transporter mediates efflux of nonpolar amino acids from the lysosome to be utilized in processes such as protein synthesis [15]. A recent lysosomal metabolomics study identified both V-ATPase and mTORC1 as important mediators of amino acid efflux from the lysosome [16].

Evidence highlighting the importance of the V-ATPase and the mTORC1 pathways in aging is also growing. Acidification by V-ATPase or overexpression of V-ATPase components promoted long-term mitochondrial stability [17] and extended lifespan in yeast, respectively [18]. Similarly, mTORC1 inhibition was shown to increase lifespan in a range of organisms including yeast, flies, and mice [2].

Calcium

Lysosomes, along with the endoplasmic reticulum (ER), serve as main cellular reservoirs for Ca^{2+}, an ion implicated in several signaling pathways and organelle homeostasis [19]. Although the mammalian lysosomal Ca^{2+} importer is unknown, various efflux channels have been characterized including transient receptor potential channel mucolipin-1 (TRPML1) and two-pore channels 1 and 2 (TPC1/2) [20]. Importantly, Ca^{2+} level has been correlated with lifespan as elevation of either intracellular or extracellular Ca^{2+} shortens replicative lifespan in yeast []. Moreover, loss of lysosomal function results in a rapid increase in cellular Ca^{2+} in yeast [22], and activation of the TRPML1 channel facilitates Ca^{2+}-induced Ca^{2+} release from the ER, further increasing intracellular Ca^{2+} [23, 24]. TRPML1 has also been linked to V-ATPase function as blockage of V-ATPase or knockdown of its V0a1 subunits results in an increase in TRPML1-mediated Ca^{2+} efflux from lysosomes, suggesting a close interplay of the 2 proteins in Ca^{2+} homeostasis [25]. Regulation of cellular Ca^{2+} also has important implications for overall cell survival as Ca^{2+} overload and subsequent accumulation in the mitochondria can induce mitochondrial permeability transition pore opening, promoting the release of pro-apoptotic factors [26].

In the central nervous system, Ca^{2+} plays an important role in modulating electrical activity and neurotransmitter release [27]. Aged neurons show a variety of alterations in Ca^{2+} homeostasis including diminished efflux from the plasma membrane [28], decreased mitochondrial buffering capacity [29], and enhanced release from the ER [30, 31]. In the aging brain, this results in a net increase in cellular Ca^{2+}, which perturbs neuronal excitability and long-term processes such as synaptic plasticity [27]. Although the function of lysosome-dependent Ca^{2+} homeostasis in the aging brain remains poorly understood, the ability of lysosomes to regulate cellular Ca^{2+} in non-neuronal cells suggests the possibility of a similar role for lysosomes in neurons.

Iron

Another key ion regulated by lysosomes is Fe^{2+} which enters the cell via the endolysosomal system [32, 33]. Tight regulation of cellular Fe^{2+} is crucial as it is an important cofactor for enzymes in the electron transport chain [34]; however, high levels may promote the formation of toxic reactive oxygen species (ROS) [35]. Of note, Fe^{2+} levels have been reported to be increased with age, thereby promoting mitochondrial protein aggregation and decreased lifespan in *Caenorhabditis elegans* [36].

Fe^{2+} is also important for central nervous system function via its involvement in myelin and neurotransmitter synthesis [33]. Fe^{2+} levels in the brain increase with age due to increased blood–brain barrier permeability, alterations in Fe^{2+} homeostasis, and neuroinflammation [37]. Moreover, changes to brain Fe^{2+} distribution in areas like the substantia nigra and basal ganglia occur with age [38]. In non-nervous tissues, free Fe^{2+} remains low via binding to ferritin and transferrin, whereas in catecholaminergic neurons, free Fe^{2+} is regulated through the formation of neuromelanin–iron complexes, which have been found to increase with age [39, 40]. A connection between lysosomes, Fe^{2+}, and neuromelanin was recently highlighted with the characterization of neuromelanin-containing organelles as specialized autolysosomes that accumulate undegraded proteins and lipids in substantia nigra neurons [41]. The importance of iron homeostasis in the brain is also supported by a group of inherited disorders called neurodegeneration with brain iron accumulation (NBIA). Several NBIA genes are related to lysosomal function [42], and it was recently reported that iron overload in NBIA mutant cells resulted in both lysosomal and mitochondrial dysfunction [43].

Lipids and Glycoproteins

The role of lysosomes in glycoprotein and lipid homeostasis is evidenced by the plethora of lysosomal storage disorders (LSDs) that result from complete loss or decreased activity of specific lysosomal hydrolases. In LSDs, undigested lipids, glycoproteins, and mucopolysaccharides accumulate in the lysosome, leading to cellular toxicity and compromised survival [44]. A comprehensive description of the LSDs can be found in previously published reviews [45, 46]. Importantly, lysosomal dysfunction due to storage of accumulated substrates in LSDs results in decreased lysosomal degradation and various pathologies in the CNS that resemble adult-onset neurodegenerative diseases—for example, Parkinson's disease (PD)-like Lewy body accumulation in Gaucher disease and Alzheimer's disease (AD)-like plaques and tangles in Niemann–Pick disease type C [46]. This suggests that alterations in lysosomal degradative capacity may contribute to age-related neuropathologies.

Autophagy

Autophagy is a critical physiological process that maintains intracellular homeostasis by degrading and recycling cytoplasmic material, including damaged organelles, long-lived proteins, and protein aggregates, via delivery to the lysosome. Though the process was previously thought to occur nonselectively, studies have shown that autophagy can be stimulated under stress conditions and by cellular insults. Additionally, dysfunctional organelles like the mitochondria may also undergo degradation by the lysosome via selective autophagy.

Mechanism of Autophagy

Macroautophagy, hereafter referred to as autophagy, is 1 of 3 modes of autophagy alongside microautophagy and chaperone-mediated autophagy. It is the primary mode for degrading large parts of cytoplasm through sequestration inside double-membrane vesicles, termed autophagosomes, which then fuse with lysosomes [47]. Several highly conserved AuTophaGy-related (Atg) genes have been identified as directly involved in autophagosome formation, maturation, and fusion with the lysosome.

Autophagosome formation is initiated by the phagophore, an open double-membrane structure that forms around proteins and organelles in the cytoplasm [47]. The membrane is derived from proximal structures including the ER, endosomes, mitochondria, Golgi, and plasma membrane [48, 49]. Unc-51-like kinase 1 (ULK1) is a critical protein in phagophore induction through its interaction with autophagy proteins including mAtg13 and the scaffolding protein FIP200 [50]. Other players in autophagosome formation include a transient interaction with mAtg9 [51], which may provide additional membrane sources to the growing phagophore.

In humans, the class III phosphatidylinositol 3-kinase, vesicular protein sorting 34 (hVps34), and its complex 1 binding partners—Beclin 1, p150, and Atg14L—are also necessary for phagophore formation [52–55]. hVps34 complex 1 interactions stimulate hVps34 activity, promoting phosphatidylinositol to phosphatidylinositol 3-phosphate conversion, which facilitates recruitment of other Atgs to the forming autophagosome [52, 55]. hVps34 also has a set of complex 2 interactors, in which the conserved hVps34–Beclin 1 complex backbone interacts with ultraviolet irradiation resistant-associated gene (UVRAG) [56, 57]. UVRAG association with complex 2 modulates several functions including membrane bending, negative and positive regulation of autophagy, and promotion of autophagosome fusion with endosomes/lysosomes [58].

Elongation of the phagophore membrane involves 2 ubiquitin-dependent conjugation systems. In the first, Atg7 activates Atg12, which is transferred to Atg10 to stimulate Atg12–Atg5 covalent linkage [59–61]. The Atg12–Atg5 complex then interacts with Atg16L to form the Atg16L complex, which drives phagophore elongation [60, 61]. The second system involves the microtubule-associated protein light chain 3 (LC3) [62]. Upon autophagy induction, LC3 is cleaved to cytosolic LC3-I which is then conjugated to phosphatidylethanolamine by Atg7 and Atg3 to generate lipidated LC3-II [59, 60]. LC3-II associates with both sides of the phagophore membrane to mediate cargo selection through its recruitment of the autophagy receptor, p62, and components involved in autophagosome–lysosome fusion [63–67]. The final step involves fusion of the outer membrane of the autophagosome with the lysosome. Various SNAREs and tethering/adaptor proteins on late endosomes/lysosomes and autophagosomes have been identified and have been discussed in previous reviews [68].

Regulation of Autophagy

Studies have revealed a close interplay between lysosomes and mTORC1 in the regulation of autophagy. Under a nutrient-rich environment, mTORC1 is recruited to the lysosomal membrane via a complex consisting of the membrane-tethered Ragulator complex and Rag GTPases [12, 69], where it is activated by Ras homolog enriched in brain (Rheb) [70]. Activation of mTORC1 along with suppression of its inhibition by tuberous sclerosis tumor suppressor complex (TSC1–TSC2) in nutrient-replete conditions inhibits autophagy [71]. Autophagy initiation and lysosome biogenesis are also controlled at a transcriptional level through the transcription factor EB (TFEB). Nutrient deprivation induces nuclear translocation of TFEB to enhance expression of autophagosome-related genes [72, 73]. However, under basal conditions,

activated mTORC1 phosphorylates TFEB, resulting in cytoplasmic retention of the transcription factor and inhibition of autophagy [74, 75].

mTORC1-independent mechanisms of autophagy induction have also been identified, including inhibition of inositol-1,4,5-trisphosphate (IP_3) levels and attenuation of Ca^{2+}-related pathways [76, 77]. IP_3 is released from the plasma membrane upon phospholipase C processing of phosphatidylinositol 4,5-bisphosphate (PIP_2) [78]. High levels of IP_3 can inhibit cellular clearance [79, 80] likely by stimulating Ca^{2+} release through IP_3 receptors and disrupting Ca^{2+} gradients needed for autophagosome–lysosome fusion. Elevated cytosolic Ca^{2+} also regulates autophagy via activation of the Ca^{2+}-dependent cysteine protease, calpain. A negative correlation likely exists between calpain activity and autophagy initiation as calpain inhibition has been shown to promote autophagosome formation [77, 81, 82]. In addition, calpain activity could increase IP_3 levels via G-protein signaling downstream of GPCRs [77]. Lastly, inhibition of TFEB through phosphorylation by the serine–threonine kinase, Akt, can block autophagy independent of mTORC1 [83]. Conversely, TFEB dephosphorylation by the Ca^{2+}-dependent phosphatase, calcineurin, enables nuclear translocation, thereby inducing autophagy [84]. Notably, it was also shown that calcineurin is activated by local Ca^{2+} release from lysosomes [84].

Mitophagy

Mitophagy is a selective form of autophagy in which damaged mitochondria are eliminated, and serves as a quality control mechanism to preserve cellular homeostasis [85–87]. The best characterized pathway for mitophagy involves the Parkinson's disease-linked proteins, PTEN-induced kinase 1 (PINK1) and Parkin [85–87]. When mitochondria are damaged, PINK1 stabilizes on the outer mitochondrial membrane to promote Parkin translocation and E3 ubiquitin ligase activity, leading to the incorporation of ubiquitinated cargo into autophagosomes [88]. In addition, PINK1 and Parkin are involved in a mechanism of mitochondrial clearance that delivers mitochondrial-derived vesicles (MDVs) enriched in oxidized proteins to lysosomes for degradation [89].

Additional pathways of mitophagy have also been identified. Transmembrane receptor-mediated mitophagy, in which LC3-interacting regions mediate recognition of damaged mitochondria, have been described for several outer mitochondrial membrane proteins including Nix [90] and FUN14 Domain containing 1 [91, 92], which were observed to be induced to drive mitophagy in response to hypoxia. Moreover, cardiolipin-mediated mitophagy, where exposure of the inner mitochondrial membrane phospholipid, cardiolipin, on the mitochondrial surface allows its interaction with LC3, has been shown to activate mitophagy independent of PINK1/Parkin [93]. Finally, other stimuli, such as iron chelators [94] and increased mitochondrial fission [95, 96], also induce mitophagy in a PINK1/Parkin-independent manner.

Although several mechanisms of mitophagy, such as the PINK1/Parkin and cardiolipin-mediated pathways, have been observed in neurons, whether additional neuronal mitophagy pathways exist and how different pathways interact and are regulated is still incompletely understood. Nevertheless, the elimination of damaged mitochondria is critical for neurons, which cannot alleviate cellular stress by division and require functional mitochondria for energy and Ca^{2+} homeostasis. The importance of mitophagy in neurons is also demonstrated by the various mutations in mitophagy-related genes that are causal or linked to neurodegenerative diseases such as PD and amyotrophic lateral sclerosis–frontotemporal dementia (ALS–FTD) [97] as well as by evidence suggesting that decreased lysosomal acidification impairs mitophagy in cellular models of AD [98]. Thus, understanding how mitophagy is dysregulated in neurodegeneration may further elucidate how lysosomal function in neurons is altered in age-related pathologies.

Interactions with Mitochondria

The regulation of nutrient homeostasis by lysosomes is intimately associated with mitochondrial function, and the modulation of mitochondrial function by lysosomes has various connections to aging. For example, vacuolar acidity is decreased with age in yeast, resulting in reduced lifespan and impaired mitochondrial function [17]. Similarly, lifespan and mitochondrial function are compromised in cells lacking certain subunits of the V-ATPase, leading to altered mitochondrial morphology, protein import, and potential [17, 99, 100].

Recent studies have elucidated the physical interaction between lysosomes and mitochondria. A tethering complex, vCLAMP, was identified between the yeast vacuole and mitochondria and was hypothesized to regulate lipid and metabolite transport between the 2 compartments [101, 102]. Importantly, mitochondria–lysosome contact sites were recently identified in mammalian cells and were found to be regulated by GTP hydrolysis of the lysosomal GTPase, Rab7 [103]. These contact sites allow for bidirectional regulation of mitochondrial and lysosomal dynamics, whereby 1) mitochondria regulate lysosomal dynamics by modulating the GTP-binding status of Rab7 via the mitochondrial-localized Rab7-GAP (TBC1D15), and conversely 2) lysosomes regulate mitochondrial dynamics by marking the majority of mitochondrial fission sites [103]. Mitochondria–lysosome contacts may have additional functions including the transfer of metabolites such as lipids or ions [103, 104].

Lysosomes may also affect mitochondrial function indirectly through the modulation of amino acid homeostasis. High levels of cytosolic basic amino acids have been shown

to induce cellular toxicity, impair mitochondrial function, and increase ROS generation [105]. Similarly, increased levels of nonpolar, unbranched amino acids in the cytosol can accumulate in the mitochondria and overload mitochondrial metabolic pathways [106, 107].

A hallmark feature of the aging brain is reduction in mitochondrial function with a concurrent increase in ROS and oxidative damage. The ability of ROS to modulate lysosomal function is mediated in part by the lysosomal TRPML1 channel, which acts as a ROS sensor. When cellular ROS is increased, activated TRPML1 releases Ca^{2+} into the cytosol, inducing both TFEB nuclear translocation for lysosome biogenesis and autophagy [108]. Because both TRPML1 activation and mitochondrial ROS production have been shown to increase in response to inhibition of V-ATPase, lysosomal deacidification may serve as an upstream pathophysiological event modulating lysosomal Ca^{2+} release and mitochondrial function [109]. Further evidence linking ROS to lysosomal dysfunction include studies showing that loss of mitochondrial function in brain tissue following deletion of mitochondrial proteins or inhibition of oxidative phosphorylation is sufficient to impair lysosomal function in a ROS-dependent manner [110]. Although there is still debate as to whether ROS is a cause or consequence of aging, it is likely that it contributes to both; initial increases in ROS due to age further exacerbate aging by enhancing lysosomal dysfunction.

Lysosomal Function in Neurodegenerative Disease

Several studies have revealed the importance of lysosome-related functions, such as autophagy, in neuronal homeostasis. Mouse models with neural cell-specific knockout of Atg5 or Atg7, leading to impaired autophagosome-lysosome clearance, exhibited neurodegeneration with motor deficits and shorter lifespan [111, 112].

Conversely, induction of autophagy has been linked to longevity and resistance to protein aggregation and oxidative stress in neurons [71, 113]. Selective activation of lysosomal activity via TFEB was also found to clear protein aggregates and increase activation of quiescent neural stem cells during aging [114]. Although the role of lysosomes in the aging brain is still being elucidated, our understanding of age-dependent neurodegenerative diseases, many of which implicate lysosome-related genes, has provided insight into the changes in lysosomal function with age (Fig. 1).

Alzheimer's Disease

AD is the most common neurodegenerative disease affecting more than 24 million individuals over the age of 65 [115]. The major pathological hallmarks include the accumulation of extracellular amyloid-beta (Aβ) plaques and intracellular tau-induced neurofibrillary tangles (NFTs) [115]. Normally, Aβ is trafficked to the lysosome via the autophagy pathway and degraded by cathepsin D [116]. However, recent studies suggest that release of Aβ into the extracellular space may also be in part regulated through autophagy [117]. Disruptions in autophagy were first linked to AD when autophagosome/lysosome accumulation was observed in dystrophic neurites [118]. Follow-up studies were able to show the presence of Aβ sequestered in the retained autophagosomes [119], thus highlighting the importance of the autophagy pathway in AD progression.

Clusterin, encoded by CLU, is 1 of the top candidate genes for AD development and is linked to autophagy through its involvement in autophagosome biogenesis via interaction with LC3 [120, 121]. In human studies, clusterin expression positively correlates with increasing levels of Aβ40/42 in brain areas normally afflicted in AD [122]. Furthermore, clusterin interacts directly with Aβ and reduces its aggregation, thereby protecting against toxic effects [123]. Although associations between clusterin and tau are still debated, 1 study found that tau overexpression increases clusterin expression [124], which could potentially stimulate autophagy-mediated degradation of tau.

PSEN1, another causal AD gene which encodes presenilin 1, is directly linked to autophagy through an identified role in lysosomal acidification [25, 125, 126]. Presenilin 1 is responsible for regulating gamma-secretase, which processes amyloid precursor protein (APP) into Aβ [127]. Mutations affecting presenilin 1 function may disrupt lysosomal proteolysis and promote accumulation of autophagic cargo within the cell [128, 129]. In support of this, cerebrospinal fluid (CSF) from PSEN1 patients exhibited less Aβ than normal individuals [130], suggesting that Aβ retention may lead to cellular toxicity when autophagy is impaired.

Several risk genes for AD also have links to autophagy. Atg7, which mediates the formation of the Atg12–Atg5 complex during phagophore maturation [131], is involved in incorporation of Aβ into multivesicular bodies (MVBs)/late endosomes [132, 133]. Increased transport of Aβ into MVBs could affect downstream fusion with autophagosomes. Recent studies suggest that release of Aβ into the extracellular space may be in part regulated by autophagy [117]. Inhibition of autophagy through genetic ablation of Atg7 decreases Aβ plaque formation in mouse models [117]. However, complete loss of autophagy also led to the accumulation of Aβ inside neurons [117], which can potentiate neurotoxicity. Atg7 is also linked to the tau degradation as knockout mice exhibited increased phosphorylated tau [134]. Altogether, this suggests that extreme modulation of autophagy may protect against 1 AD phenotype but exacerbate another.

Associations between Bcl-2 expression and memory have been reported in AD patients [135], highlighting an important

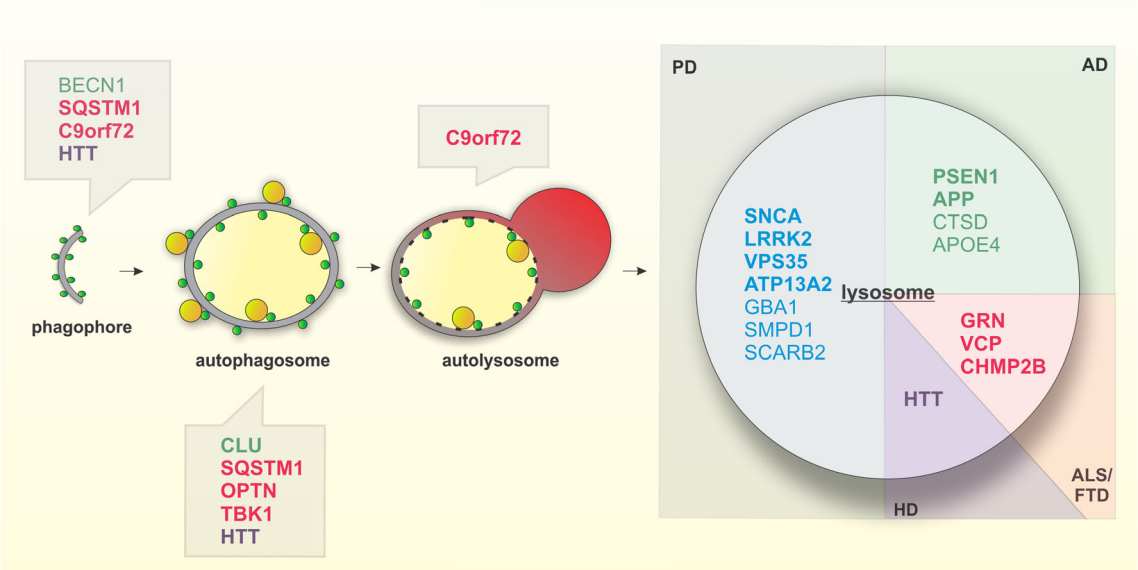

Fig. 1 Genes implicated in neurodegenerative disease related to autophagic and lysosomal function. Genes involved in several neurodegenerative diseases affect various steps of autophagy as well as have direct impacts on lysosomal function. Bolded correspond to causative genes, unbolded correspond to risk genes. Genes implicated in PD (blue), AD (green), ALS/FTD (red), and HD (purple)

role for Bcl-2 in AD pathogenesis. Treatment with Aβ decreases Bcl-2 expression and overexpression of Bcl-2 in APP mutant mice was found to be neuroprotective [135, 136]. Bcl-2 overexpression also decreases APP processing, extracellular Aβ deposits, and intracellular NFTs [137]. As stated previously, Beclin 1 is responsible for initiating autophagy and regulating autophagosome formation [138]. Beclin 1 heterozygous knockout mice displayed disruptions in autophagy [139], suggesting that alterations in Beclin 1 levels and function can contribute to AD progression. In agreement with this, presenilin-null cellular models and postmortem analysis of AD brains reported a decrease in Beclin 1 level [140]; however, a recent large-scale quantitative study found no significant changes in Beclin 1 gene expression or protein levels across multiple stages of AD [141].

Cathepsin D, the lysosomal protein involved in Aβ processing [116], is encoded by CTSD which confers risk in AD [142, 143]. Although the mechanism of cathepsin D in AD pathology is not well understood, 1 potential mechanism is through altered functioning of lysosomal proteolysis potentially mediated by presenilin 1 mutations which ultimately result in disrupted lysosomal acidification [25, 125, 126]. Cathepsin D levels were reportedly lower in bone marrow-derived monocytes, fibroblasts, and peripheral blood lymphocytes in AD patients [144–146], whereas elevated CTSD gene expression and protein levels were observed in AD brains and neurites [141, 147]. Interestingly, 1 additional study found that cathepsin D levels were increased in postmortem CSF albeit in an inactive form of the protein [148]. This suggests that like Beclin 1, alterations in cathepsin D function can be detrimental in AD pathogenesis when compounded with disease-associated mutations like those found in PSEN1.

Another hallmark of AD is dysfunction in the endosomal–lysosomal system, such as endosomal enlargement, which is 1 of the earliest pathological features of disease [149]. APP, in which mutations cause autosomal dominant, early-onset AD, has been implicated in AD-related endosomal aberrations. It was demonstrated that the APP β C-terminal fragment directly interacts with the adaptor protein containing pleckstrin homology domain, phosphotyrosine binding domain, and leucine zipper motif (APPL1), which also stabilizes active Rab5 on the endosomal membrane [150]. Overactivation of Rab5 by APP β C-terminal fragment mediates downstream events including altered endosomal motility and signaling and may also contribute to AD phenotypes such as defects in long-term potentiation [149, 151–153] and cholinergic degeneration. In addition, APP and Rab5 overactivation has been linked to defective lysosomal morphology and proteolysis as well as autophagy in various cellular and mouse models of AD [151, 152, 154].

The E4 allele of apolipoprotein E (ApoE4), a major risk factor for sporadic AD, has similarly been linked to endolysosomal dysfunction including increased endocytosis of APP [155] and impaired endosomal recycling [156, 157]. ApoE4 intermediates have also been shown to disrupt lysosomal membranes in neuronal cultures, leading to leakage of hydrolases and induction of apoptosis, which is further potentiated by Aβ(1-42) [158, 159]. A recent transcriptomics analysis in ApoE4-targeted replacement mice showed significant enrichment of genes involved in endosomal–lysosomal

processing, further implicating ApoE4 in endolysosomal function [160].

Parkinson's Disease

PD is the most common movement disorder with an estimated global prevalence of ~400 out of 100,000 by the age of 65 [161]. The cardinal motor symptoms—bradykinesia, rigidity, and tremor—are primarily attributed to dopaminergic neuron deterioration. In the majority of PD cases, Lewy bodies composed of proteins, lipids, and undegraded organelles including autolysosomes and damaged mitochondria accumulate in several brain regions [162–164].

Several autosomal dominant PD genes are directly linked to lysosomal function including SNCA, leucine-rich repeat kinase 2 (LRRK2), and vacuolar protein sorting-associated protein 35 (VPS35). The first PD gene identified, SNCA, encodes for alpha-synuclein (αSyn) [165], a major constituent of Lewy bodies [166]. Phenotypically, multiplication of the SNCA gene leads to earlier onset PD, indicating that increased expression of SNCA promotes disease pathogenesis [167, 168]. αSyn overexpression impedes autophagy by prohibiting Atg9 from initiating phagophore formation [169, 170]. Moreover, high endogenous αSyn in human iPSC-derived dopaminergic neurons reduces lysosomal proteolysis by delaying the delivery of hydrolases such as cathepsin B and glucocerebrosidase (GCase) to the lysosome [171]. SNCA missense mutations, which have an increased propensity to oligomerize, are also linked to familial PD [172, 173]. These aberrant forms of αSyn are resistant to degradation and reduce the efficiency of autophagy [174, 175]. Inhibition of autophagy and lysosomal function increase αSyn release, potentially as a compensatory mechanism [176–183].

Gain-of-function mutations in LRRK2 are the most common cause of familial late-onset PD, and polymorphisms in the locus increase PD risk [184]. LRRK2 can be localized on membranes of synaptic vesicles, late endosomes, and lysosomes [185, 186], and several Rabs involved in vesicle formation, trafficking, and docking/fusion are phosphorylated by LRRK2 [186, 187]. The consequences of pathogenic LRRK2 mutations on synaptic integrity, autophagy, and lysosomal function require further elucidation. Studies have described that patient G2019S LRRK2 fibroblasts exhibited higher lysosomal abundance and protein clearance at baseline [188], and several LRRK2 mutant fibroblasts showed decreased autophagic flux following serum/amino acid deprivation [189]. Moreover, G2019S LRRK2 iPSC-derived dopaminergic neurons were more susceptible to oxidative stress and retained higher loads of autophagic vacuoles and lipid droplets [190, 191].

VPS35, causally linked to autosomal dominant PD, is part of the retromer complex and is important for trafficking of cathepsin D, Lamp2A, and Atg9a [172, 192–194], thereby regulating autophagy and endolysosomal processing. Mitochondrial quality control also requires VPS35 for both fusion/fission dynamics and trafficking of MDVs to the lysosome for degradation [89, 195].

Loss-of-function of the cytosolic E3 ubiquitin ligase, Parkin, and the mitochondrial kinase, PINK1, are linked to early-onset autosomal recessive PD [172], and both proteins are also involved in mitochondrial quality control via mitophagy and MDVs [88, 89]. Parkin deficiency has been shown to destabilize Rab7 [196], which is crucial for both mitochondrial fission and autophagosome–lysosome fusion.

DJ-1, a chaperone with antioxidant functions, has been shown to protect dopaminergic neurons against oxidative stress in PD pathogenesis [197, 198]. iPSC-derived dopaminergic neurons from DJ-1 patients show an accelerated pathological cascade beginning with increased oxidative stress which further induces dopamine oxidation [199]. Oxidized dopamine in turn inhibits the lysosomal enzyme glucosylceramidase (GCase) and is paralleled by increased levels of soluble and insoluble αSyn [199].

The juvenile-onset atypical parkinsonism, Kufor–Rakeb syndrome, is caused by autosomal recessive mutations in PARK9, which deplete P5-type ATPase 13A2 (ATP13A2) from endolysosomes [200]. Studies using patient fibroblasts, iPSC-derived neurons, and knockout mice report mitochondrial dysfunction, lysosomal acidification and macroautophagy deficits, as well as lipid deposition preceding αSyn accumulation [201–204]. Moreover, ATP13A2 is considered to be important for maintaining intracellular ion homeostasis [205–208], a function which may be critical for autophagy/endolysosomal processing efficiency.

Various PD risk factors further implicate lysosomal dysfunction in PD pathogenesis. Autosomal recessive mutations in GBA1, which encode GCase, increase the risk for PD and cause the LSD, Gaucher disease [209, 210]. Some Gaucher patients present with parkinsonism as well as dopaminergic neurodegeneration and Lewy body pathology [209]. iPSC-derived neurons from GBA1-associated PD patients show reduced lysosomal GCase activity, macroautophagy, and Ca^{2+} buffering [211, 212]. Reduced GCase activity is linked to elevated glucosylceramide levels, which further promote αSyn accumulation and higher-order assembly within lysosomes [211, 213, 214]. Variants of the acid sphingomyelinase-encoding SMPD1 gene are linked to the LSDs, Niemann–Pick disease types A and B, and may follow a similar mechanism to GBA1 variants [215–217]. Furthermore, the PD risk factor SCARB2 encodes the lysosomal integral membrane protein type 2 (LIMP2), which sorts GCase to the lysosome and is important for autophagy, αSyn proteostasis, and dopaminergic viability [218–220]. Additional PD risk loci linked to lysosome-related genes (e.g., GALC, CTSB, ATP6V01A) were recently revealed by a large meta-analysis of genome-wide association studies [210]. Collectively, this points to the necessity of improving lysosomal function in PD.

Amyotrophic Lateral Sclerosis and Frontotemporal Dementia

ALS is characterized by the progressive degeneration of motor neurons. Like other neurodegenerative diseases, ALS is a proteinopathy where pathological DNA-binding protein 43 (TDP-43) is found accumulated in the cytosol of affected neurons [221, 222]. Aggregated TDP-43 plays a pathogenic role in both ALS and FTD [221], indicating that both ALS and FTD belong to a spectrum of the same disease. To date, a large number of genes have been linked to familial ALS, but strikingly, many of these genes are directly linked to autophagic function, suggesting that improving autophagy may alleviate ALS-related pathologies.

Superoxide dismutase 1 (SOD1) was 1 of the first enzymes associated with ALS. Inhibition of autophagy led to aggregation of mutant SOD1 [223], indicating that dysfunctional SOD1 is largely degraded by autophagy. The autophagy receptor protein p62, encoded by the ALS-linked gene SQSTM1, is responsible for loading of cargo into the extending phagophore/autophagosome [224–226]. Interestingly, p62 has been shown to interact with polyubiquitinated mutant SOD1 [227, 228], which enhances its interaction with lipidated LC3-II on the phagophore membrane [228], suggesting that autophagy is activated in the presence of aggregated mutant SOD1. These interactions lead to the formation of protein inclusions within motor neurons [228]. Autophagy was also reportedly increased in mutant SOD1 mice potentially due to decreased phosphorylation of ULK1 [229], which activates autophagy [230]. In addition, proteins involved in autophagy regulation such as TFEB and Beclin 1 were found upregulated in the spinal cords of mutant SOD1 mice [231].

Several genes associated with ALS also encode RNA-binding proteins that aggregate when mutated. Both TDP-43 and fused in sarcoma (FUS) are found in intracellular aggregates in brain autopsy [232, 233]. In order to maintain homeostasis, autophagy is known to degrade aggregated TDP-43 and FUS [234–236]. Not surprisingly, increased expression of TDP-43 and FUS promote aggregation and stall autophagy in ALS models that could be rescued upon autophagy induction [234, 237, 238]. Furthermore, TDP-43 depletion increases translocation of TFEB into the nucleus, thereby stimulating autophagy [239]. However, downregulation of TDP-43 has also been shown to decrease Atg7 expression leading to reduced autophagic flux and autophagosome–lysosome fusion [240]. Thus, studies on the mechanism of action for TDP-43 in relation to autophagy have given both gain and loss of function results.

A subset of ALS-linked genes encode autophagy receptors, such as p62 and optineurin (OPTN) [241], or regulators of such receptors like Tank binding kinase 1 (TBK1) [242, 243]. As autophagy receptors, both p62 and optineurin contain a ubiquitin-associated domain (UBA) and an LC3-interacting region [241]. Generally, the proteasome is responsible for the degradation of ubiquitinated proteins. However, the discovery of autophagic receptors with UBA domains suggests that ubiquitinated cargo may also be targeted to and degraded by lysosomes. Many ALS-associated mutations affecting p62 and optineurin activity fall within the UBA domain [244, 245]. Furthermore, mutations located in the UBA domain may affect the protein's ability to be phosphorylated by TBK1 [242, 243], suggesting that loss of TBK1 activity can enhance autophagic dysfunction in ALS.

The most commonly mutated gene in ALS, C9orf72 [246], has also been linked to autophagy via its interaction with endocytic proteins, Rab5 and Rab7 [247, 248], which regulate endosomal maturation [249]. Proper maturation and positioning of late endosomes is critical for autophagosome fusion [249]. C9orf72 also interacts with the ULK1 complex directly, and loss of C9orf72 prevents autophagy in an ULK1-mediated manner [250, 251]. As C9orf72 was previously shown to interact with Rab1a, an early autophagic regulator involved in phagophore formation [247, 251], C9orf72 may also regulate the recruitment of ULK1 to the initiation complex. Conversely, loss of C9orf72 leads to inactivation of mTORC1, increased TFEB nuclear translocation, and subsequent activation of autophagy [250, 252]. Therefore, C9orf72 is involved in several conflicting steps of autophagy but ultimately has been implicated in the maintenance of lysosomal function.

FTD is the second most common young-onset dementia and is characterized by atrophy of the frontal and temporal lobes [253, 254]. FTD is divided into subgroups based on the pathological hallmarks that develop with disease progression, including FTD with tau (FTD-tau) or TDP-43 (FTD-TDP)-positive inclusions [253]. The majority of FTD cases are attributed to mutations in microtubule-associated protein tau, progranulin (GRN), and C9orf72 [253, 254]. The remaining familial-associated FTD cases are linked to mutations in valosin-containing protein (VCP), FUS, TDP-43, and charged multivesicular body protein 2B (CHMP2B), which play distinct roles in the endolysosomal pathway [253, 254]. This suggests that disruptions in normal endosome to lysosome maturation may delay the degradation of autophagic cargo or impair the delivery of lysosomal hydrolases. For example, it was previously shown that VCP patients exhibited autophagic defects including an increase in the number of enlarged cathepsin B-positive autophagic vesicles [255]. Moreover, patients with mutations in VCP and CHMP2B were also reported to have decreased autophagic clearance and lysosomal function [255–259].

It was previously posited that FTD and LSDs were related diseases as they both shared similar common pathophysiological phenotypes. However, a concrete link was established with the discovery of a family affected by both neuronal ceroid lipofuscinosis (NCL), a young-onset LSD, and FTD

[260]. Linkage analysis revealed independent heterozygous and homozygous mutations in the GRN gene [260], which encodes granulin, thus linking lysosomal dysfunction to GRN mutants and FTD. The proprotein, progranulin, is cleaved into individual granulins in the endolysosomal pathway [261, 262]. Recent work further demonstrated granulin E is responsible for modulating cathepsin D activity [263, 264], suggesting that GRN can directly alter lysosomal function through resident hydrolases. In agreement with this, GRN knockout mice displayed an increase in the levels of TMEM106B, as well as an accumulation of lipofuscin puncta in lysosomes [265], both of which are indicators of lysosomal dysfunction commonly found in NCLs. Furthermore, CTSD knockout mice exhibited increases in progranulin and TMEM106B along with saposin D, which is normally found accumulated in NCL10 patients [265]. Altogether, the genetics of ALS/FTD strongly implicate dysfunctions in both autophagy and endolysosomal systems in disease pathogenesis.

Huntington's Disease

Huntington's disease (HD) is an autosomal dominant, trinucleotide repeat disorder characterized by cognitive dysfunction, psychiatric disturbances, and loss of motor control [266]. The brain region most affected in HD is the striatum, an area critical to initiating and controlling movement, although other areas of the brain such as the cortex and cerebellum are also affected as the disease progresses [267]. Although defects in various pathways have been associated with HD, including alterations in Ca^{2+} handling, vesicle transport, and ER homeostasis [268], recent studies have implicated defective autophagy as a key feature of the disease, highlighting the potential of lysosomes and other autophagy machinery as druggable targets in HD.

The genetic basis of HD is a polyglutamine (polyQ) repeat expansion near the N-terminus of the huntingtin (htt) protein [269]. A repeat expansion of greater than 36Q is causative of disease, and like in other repeat disorders, greater repeat lengths correlate with earlier disease onset [268]. The hallmark feature of HD pathology is the accumulation and aggregation of mutant htt protein (mhtt). These aggregates form both intranuclear and intracytoplasmic neuronal inclusions, although whether these aggregates are pathogenic is still debated [270, 271]. Furthermore, htt can be proteolyzed to smaller fragments, which may also contribute to disease pathogenesis [272–275].

Although the function of wild-type htt is not fully understood, htt has been shown to interact with autophagy machinery. For instance, human and mouse HD samples exhibit an increase in autophagosomes [276, 277], and mhtt was shown to activate autophagy via sequestration and inactivation of mTORC1 [278]. Despite increasing autophagosome formation, mhtt appears to alter cargo recognition and loading through an unidentified mechanism, ultimately leading to accumulation of damaged organelles and protein aggregates [279]. Additional aberrations in autophagy are linked to effects of mhtt on vesicle trafficking including deficits in autophagosome motility [280] and autophagosome–lysosome fusion [281]. Silencing of either htt or its binding partner, huntingtin-associated protein 1, blocked retrograde transport of autophagosomes along the axonal compartment, whereas depletion of mhtt also resulted in an accumulation of autophagosomes containing undegraded cargo [280]. Alterations in mRNA of autophagy-associated proteins were also observed in the striatum of HD patients. Notably, LC3A and LAMP2 mRNA are increased, consistent with an overall increase in autophagic flux, whereas PINK1 is decreased, suggesting defects in mitophagy [282]. Finally, a polymorphism in Atg7, which is required for LC3 lipidation, is associated with earlier HD onset, thus providing strong genetic evidence for the close interplay between HD and autophagy [283].

Another important feature of htt is its ability to modulate autoclearance through posttranslational modifications. Ubiquitination of htt at K6, K9, and K15 is associated with increased degradation, whereas SUMOylation of these residues leads to decreased clearance [284]. Wild-type htt is further ubiquitinated at the K48 linkage, which is a classical marker of proteosomal degradation, whereas mhtt is ubiquitinated at K63, which is correlated with increased aggregation [275]. The ubiquitination and SUMOylation of htt is regulated through phosphorylation of S13 and S16, which are critical for mediating mhtt toxicity [285]. Acetylation at K444 is also important for regulating htt degradation and has been shown to increase mhtt clearance through enhanced binding to p62 [286].

In addition to the effects of mhtt on the autophagic pathway, recent studies have suggested a direct interaction of mhtt with lysosomes. In mouse striatum, mhtt alters lysosomal positioning via increased perinuclear localization which correlated with increased autophagic flux in response to nutrient deprivation [287]. Furthermore, it was recently shown that mhtt is able to be secreted from neurons through an unconventional endolysosomal pathway, suggesting that interactions of mhtt with the endolysosomal system may also contribute to disease pathogenesis [288].

Targeting Lysosomal Pathways in Neurodegenerative Disease

As discussed, several neurodegenerative diseases present with changes to lysosomal and autophagic function, and these alterations may in turn correlate to physiological aging processes. At present, several therapeutic approaches

converge on targeting autophagy and lysosomes in neurodegenerative diseases. Therefore, combating the pathophysiological mechanisms implicated in these diseases will improve our knowledge and assist in the development of lysosome-targeted therapies to reduce disease burden and ultimately preserve lysosomal function in the aging brain.

Targeting Autophagy in Neurodegeneration

The strong association between neurodegenerative diseases and aberrations in autophagy implicates the autophagic pathway as a prime therapeutic target for the treatment of these diseases. Various therapeutic approaches targeting both mTOR-dependent and mTOR-independent pathways of autophagy have now been tested preclinically and clinically (Fig. 2, Table 1).

mTOR-Dependent Targets

The macrolide allosteric mTOR inhibitor, rapamycin, and its analogue, temsirolimus, have been tested in multiple neurodegenerative disease models. mTOR inhibitors were shown to induce autophagy, prevent dopaminergic loss, and revert motor, cognitive, and affective symptoms in several PD mouse models [289–292]. Moreover, rapamycin was able to improve pathology and disease phenotypes in AD, ALS, and HD models [237, 278, 333–337]. Other mTOR-dependent stimulators of autophagy, including GTM-1, carbamazepine, and latrepirdine, were shown to decrease Aβ accumulation and APP metabolites in AD cell models and also protect against memory dysfunction in AD patients [310–313].

mTOR-dependent stimulators have been most extensively tested in PD. The GSK3B inhibitor 6-Bio was neuroprotective in PD mouse model, and reduced αSyn aggregation in vitro

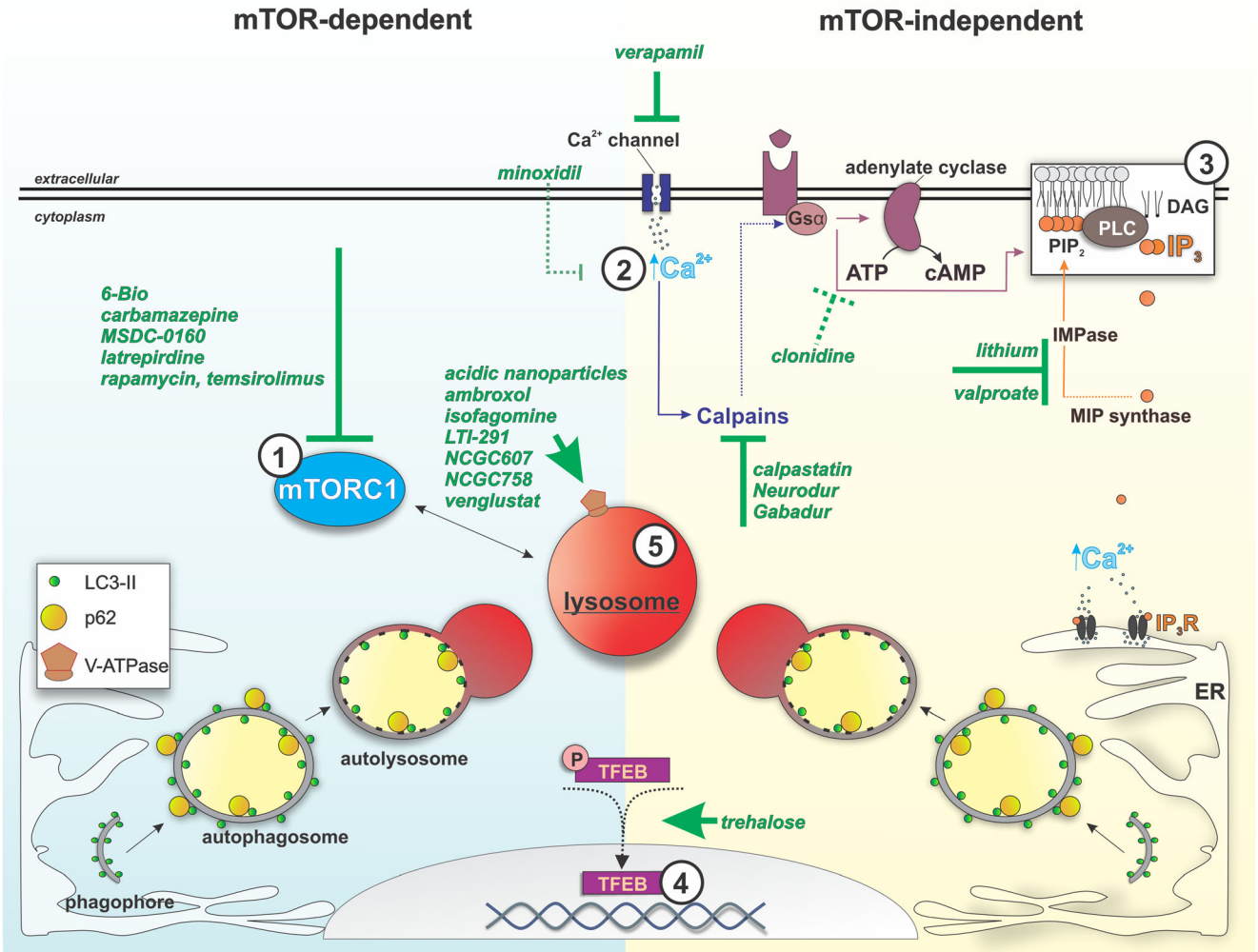

Fig. 2 Pharmacological agents used in preclinical and clinical investigational therapies for neurodegeneration and their known or proposed targets related to autophagy and lysosomes. Agents whose action relates to suppression of mTORC1 activity (1), reduction of cytosolic calcium and IP_3 levels as well as enhancement of TFEB activity (2-4) are expected to generally induce cellular autophagy. Targeting lysosomal acidity or glucocerebrosidase activity (5) can specifically induce lysosomal clearance efficiency

Table 1 Therapies for neurodegenerative diseases targeting lysosomal pathways. Various therapies have been used to stimulate autophagy, both mTOR-dependent and mTOR-independent, as well as promote lysosomal function in neurodegenerative diseases

Disease	Therapy	Pathway	Proposed mechanism	Reference(s)
PD	Rapamycin Temsirolimus	mTOR-dependent	mTORC1 inhibition	[289–292]
	MSDC-0160			[293]
	6-Bio		GSK3B inhibitor	[294]
	Verapamil Minoxidil	mTOR-independent	attenuation of cytosolic Ca^{2+} increase	[77]
	Clonidine		cAMP levels reduction	
	Lithium, valproate		IP_3 reduction	[76, 295]
	Calpastatin		Calpain inhibition	[77]
	Trehalose		Akt inhibitor	[296–298]
	AUTEN-99		MTMR14/Jumpy inhibitor	[299]
	Acidic nanoparticles	Lysosomal	Lysosomal acidification	[300]
	Ambroxol** Isofagomine		GCase chaperone	[301–304]
	NCGC758		GCase chaperone	[214, 305]
	NCGC607		GCase chaperone	[306]
	Venglustat**		Glucosylceramide synthase inhibitor	[307]
	LTI-291**		GCase activator	[307]
AD	Carbamazepine Latrepirdine	mTOR-dependent	mTORC1 inhibition	[308–311]
	Temsirolimus			
	Lithium	mTOR-independent	IP_3 reduction	[312]
	Resveratrol**		Unknown	[313–316]
	Memantine*		NMDA receptor agonist	[317, 318]
	Metformin**	Lysosomal	TFEB activation	[319, 320]
ALS/FTD	Rapamycin	mTOR-dependent	mTORC1 inhibition	[237]
	Trehalose	mTOR-independent	Akt inhibitor	[321–323]
	Resveratrol		Unknown	[324]
HD	Rapamycin	mTOR-dependent	mTORC1 inhibition	[278]
	Trehalose	mTOR-independent	Akt inhibitor	[321, 325]
	Lithium, valproate Verapamil		IP_3 reduction attenuation of cytosolic Ca^{2+} increase	[326, 327]
	Clonidine, rilmenidine		cAMP levels reduction	[77, 328]
	Calpastatin		Calpain inhibitor	[77, 322]
	Zn finger repressors	Various	Correction of mhtt	[329]
	CRISPR-Cas9			[330]
	ASOs (IONIS-HTT_{Rx})**		mhtt RNA degradation	[331, 332]

*Memantine is FDA-approved for AD

** Currently in clinical trials

[294]. The insulin sensitizer, MSDC-0160, prevented and restored dopaminergic loss and motor deficits in lesioned mice while additionally exerting an anti-inflammatory effect [293]. Similarly, pathways targeted by the anti-diabetic drug, exendin-4/exenatide are implicated in the preservation or restoration of dopaminergic neuron integrity in lesioned PD rodent models [338, 339], yet autophagy-related effects are unclear. Exenatide, now in phase I clinical trials for early stage PD, showed potential for sustainable motor score improvement for PD patients in a previous Phase II study [340]. Lastly, the c-Ab1 tyrosine kinase inhibitor and AMPK activator nilotinib, was shown to induce autophagy, enhance dopaminergic viability, and

improve motor function in PD mouse models [341–343], yet mTOR involvement is unclear. The compound, which is currently in phase II clinical trials, showed potential to improve symptoms in both late-stage PD and Lewy body dementia patients [344].

mTOR-Independent Targets

Despite the promise of mTOR-dependent activation of autophagy, side effects associated with modulating mTOR have led to the emergence of mTOR-independent activators of autophagy as possible alternatives. Trehalose, a disaccharide, has been proposed to promote lysosomal clearance via inhibition of Akt and prevention of inhibitory TFEB phosphorylation [83]. The compound was shown to enhance clearance of mutant αSyn and Htt aggregates in cell lines, attenuate dopaminergic loss in PD mice, and protect mitochondrially challenged human iPSC-derived dopaminergic neurons [296–298, 345]. Moreover, trehalose delayed neurodegeneration while improving pathology and phenotypes in both HD and SOD1 ALS mouse models [323–325, 327].

Mood-stabilizing agents, such as lithium and valproate, also function as mTOR-independent autophagy activators through inositol depletion via inhibition of inositol monophosphatase and inositol 3-phosphate synthase, respectively [78]. These agents have been shown to enhance clearance of aggregate-prone htt and protect dopaminergic integrity in PD mouse models [76, 295], alleviate pathology and motor phenotypes in HD models [328, 329], and improve cognitive decline in AD patients with few adverse effects [314]. Resveratrol, another autophagy stimulator, was found to delay symptoms and pathology in SOD1 ALS mice [326], as well as decrease Aβ pathology in AD models in an AMPK-dependent manner [313, 314]. Although 1 study found that AD patients treated with resveratrol show an increase in plasma Aβ40 levels [317], another study demonstrated decreased levels of Aβ42 in the CSF with long-term treatment in patients [318]. Therefore, whether resveratrol is beneficial for AD patients in the long term remains to be determined. Calpain inhibitors such as calpastatin, Gabadur, and Neurodur have also been shown to be beneficial in PD and HD mouse models in an mTOR-independent manner [346, 347].

Other putative mTOR-independent autophagy inducers have been tested in specific disease models. AUTEN-99, which inhibits the myotubularin-related phosphatase MTMR14/Jumpy, a negative regulator of autophagic membrane formation, phenotypically rescues PD-associated outcomes in Drosophila models [299]. Memantine, an NMDA receptor agonist, displays efficacy in patients with moderate to severe AD [319, 320], though the compound's effects on autophagy remain to be explored. Rilmenidine, an antihypertensive, attenuates HD pathology and accumulation of mhtt in a mouse model via an mTOR-independent mechanism [330]. Lastly, L-type Ca^{2+} channel antagonist verapamil, ATP-sensitive potassium channel opener minoxidil, and the G-signaling modulator clonidine, have been proposed to promote autophagy via modulation of IP_3 levels, intracellular Ca^{2+}, and calpain activity, and have been suggested as potential therapeutic agents in HD and other neurodegenerative diseases [77].

It is not currently known whether induction of autophagy can confer long-term improvements in neuropathology, delay disease progression, or ideally prevent neurodegeneration. Apart from its central role in cellular degradation and recycling, autophagy has emerging roles in DNA repair, inflammation, and protein secretion [348–350], suggesting that upstream targeting of this mechanism can have multiple effects which cannot be precisely predicted based on current knowledge. Though autophagy is an attractive target in neurodegeneration, the prolonged and systemic administration of upstream autophagy stimulators comes with the risks. For instance, although rapamycin shows positive outcomes in preclinical studies, higher doses of rapamycin inhibit mTORC2 thereby potentially compromising survival, whereas immunosuppression and other side effects have also been reported [351]. For several other compounds, the mode of action is not thoroughly characterized, and the effect on autophagy may not be specific or direct. In addition, a very recent study in *C. elegans* demonstrated that increasing autophagy can also be detrimental and compromise lifespan, depending on the status of the mitochondria [352]. This becomes a concern for the systemic induction of autophagy because different cell types or tissues exhibit baseline metabolic differences and could be differentially affected by aging and neurodegeneration. An additional consideration is the timing of intervention as well as the genetic background of patients. Indeed, enhancing autophagy at early disease stages may be effective in delaying the accumulation of disease-related proteins. On the contrary, increasing autophagosome formation at later disease stages or in patients whose lysosomal function is likely already compromised may exacerbate neurodegenerative pathology due to exceeding the cellular capacity for degradation. For these reasons, more targeted approaches could have more efficacious, powerful, and specific outcomes for patients.

Targeting Lysosomes in Neurodegeneration

More direct lysosome-targeted therapies have been proposed and tested in PD, AD, and HD (Table 1). Given the selective vulnerability of substantia nigra neurons in PD, direct gene targeting is a potentially relevant therapeutic strategy. Preclinical models suggest that overexpression of Beclin 1, Lamp2A, and TFEB could induce lysosomal function and confer neuroprotection [290, 353, 354]. Another approach to target lysosomes in PD involves the direct delivery of nanoparticles to enhance lysosomal acidification, which restored lysosomal activity in ATP13A2 and GBA1 PD models [300].

Decreased lysosomal hydrolase activity has been attributed to many neurodegenerative diseases. Thus, patients may also benefit from direct targeting and enhancement of these hydrolases. For example, AAV delivery of GRN to progranulin knockout mice significantly reversed lysosomal dysfunction associated with both FTD and NCL [355]. In addition, viral expression of GBA1 or reduction of GCase substrate levels via pharmacological glucosylceramide synthase inhibition reduced αSyn pathology and rescued dopaminergic integrity and cognitive function in rodents [308, 309, 356]. Venglustat, which inhibits glucosylceramide synthase, is currently in phase II clinical trials for early-stage GBA1-PD patients [307]. Reduction of glucosylceramide levels can also be achieved via small molecule non-inhibitory chaperones. NCGC758, which activates GCase specifically in the lysosomal compartment, preserved physiological αSyn conformation and increased the efficiency of trafficking and maturation of GCase in the endolysosomal compartment in PD iPSC-derived midbrain neurons [214, 305]. Similarly, NCGC607, also a small molecule non-inhibitory chaperone was found to reduce glucosphingolipids and αSyn levels in iPSC-derived dopaminergic neurons from Gaucher patients with parkinsonism [306]. Other compounds, such as the pharmacological chaperones ambroxol and isofagomine, have also been shown to increase GCase levels and activity and preserve αSyn homeostasis in PD/GBA1-PD and also wild-type cell and animal models [301–304]. Currently, ambroxol is in phase II clinical trials for GBA1-PD and PD with dementia patients [307]. Furthermore, the compound LTI-291, which stimulates GCase activity in the brain, is being assessed in phase II trials in GBA1-PD patients [307]. Unlike enzyme replacement therapies which would require invasive CNS delivery, pharmacological chaperones target specific lysosomal hydrolases, such as GCase, and could be orally administered and cross the blood–brain barrier. Examining the distribution of these compounds in disease-relevant CNS regions is important, to which end both wild-type and lysosomal enzyme-deficient animal models are necessary. Furthermore, GCase activation via pharmacological chaperones such as isofagomine binding to the active site of the enzyme, requires washout in order to achieve maximal lysosomal activity induction [357, 358]. The administration to patients should therefore be optimized so that the chaperone is rapidly removed from the lysosome, allowing the enzyme sufficient time to interact with its endogenous substrate. Future studies focusing on the efficacy of non-inhibitory or allosteric chaperones [305, 306, 359] could provide significant advancements, because the enzyme could interact with the endogenous substrate without the necessity of compound removal.

In AD, a class of interventions is focused on directly improving lysosomal function. Current clinical trials suggest that protein phosphatase 2A agonists, such as metformin, have beneficial effects by inhibiting hyperphosphorylation of tau [321, 322]. As phosphorylated tau constitutes a major component of NFTs in neurons [321, 322], metformin in conjunction with other treatments that reduce Aβ pathology may be useful for improving both pathological hallmarks of AD. Furthermore, TFEB transduction in the hippocampi of AD mouse models reduced AD-associated pathologies by upregulating lysosome biogenesis [360].

Because of growing evidence for htt regulation of autophagy and lysosomal function in HD, direct targeting of htt was proposed as a therapeutic approach in disease. The monogenetic etiology of HD makes it particularly attractive for gene therapies, specifically those targeting mhtt DNA and mRNA. DNA-targeting therapies, such as zinc finger proteins and CRIPSR-Cas9, to transduce cells with functional htt, have been tested in animal models. Zinc finger repressors reduced mhtt expression in the brains of R6/2 mice [331], and CRISPR-Cas9 gene editing ameliorated neurotoxicity in an HD mouse model [332]. Moreover, mRNA-targeting therapies against mhtt, such as antisense oligonucleotides (ASOs), nucleotide-based therapies that bind mRNA to trigger degradation, have already entered clinical trials [361]. The first of these, IONIS-HTTRx, was well-tolerated and resulted in dose-dependent reductions in mhtt levels in the CSF of early HD patients [362] and has now entered phase III clinical trials. The development of therapies directly targeting mhtt is advantageous in that it may not only mitigate lysosomal defects in disease but improve other aspects of disease pathophysiology and symptomology as well.

Overall, lysosomal function is a promising and specific target in neurodegeneration, with the possibility to benefit patients of various genetic backgrounds at early or even later stages of disease. Further elucidation of lysosomal functions and regulation of lysosome-related pathways will be instrumental to shaping the design and specificity of future therapeutic interventions. In this endeavor, a significant step forward has been made by the development of iPSC and CRISPR/Cas9 technologies, which in combination have improved our ability to assess the efficacy of therapies targeting lysosomal function in patient-derived neurons in both sporadic and familial disease. Nevertheless, a major challenge of the future will be the identification of sensitive and specific biomarkers for both diagnosing neurodegenerative diseases early and evaluating patient responses to therapies, which may include assessment of lysosomal efficiency in disease-relevant regions of the CNS.

Conclusion

Lysosomal function in the aging brain is an area that is just beginning to be explored. The lysosome is cardinal to various cellular functions including autophagy, nutrient and ion homeostasis, and inter-organelle interactions. As our understanding of the role of lysosomes in aging continues to take shape, it

will be necessary to identify how various lysosomal nutrient storage, sensing, and processing pathways are engaged in brain-specific cell types in order to facilitate the discovery of therapeutic targets. For now, the best insight into lysosomal alterations with age will come from the detailed investigation of pathophysiological mechanisms in age-related neurodegenerative diseases that often present with hallmarks of lysosomal and autophagic dysfunction. Several therapeutic strategies to combat these diseases have already emerged and entered into clinical trials, including those promoting the upregulation of autophagy and those directly aimed at rescuing lysosomal function. The promise of these treatments in neurodegeneration beckons the question of whether these therapies will be similarly beneficial in preserving lysosomal function in the aging brain.

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