

Review

Emerging Potential Role of Autophagy to Modulate Aggresome Formation: insights Molecular Treatment of Alzheimer's Disease

Md. Ataur Rahman^{1,2*}, MD. Hasanur Rahman³, ANM Mamun-Or-Rashid⁴, Hongik Hwang¹, Hyewhon Rhim^{1,5*}

¹Center for Neuroscience, Brain Science Institute, Korea Institute of Science and Technology (KIST), Seoul 02792, Republic of Korea.

²Global Biotechnology & Biomedical Research Network (GBBRN), Department of Biotechnology and Genetic Engineering, Faculty of Biological Sciences, Islamic University, Kushtia 7003, Bangladesh.

³Department of Biotechnology and Genetic Engineering, Bangabandhu Sheikh Mujibur Rahman Science and Technology University, Gopalganj-8100, Bangladesh.

⁴Anti-Aging Medical Research Center and Glycation Stress Research Center, Graduate School of Life and Medical Sciences, Doshisha University, Kyoto 602-8566, Japan.

⁵Division of Bio-Medical Science and Technology, KIST School, Korea University of Science and Technology (UST), Seoul 02792, Republic of Korea.

***Correspondence address to:** Center for Neuroscience, Brain Science Institute, Korea Institute of Science and Technology (KIST), 5 Hwarang-ro 14-gil, Seongbuk-gu, Seoul 02792, Republic of Korea. Md. Ataur Rahman, E-mail: ataur1981rahman@hotmail.com; Hyewhon Rhim, Tel.: +82-2-958-5923, Fax: +82-2- 958-6937, E-mail: hrrhim@kist.re.kr

Abstract

Alzheimer's disease (AD) is one of the most prevailing neurodegenerative diseases in the world, which is characterized by memory dysfunction and the formation of tau and amyloid β ($A\beta$) aggregate in multiple brain regions, including the hippocampus and cortex. The formation of senile plaques involving tau hyperphosphorylation, fibrillar $A\beta$, and neurofibrillary tangles (NFTs) are used as pathological markers of AD, and eventually produces aggregation or misfolded protein. Importantly, it has been found that failure to degrade these aggregate-prone proteins leads to pathological consequences, such as synaptic impairment, cytotoxicity, neuronal atrophy, and memory deficits associated with AD. Recently, increasing evidences have been suggested that autophagy pathway plays a role as a central cellular protection system to prevent the toxicity induced by aggregate or misfolded proteins. Moreover, it has also been related that AD-related protein aggresomes could be selectively degraded by autophagosome and lysosomal fusion through autophagy pathway which is known as aggrephagy. Therefore, the regulation of autophagy might be served as a useful approach to modulate the formation of aggresome associated in AD. This review focuses on the recent improvements in the application of natural compounds and small molecules as a potential therapeutic approach for AD prevention and treatment via aggrephagy.

Key words: Alzheimer's disease (AD); aggregation; autophagy; aggresome; autophagosomes; aggrephagy.

Introduction

Aggresomes are inclusion bodies consist of aggregated cytoplasmic proteins induced by the overexpression or inhibition of certain proteins of the proteasome system [1], and the accumulation of incorrectly folded proteins is thought to contribute to the etiology of various neurodegenerative diseases [2]. Misfolded protein molecules processing has an important to maintain normal cellular function as well as homeostasis. There are three protein quality systems have been found to degrade misfolded or aggregated protein, such as ubiquitinated proteasomal degradation, chaperone-mediated degradation, and selective autophagy or aggrephagy [3]. Previous studies have been demonstrated that an increasing trend in the number of AD-related proteins are associated with aggresome [4], and non-pathological proteins could form invasive inclusion bodies as well. It has been found that silencing the expression of ubiquitin ligase HRD1 in SH-SY5Y human neuroblastoma cells prevents the formation of APP [5], and ubiquitin 1 linked to AD is known to regulate presenilin-1, which plays a key role of invasiveness in AD pathogenesis [6]. It has been hypothesized that autophagy manipulations might be a potentially promising therapeutic strategies target to modulate protein aggregation-related diseases and toxicity.

In this review, we would like to emphasize the susceptibility of AD-associated proteins to autophagy. New evidences have been suggested that macrolysis (henceforth mentioned as autophagy) of the hemolysate-mediated degradation system is the main regulator of internal kinetics [7]. For instance, independent reports have demonstrated the significance of autophagy in neurodegenerative diseases and inclusion body formation [8,9]. Furthermore, in other studies, the pharmacological initiation of autophagy has promoted the breakdown of aggregated proteins, such as Huntington's protein (Htt) and tau mutants, while increasing cell viability [10,11], thus suggesting that targeting the autophagy pathway may provide innovative methods for treating diseases related to protein conformational changes. Attack formation promotes the delivery of decentralized proteins aggregation to the autophagic pathway [12]. Pharmacological inhibition of autophagy delayed the elimination of the attack formed by the mutant peripheral myelin protein PMP22, a Schwann cell protein associated with demyelinating neuropathy [13]. It has been exhibited that aggresomes produced by the Htt mutant positively stained certain proteins related to autophagy and sensitive to eliminate by autophagy [14]. However, it has not been determined whether autophagy easily eliminates invasion caused by other proteins associated with the disease.

Mechanism of aggresome formation

Aggrephagy is a selective form of autophagy that eliminates aggregated and ubiquitinated proteins [15]. The first stage of aggresome formation involves the accumulation of irregularly folded or unfolded proteins, packed into larger insoluble aggregates and transported to the microtubule-organizing center (MTOC) [15,16], and aggresomes can cause autophagic degradation. In autophagic process, autophagosomes undergo lysosomal degradation through dual autophagy membranes encompassing ubiquitin-labeled proteins [17,18]. The autophagy pathway plays an important role in the cytoplasm, but it is not efficient in the nucleus because autophagy-related molecules do not have nuclear inclusions. Aggresomes are membrane-free microtubule-dependent cytoplasmic inclusion bodies that form oligomer complexes with amorphous structure containing unfolded or misfolded proteins which are generally stable and insoluble under physiological conditions [19,20]. Typically, aggregated proteins continue to grow and develop, and then oligomerize to form longer insoluble inclusions bodies or aggregates [21]. These aggregates can either link with themselves or join with existing aggresomes located at the MTOC, residing near the cellular centrosome. These aggregates are labeled with ubiquitin alone with heat shock proteins (Hsps), such as Hsp70, and their development in neurodegenerative diseases is demonstrated in Figure 1 [3,22],[23,24]. Also, aggresomes are primarily surrounded by the cytoskeleton of intermediate filament, and a cage-like structure appears after collapsing with cytoskeleton, such as vimentin and keratin, along with neurofilaments [19,25] (Figure 1). This cage-like structure is known to promote structural stability and inhibit non-specific interactions. These aggresomes exert cellular cytotoxicity, but at a later stage, matured bilayer autophagosomes lose their activity [26]. The components of the ubiquitin-proteasome systems are concentrated or grouped into these assemblies before discarding of aggregates molecules through degradation or refolding [27]. Recently, it has been reported that the modulation of aggresome formation and assembly may serve as a novel approach to treat diseases associated with a defect in protein conformation.

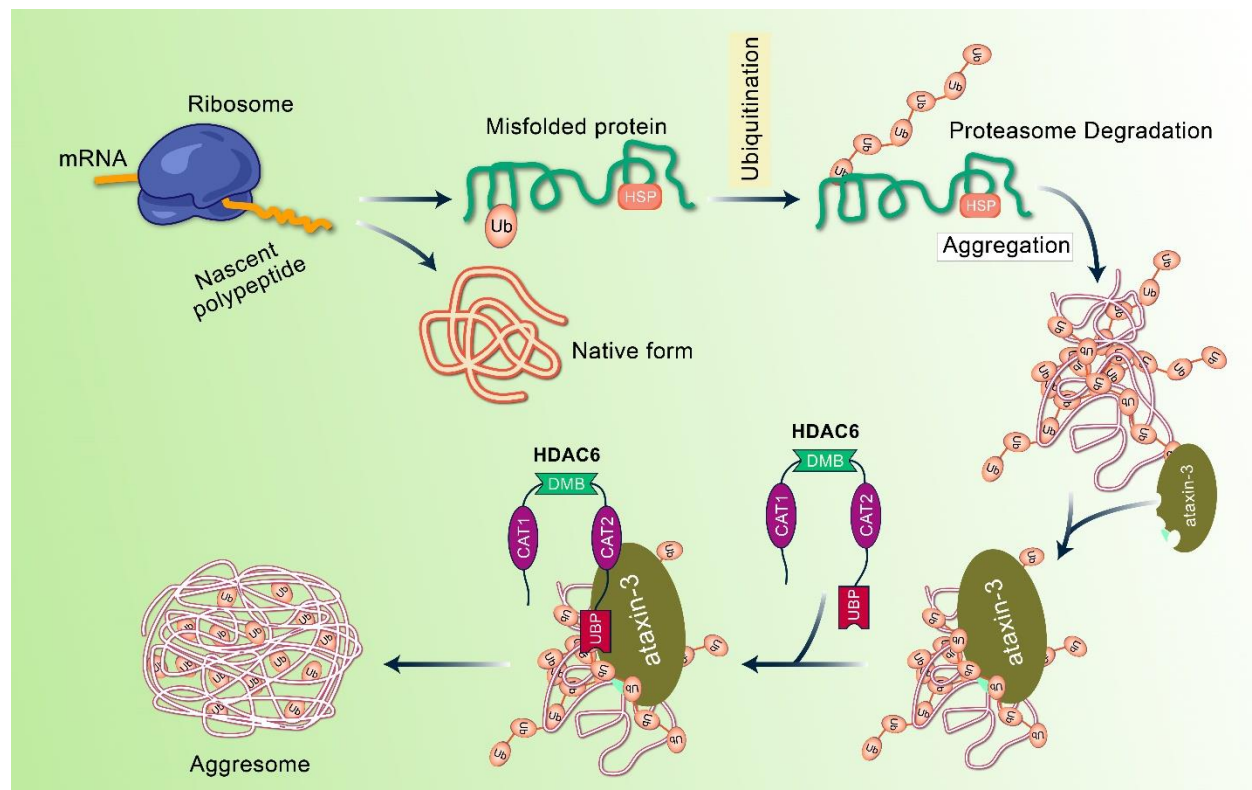


Figure 1: Molecular mechanism of aggresome development and formation. Under normal conditions, misfolded and polyubiquitinated proteins are fragmented via the ubiquitin proteasomal system. When ubiquitin proteasomal system is altered or overhauled, misfolded polyubiquitin proteins accumulate and form to aggregate. In this case, ataxin-3-dexiquitinase interrelates and aggregates with polyubiquitinated proteins to form ubiquitin chains structure. In addition, HDAC6 binds these non-anchored C-terminal tails of ubiquitin to form aggregates and recruits them into the dynein motor complex.

Clearance of aggresomes through autophagy

When the proteasomal degradation system is overwhelmed, the autophagy pathway is activated as an essential cellular defense system to resist incorrect folding and to prevent the accumulation of aggregated proteins [2,28]. In this system, poorly folded and aggregated proteins are selectively identified and transferred to the MTOC (center of microtubule tissue) around the central body by microtubule-based and microtubule-based retrograde transport [29]. Accumulating evidences suggest that autophagy not only protects aggressive cells by chelating poorly folded cytotoxic aggregates, but also enriches poorly folded aggregates for subsequent removal [12]. Autophagy is

a multi-step progression well-characterized by the construction of an insulating membrane, named phagophore, which swells to form the double-membrane autophagosome and fuses with the lysosome to degrade the sequestered cytoplasmic cargo [28,30]. Unlike proteasomes, autophagy does not require substrate expansion, and is capable of degrading large protein complexes, protein aggregates, and even entire organelles [31,32]. Moreover, autophagy is a precisely controlled process involving multiple proteins encoded by the Atg (autophagy-related) genes [33,34], and previous studies revealed that autophagy is induced in response to the oxidative stress or proteasomal damage, and is directly involved in the elimination of aggresomes [35,36].

Emerging evidences suggest that autophagy is responsible for the removal of aggregated and misfolded proteins, and the inhibition of autophagy preferentially affects the degradation of mutant proteins associated with neurodegenerative diseases while leaving their wild-type counterparts unaffected [37]. Although the mechanism of autophagy for the specific elimination of misfolded proteins is not yet clear at the molecular level, selective isolation of incorrectly folded proteins in aggresomes can facilitate the preferential removal of abnormal autophagy proteins [38]. By chelating the endogenous autophagy inhibitor mTOR (mammalian target of rapamycin) kinase [39], the aggresome can also contribute to the initiation of autophagy [40,41]. Importantly, the conventional concept of autophagy only involves the non-selective removal of misfolded proteins and regular cellular proteins, whereas the aggresome-autophagy pathway is specific to aggregated and misfolded proteins. Therefore, a special type of autophagy induction is thought to be related to the specific elimination of toxicity linked to the aggresome formation. One potential mechanism has been found in parkin-mediated Lys⁶³-linked polyubiquitination which promotes the elimination of misfolded proteins via autophagy, and autophagy occurs by binding to the adapter protein p62 [42], an Ub binding protein interacting with the ubiquitinated proteins via the autophagic mechanical component LC3 through its UBA domain (connected to Ub) and the 22 amino acid LIR (LC3 interaction region) [43]. p62 promotes the binding of a polyubiquitin chain linked to Lys63 [30,31], and the inhibition of its UBA or LIR domain changes the conditions of the ubiquitinated aggregates in the autophagosomes [44,45]. According to recent evidences, p62 may also promote the formation of protein aggregates in addition to promoting the elimination of autophagy [46]. Further studies are required to determine whether p62 is indeed an Ub receptor,

which regulates the processing of Lys63-linked folded polyubiquitinated proteins via an active aggresome-autophagy pathway [47] (Figure 2).

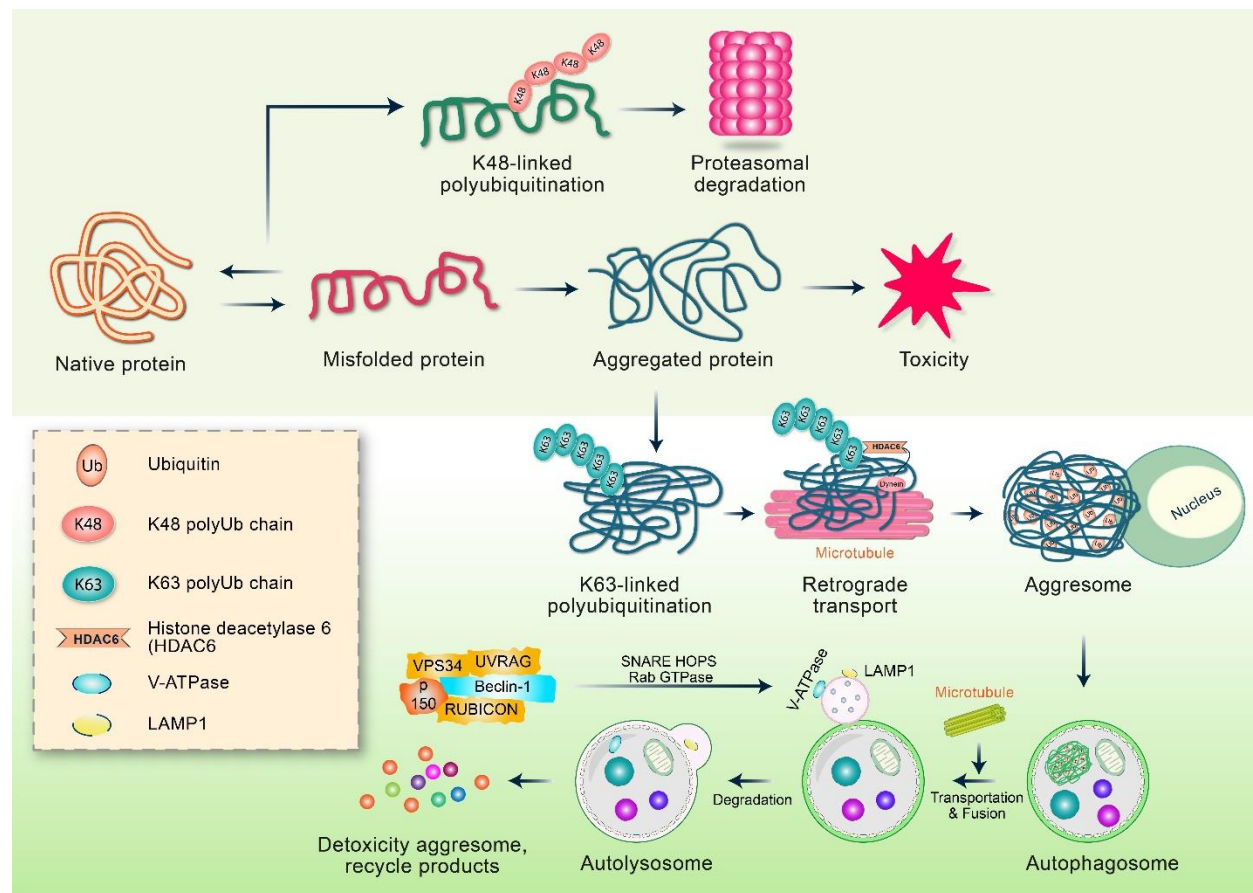


Figure 2: The regulation of aggresome-autophagy pathway. Aggresome and autophagy are regulated by Lys63-associated polyubiquitination mediated pathway. Oxidative damage or genetic mutations are responsible to cause protein folded. After folding, the misfolded proteins are labeled with polyubiquitin chains linked to Lys48, which is subsequently degraded via either the proteasomal system or chaperones-mediated pathway. However, when proteasome and chaperone systems are overwhelmed, misfolded proteins form oligomers or aggregates with cellular toxicity. Moreover, PD-related parkin ligase E3 acts with the E2 enzymes Ubc13/Uev1a to facilitate Lys63-associated polyubiquitination of misfolded proteins in proteasome injury conditions. Polyubiquitin chain stimulates the binding with HDAC6 and the misfolded proteins bind to dynein motor complex which retrograde transports aggresome to MTOC. Polyubiquitination encourages p62 binding in addition to recruiting autophagic membranes to form autophagosomes. Consequently,

the fusion of autophagosomes and lysosomes facilitates the degradation of misfolded and aggregated proteins through the lysosomal hydrolases.

Molecular mechanism of the fusion of aggresome and lysosome

In the final stage of degradation, fully matured and closed membrane aggresome fuses with lysosomes to form hybrid compartment organelles called "autolysosomes" which digests their contents [16] (Figure 2). Although the fusion process is morphologically characterized well, its mechanism remains relatively elusive at the molecular level. Typically, the separation membrane is fused with the lysosomal membrane, and then the lysosomal hydrolase breaks down the content of the fused (or aggresome/lysosome) autolysosome. The substance (amino acid, etc.) is eventually degraded, which is then either recycled or removed into extracellular space [48]. Subsequently, the lysosomal fraction of the autolysosomes is recovered to produce new lysosomes [49]. Rab7A (Ypt7 in yeast), mature autophagosome, HOPS (homologous protein fusion and classification complex) and SNARE receptor (SNAP (soluble adhesion protein NSF)) (NSF, fusion-sensitive) labeled (ethyl-maleimide) proteins are essential for the fusion of autophagosomes and lysosomes [48]. The SNAREs increases the permeability of the membrane, forming the membrane opening and fusing the content of two adjacent organelles [50]. The Rab7A is transformed into an active form linked to GTP through the action of HOPS (Figure 2). The fusion of organelle binding is dependent on the complex of Rab7A and HOPS (Rab7A downstream effector), which is present in two membranes fused, and SNARE and Rab7A are enriched at the fusion site [51]. SNAREs are also required for the expansion and closure of autophagosomal membranes, the early stages of autophagy, and for the transportation of Atg9 (a transmembrane protein necessary for membrane development) [52]. Recently, it has been found that the SNARE protein syntaxin 17 (STX17) is employed in the outer membrane of the autophagosomes and mediates the lysosomal fusion [53,54]. The depletion of STX17 prevents the destruction of autolysosomes and leads to the accumulation of autophagosomes in the process of basal and starvation-induced autophagy [55]. It is currently unknown whether STX17 is also involved in selective aggrephagy [56]. Furthermore, when autophagosomal membrane is formed around the materials to be submerged in the endoplasmic reticulum, STX17 interacts with the autophagosome-labeled Atg14L protein at a very early stage [57,58]. These findings together suggest that STX17 plays an important role at both ends of the autophagy decomposition pathway.

The role of autophagy to modulate the aggregation in AD

The crucial function of autophagy in preserving neuronal homeostasis is well established in *in vivo* studies conducted in mice, in which the inhibition of autophagy results in protein deposition and neurodegeneration over time [59]. Decreased autophagy is related to an increase in age-associated neurodegenerative diseases, such as AD [60]. Defective autophagy typically involves decreased expression of major receptors for autophagy, and altered orientation substrate and autophagosome formation. Emphasis has been placed on several aspects, such as the lack of maturation, degradation and lysosomal alteration [61]. Interestingly, several genes involved in protein homeostasis in neurodegenerative diseases are mutated, such as the lysosome-autophagy system and ubiquitin-proteasome system [62]. In the cases of neurodegenerative diseases, the first marker of altered autophagy is abnormal quantity of autophagosomes or amphisomes, which can lead to the generation of ROS and other cytotoxic elements. Incidentally, the accumulation of autophagosomes is characterized by an endogenous pool of amyloidogenic A β peptides in animal models with AD [63].

The potential function of SQSTM1/p62 in AD progression and other neurodegenerative disorders has been receiving more attention [64]. Compared to the control group, cytoplasmic SQSTM1/p62 protein levels were found to be reduced in the frontal cortex of AD patients [65,66], indicating that SQSTM1/p62 expression is downregulated and the protein is retained in the pellet [66]. Interestingly, oxidative impairment to the promoter of SQSTM1/p62 leads to the reduction in gene transcription, which also appeared in the brain of AD patients [66]. Histopathological analysis of hippocampal and cortical samples from patients with AD showed that the SQSTM1/p62 protein is contained in inclusion bodies mainly consisted of phosphorylated tau, TRAF6 (tumor necrosis factor receptor associated 6), and ubiquitin, promoting the formation of aggresome [67]. Moreover, co-localization of SQSTM1/p62 and Keap-1 insoluble deposits was observed in AD brain extracts [68], and the impairment to the promoter of SQSTM1/p62 as well as co-localization of SQSTM1/p62 with protein aggregation have been observed in other neuropathies, such as Huntington's disease, tauopathy and α -synucleinopathies [64,66]. Several evidences suggested that SQSTM1/p62 plays a central role in the transport of tau protein to proteasome [67], and that there is a negative connection between the levels of SQSTM1/p62 and p-tau [69], indicating that reduced SQSTM1/p62 function likely causes the accumulation of tau positive aggregates with age. Notably,

a SQSTM1/p62-deficient mouse model has typical AD phenotype, including p-tau neurofibrillary tangles, memory impairment, and synaptic depletion [70]. In addition to protein homeostasis, these last two effects are consistent with the assumption that autophagy is necessary for cell remodeling and neuronal plasticity, and autophagy is a prerequisite for neurogenesis and memory processes [71]. However, it is unknown whether a change in the levels of SQSTM1/p62 is correlated with the elimination of abnormal aggresomes in AD patients. Collectively, current findings together suggest that it would be promising to decrease the formation of aggresomes in AD via modulating autophagy.

Potential therapeutic action of autophagy to control aggresome formation in AD pathogenesis

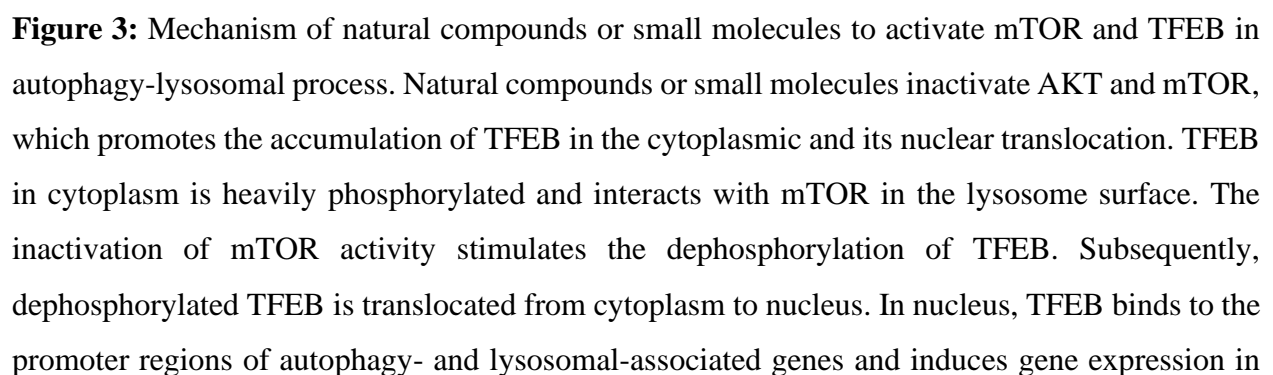
Natural compounds or small molecules have been used to induce clearance of aggresome and to restore or enhance cognitive function in patients with AD [4]. For example, liraglutide has been found to activate insulin degradation enzyme (IDE), increase cognition function and long term potentiation (LTP), and reduce A β plaque deposition and inflammation in APP/PS1 mice via mTOR-independent and JNK pathway [72,73]. Also, rapamycin, a well-known natural macrolide, was found to mitigate A β plaques, liberate cerebral amyloid angiopathy, and enhance memory impairment in the AD mice model of PDAPP, hAPP (J20) and P301S through the inhibition of mTOR activity [74,75]. Moreover, a polyphenolic compound known as curcumin, a PI3K/mTOR inhibitor, not only relieved AD pathology by decreasing A β but also repaired spatial memory function in APP/PS1 mice through the degradation of autophagic A β aggregates [76]. In clinical trials using JNPL3 mice as an AD model, autophagy induced by methylene blue was shown to decrease the aggregation insoluble tau through the inhibition of mTOR [77]. Another polyphenol, oleuropein aglycone, also promotes autophagy by releasing Ca²⁺ from reticulum and preventing the activity of mTOR in TgCRND8 mice, and considerably downregulated A β plaque [78]. A carbazole-based fluorophore, SLM, binds to A β and prevents A β aggregation in 3xTg-AD mouse model by alleviating the pathological and behavioral impairments in AD [79]. Additionally, human prolactin-releasing peptide palmitoylated analog, palm11-PrRP31, was shown to protect neuronal cells in APP/PS1 mice model of AD and decrease astrogliosis, microgliosis, β -amyloid plaque load as well. [80]. The list of representative natural compounds and small molecules that modulate aggresome formation in AD pathogenesis by enhancing autophagy is summarized in Table 1.

Table 1: Natural compounds and small molecules that modulate autophagic activity and control aggresome formation in AD models.

Natural compounds/ small molecules	AD model	Molecular mechanism	Research outcomes	References
Fisetin	Mouse and rat primary cortical neurons	mTOR inhibition, TFEB and Nrf2 activation	Autophagy induction, Decrease sarkosyl-insoluble tau phosphorylation	[81]
Ouabain	Tau transgenic fly, P301L mice	Inactivation of mTOR, Activation of TFEB	Increase autophagy, Decrease toxic tau, Increase memory function	[82]
SLM, a carbazole-based fluorophore	3xTg-AD	Activating GSK-3 β , Reduces neuroinflammation	Decreasing A β 40 and A β 42 levels, Reducing phosphorylation of tau	[79]
Aspirin	5xFAD	Activation of PPAR α and TFEB	Increase lysosomal biogenesis, Decrease A β	[83]
Liraglutide	APP/PS1, APP ^{swe} /SH-SY5Y cells	Increase IDE levels, mTOR-independent, JNK activation	Improved cognitive function, Reduced A β plaque deposition and inflammation, Enhanced LTP, Autophagy activation	[72,73]
Rapamycin	Transgenic (h)APP mice	mTOR inactivation	Improved memory, Decrease sarkosyl-insoluble tau,	[74,75]
Cinnamic acid	5xFAD	Activates PPAR α , Upregulate TFEB	Reduced cerebral A β plaque burden, Improved memory function, Stimulate lysosomal biogenesis.	[84]
Trehalose	APP/PS1, Tg2576	Increase synaptophysin, doublecortin, and progranulin	Inhibits tau, Improve cognitive and learning ability	[85,86]
Curcumin	APP/PS1	mTOR inactivation	Reduced A β plaque, Increase memory function	[76]

Oleuropein aglycone	TgCRND8 mice	Inhibition mTOR and Ca ²⁺ liberating	Reduced A β plaque, Increase synaptic plasticity	[78]
Hep-14	APP/PS1	Upregulate TFEB	Reduction of A β plaque	[87]
Palm ¹¹ -PrRP31	APP/PS1	Activation of pre-synaptic marker synaptophysin,	Decrease in Tau phosphorylation, Reduce A β plaque and microgliosis,	[80]
Methylene blue	JNPL3	mTOR inactivation	Reduces insoluble tau, Increases memory function.	[77]
Temsirolimus	P301S mice	Inhibition of mTOR	Improve motor and memory function, Sarkosyl insoluble tau reduction,	[88]

Recently, mTOR-independent transcription factor, TFEB, was shown to reduce aggresome formation and activate autophagy, and several compounds are capable of stimulating TFEB-induced lysosomal biogenesis and mTOR-independent autophagy (Figure 3). For example, ouabain has been found to activate TFEB, decrease the aggregation of p-Tau, and increase cognition function in P301L mice [82]. Cinnamic acid and aspirin are found to activate TFEB promotor and promotes lysosomal biogenesis which decreases the formation of A β plaque in 5xFAD mice model [83,84]. Trehalose, a disaccharide molecule, activates calcineurin and protein phosphatase-3 CB (PP3CB) by promoting the translocation of TFEB into the nucleus via mTOR-independent autophagy [85]. Additionally, it has been found that, in APP/PS1 mice, trehalose treatment promotes the clearance of A β plaque independent of the mTOR pathway [85,86]. Hep14, a cardiac glycoside-ingenol, increases TFEB-induced ALP by activating PKC and inhibiting GSK3 β , thus decreasing plaques formation in APP/PS1 AD mice model [87]. Jiang *et al.* has demonstrated that the treatment of temsirolimus successfully improved autophagic clearance of hyperphosphorylated tau in the brain of P301S transgenic mice and okadaic acid-incubated SH-SY5Y cells. In addition, temsirolimus administration improved memory impairments and spatial learning function in P301S mice [88]. Small molecule or natural compounds may control autophagy-lysosomal process via mTOR- and TFEB-mediated pathways, which is summarized in Figure 3.



addition to lysosome biogenesis. Aggresome is bound to phagosome, resulting in the formation of autophagosome. Eventually, autophagosome fused with lysosome degrades aggresome via autophagy process.

Future prospective of inhibiting aggresome formation as a treatment for AD

Recently, extensive resources were dedicated to the development of high-performance, automated detection platforms to identify compounds that can prevent aggresome development and endorse aggresome formation [89]. Such high-performance screening primarily uses large libraries designed for general-purpose detection, which serves as a useful approach when running screens with unknown targets or with no structural information available [89,90]. These screenings play an important role in drug discovery and the creation of new forms of chemical treatment [89,90]. However, since protein aggregation and the formation of aggresomes are complex and mediated by multi-step processes [91], special care must be taken in interpreting the results, especially when aggresome-related genes are used as reporter genes. For example, inhibiting the early stage of autophagy pathway reduces the formation of aggresome and the levels of toxic protein species [92] (Figure 4). However, although preventing the late stages of autophagic pathway reduces aggresome development, it was shown to increase the assembly of soluble-toxic protein species. This complexity highlights the importance of verifying and characterizing the target sites as well as working mechanisms of candidate compounds.

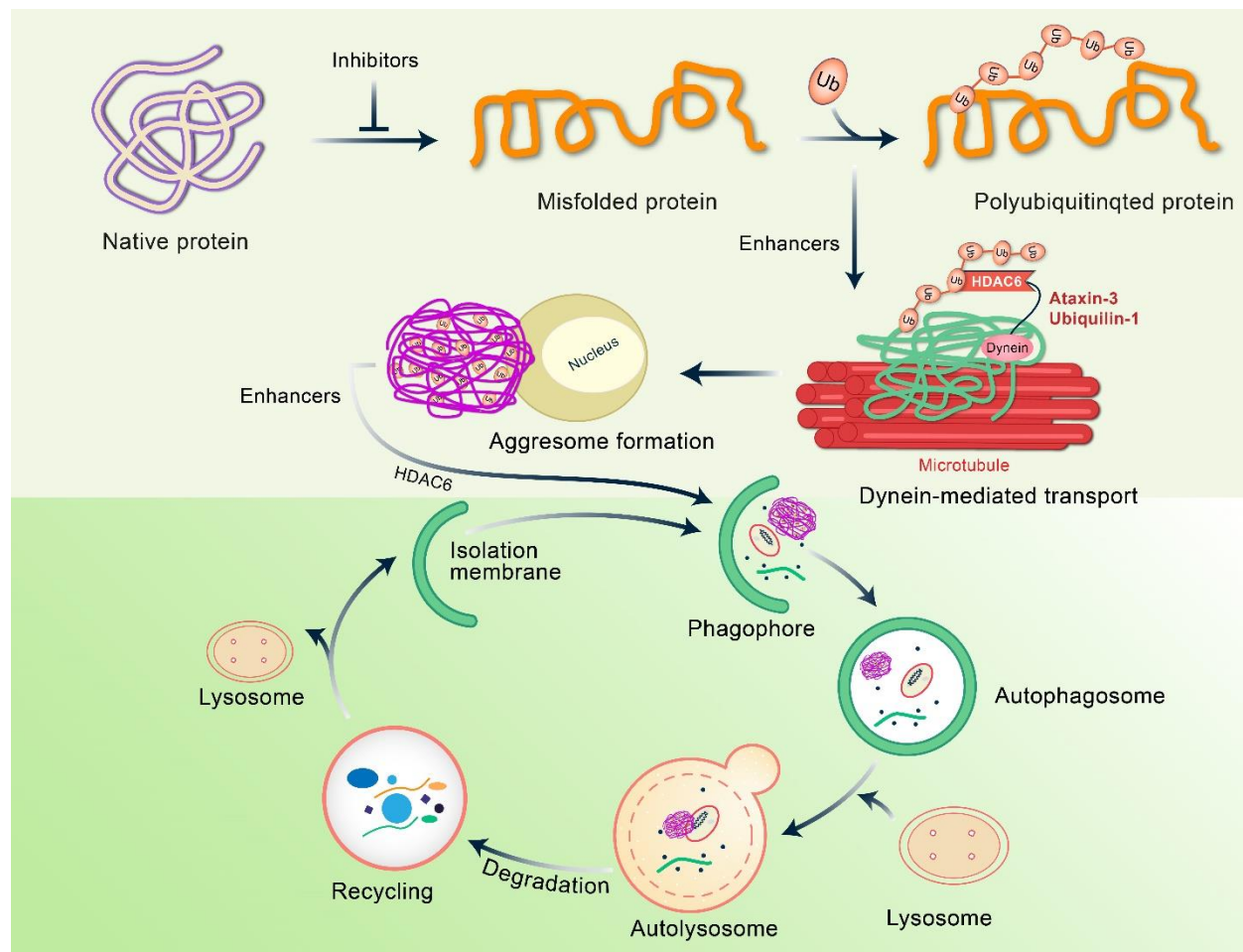


Figure 4: Regulation of misfolded protein by autophagy. Misfolded proteins are recognized and polyubiquitinated by ubiquitin E3 protein ligases. Adapter proteins, such as HDAC6, ataxin3 and ubiquitin-1, bind to polyubiquitinated proteins on the dynein motor complex for retrograde transport to the aggresome. Aggresome takes over the autophagy mechanism, including HDAC6, and breaks down aggresome. Several steps along this path enable small molecules to block incorrect protein folding and improve the coupling of folded proteins to dynein for retrograde transport, or improve the clearance of aggresome by autophagy, which can be potentially applied in the treatment of neurodegenerative diseases.

Conclusions

Autophagy enhancers that can be utilized for the potential treatment of neurodegenerative diseases is receiving growing attention in recent studies [9,18,93,94]. It has been shown that the inactivation of mTOR by the lipophilic macrolide antibiotic rapamycin promotes the induction of autophagy

[95]. Furthermore, long-term administration of rapamycin can reduce amyloid load in mouse models of AD and improve cognitive function as well as the pathology of tauopathy [96,97]. In addition, post-translational modifications, such as ubiquitination, acetylation, O-GlcNAcylation, and phosphorylation also appear to exert a positive effect on autophagy [45,98]. Acetylation is an important cellular mechanism that protects cells from stress stimuli, and can be changed in neurodegenerative pathologies, highlighting acetylation and deacetylation processes as a potential therapeutic candidate for neurodegenerative diseases. Several selective small molecule inhibitors of HDAC6 have been identified [99]. For example, tubastatin A promotes the acetylation of α -tubulin, stabilizes the microtubule network, and confers neuroprotection on neurons during *in vitro* culture and neurodegeneration. By clarifying the mechanism underlying the selection of lysosomal loads, autophagy-based approach will serve as a more effective therapeutic candidate [100]. In the case of AD, aggresome formation can occur, which can induce the inhibition of proteasome activity. Aggrephagy and its downstream signaling cascades offer promising new therapeutic targets for preventing AD, and further research is required to clarify the relationship between the mechanisms involved in autophagic activities and the formation of aggresomes.

Authors' Contributions

MAR designed and proposed the original idea of this manuscript. MHR prepared the figures and ANMMR wrote the draft. HH reviewed the manuscript. HR reviewed the scientific contents of the manuscript. All authors read and approved the final version of this manuscript.

Funding:

This work was funded by NRF Research Program (2016M3C7A1913845) and supported by the Korea Research Fellowship (KRF) Program (2016H1D3A1908615) through the National Research Foundation of Korea, Ministry of Science and ICT, Republic of Korea.

Conflict of Interest: The authors declare that they have no conflict of interest.

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