

# Analysis of Alzheimer's Disease: From Pathological Mechanism to Therapeutic Approach in Autophagy

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**Abstract:** As a “non-curable” disease, Alzheimer’s disease (AD) is the most common neurodegenerative disease in the aged population. Physical and mental pain exerts on every AD patient and their families. Even though there is no worldwide approved treatment against AD now, researchers have never given up on investigating and exploring potential approaches for curing AD. Gene therapy and drug treatment arise for alleviating AD symptoms. This paper illustrates the pathological mechanism of AD and focuses on the role of autophagy in AD pathology. Autophagy is a self-degrading mechanism to clear out dysfunctional cells; abnormal autophagy can directly trigger AD. This paper summarizes the effective and novel therapeutic approaches to treating AD by promoting autophagy activity, as well as AD diagnosis and assessment from early to severe stage, which provides promising approaches for researchers who are interested in AD treatments and feasible directions for science translational medicine.

**Keywords:** Alzheimer’s disease; Autophagy; Pathological mechanism; Treatments and nursing

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## 1. Introduction

Alzheimer’s disease (AD) is the most common and prevalent neurodegenerative disease that causes the shrinkage of the brain and the death of brain cells <sup>[1,2]</sup>. Patients have continuous decline in thinking, memory, and behavioral and social skills, affecting a person’s ability to function independently; this is a type of dementia <sup>[3]</sup>. Symptoms grow severe as the stage of disease develops which eventually interferes with daily tasks and patients can no longer live independently <sup>[3]</sup>. AD affects 10% of people over the age of 65 and 50% over the age of 85 years; the approximate annual home care costs for AD and related dementia are \$5975 per person versus \$794 for a non-AD individual in the US (according to a paper published in 2022) <sup>[1,4]</sup>. AD becomes undiminished suffering for patients and patients’ families in both physiological and social manner. As the global average life span increases, more people live long enough to have AD <sup>[5]</sup>. However, there is no worldwide approved, standard, and effective treatment for AD; current drugs alleviate and improve symptoms, but have no disease-modifying effects, causing AD to be the largest unmet medical need in neurology <sup>[5]</sup>. Thus, understanding the pathological mechanism for such disease is crucial for more treatments and potential targets to arise. Autophagy is an essential homeostatic mechanism, which keeps protein homeostasis and neuronal healthy by clearing and degrading abnormal protein aggregates <sup>[2]</sup>.

Studies have demonstrated the relationship between autophagy and AD, such that autophagy deficits occur in the early stage of AD <sup>[2]</sup>. This paper summarizes the recent targets and therapeutic approaches

regarding AD treatment in the field of autophagy deficit, and the reliable AD assessments; those suggest directions of AD treatment that can be dived into in both research and clinical aspects. With that, novel achievements in AD treatment are integrated, which provides referencing information for researchers that are interested in developing AD treatments via gene therapy or pharmaceutical targeting.

## **2. Mechanism analysis of autophagy**

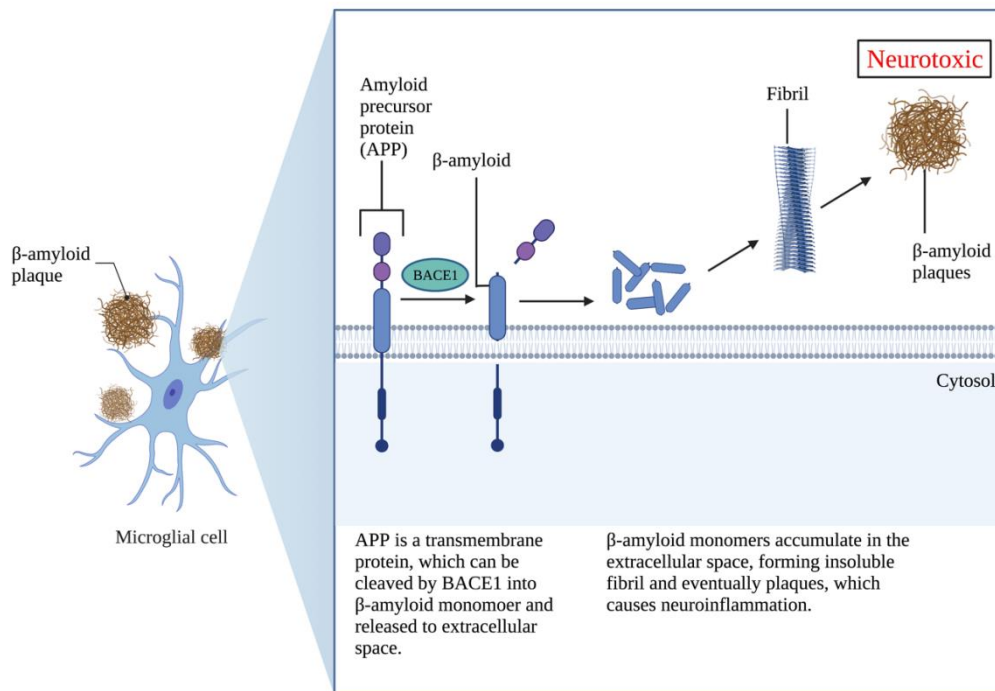
### **2.1. Mechanism-role of autophagy in AD**

It is worth mentioning the amyloid-beta precursor protein, which is a large membrane protein that contributes a lot to neural growth and repair [2]. A corrupted form of  $\beta$ -amyloid destroys nerve cells, which is a direct cause of loss of thoughts and memory deficit in AD patients [6]. The generation and metabolism of  $\beta$ -amyloid are heavily dependent on autophagy [2]. More specifically, extracellular deposits of  $\beta$ -amyloid and accumulation of abnormal filaments of tau (a protein that stabilizes neuron internal skeleton) make up the plaque and neurofibrillary tangles in the region of cognition and memory in the brain, such dysfunction leads to AD [5]. This explains the influence of autophagy in AD as its dysfunction can lead to progress and aggravation of AD directly.

### **2.2. Mechanism- $\beta$ amyloid metabolism (degradation) in autophagy**

Amyloid precursor protein (APP) can be cleaved during autophagic turnover, producing  $\beta$ -amyloid peptides [7,8]. The maturation of autophagolysosomes is a process of fusing autophagosomes with lysosomes [9]. In Dr. Nixon's study, there is an accumulation of autophagic vacuoles in neuronal dendrites of AD patient brains, which is due to the obstructed path toward the neuronal body [7]. Such accumulation is associated with ESCRT-III complex dysfunction, triggering neurodegeneration and interfering maturation of autophagolysosomes [9]. With the decreased activity of ESCRT-III complex, lysosome and autophagosome fusion is interrupted, causing reduced autolysosomes formation, which is then provoking tau and other aggregates accumulation, forming a vicious cycle [9]. Dr. Nixon observed the accretion of autophagic vacuoles from the PS-1/APP double transgenic mice, and it was proved that soma before  $\beta$ -amyloid plaques appeared compared to age-matched controls [7].

In another study that is done in 2013, Dr. Nilsson showed accumulating intracellular  $\beta$ -amyloid is neurotoxic and leads to AD pathology [10]. As  $\beta$ -amyloid releasing depends on autophagy, Dr. Nilsson developed a transgenic mouse with dysfunctional autophagy by knocking-out *ATG7*, which represents a key gene regulating autophagic conjugation systems [10,11]. With dysfunctional autophagy, the offspring is unable to secrete  $\beta$ -amyloid peptides; thus, they have fewer extracellular  $\beta$ -amyloid plaques, which co-occurs with intracellular  $\beta$ -amyloid accumulation in the brain cells [10]. The deficient autophagy is neural toxic and leads to AD since the cells have a limitation in secreting  $\beta$ -amyloid peptides [10]. Therefore, degradation and clearance of  $\beta$ -amyloid are important.  $\beta$ -amyloid can be degraded by its degrading proteases such as BACE1 (which is also known as  $\beta$ -secretase) and CTSD via the ubiquitin-proteasome pathway in neurons and the extracellular neprilysin-mediated pathway, respectively [12,13]. It can also be incorporated into the primary intracellular reservoir of toxic peptides [12,13]. Regarding clearance, the ATP-binding cassette (ABC) transporter sub-family transfers soluble  $\beta$ -amyloid crossing brain endothelial cells; or,  $\beta$ -amyloid undergoes clearance via brain interstitial fluid bulk-flow and cerebrospinal fluid (CSF) absorption involving both circulatory and lymphatic systems [13]. The graphical abstract of  $\beta$ -amyloid degradation in a pathological case is shown below in **Figure 1**.



**Figure 1.**  $\beta$ -amyloid plaques formation in Alzheimer's disease

Amyloid precursor protein can be cleaved into  $\beta$ -amyloid monomers by enzyme, BACE1, also known as  $\beta$ -secretase<sup>[12, 13]</sup>. In pathological pathway, the monomers accumulate in the extracellular space and forming insoluble fibrils, which turn into plaques and tangles, causing memory deficit in AD<sup>[13]</sup>.

### 2.3. Mechanism-mTOR pathway in autophagy

Some studies demonstrated the relationship between mechanistic target of rapamycin (mTOR) and AD; they are closely integrated for cellular metabolism to function properly<sup>[14]</sup>. mTOR is a significant pathway for sensing nutrients and regulating cell growth and metabolism, which is found by integrating signaling cascades<sup>[14]</sup>.

More specifically, mTOR complex 1 (mTORC1) initiates autophagy process<sup>[14]</sup>. It was found that the mammalian target of mTOR can be genetically reduced so that enhanced autophagy induction is observed, which was demonstrated using Tg2576 mice brains<sup>[15]</sup>. Inhibition of mTOR signaling is observed in the hippocampus in the AD mice model<sup>[16]</sup>. By inhibiting mTOR, normal hippocampal gene expression can be restored, resulting in a reduced deposit of  $\beta$ -amyloid and alleviating memory deficits<sup>[15]</sup>. Tau protein homeostasis is regulated by mTOR signaling, which mediates intra- and extra-cellular distribution of tau<sup>[17]</sup>. Therefore, reducing mTOR signaling is a pharmacological approach that improves tau pathology, and inducing mTOR-dependent autophagy can be a potential approach to treat AD<sup>[8]</sup>.

## 3. Treatments of Alzheimer's disease

### 3.1. Treatments-induction of autophagy by mTOR

As manipulating the mTOR pathway proved to be promising, there are studies using rapamycin (mTOR inhibitor) or latrepirdine as a target to treat AD, which is proved to have positive effects on cognitive deficits by lowering A $\beta$ 42 levels<sup>[17]</sup>. A study conducted in 2011 demonstrated that rapamycin reduces  $\beta$ -amyloid plaques, microglia activation and neurofibrillary tangles formation by inhibiting mTOR<sup>[18]</sup>. They built a mice model of 3xTg-AD mice, and they proved that rapamycin increased autophagy induction in the mice with age in the range of 2 to 18 months<sup>[18]</sup>. However, such treatment can prevent but not cure learning and

memory deficits <sup>[18]</sup>. More specifically, for 2 months old 3xTg-AD mice, treatment with rapamycin-induced autophagy has a significant impact, which is large enough to reduce plaques and tangles, alleviating cognitive deficits <sup>[18]</sup>. For 15 months old 3xTg-AD mice, such treatment indeed induces autophagy but since plaques and tangles have been established already, no positive effects are exerted in AD pathology <sup>[18]</sup>. Therefore, they concluded that rapamycin is a valid approach to induce autophagy, but such treatment only plays the role before the formation of plaques and tangles, suggesting rapamycin can be a therapeutic strategy only in the early stage of AD progression <sup>[18]</sup>.

### **3.2. Treatments-induction of autophagy by Beclin 1**

A novel study shows that expressing Beclin 1 via lentiviral vectors leads to induction of autophagy and reduced both extracellular and intracellular amyloid pathology in APP transgenic mice <sup>[17]</sup>. Researchers induced a knock-in point mutation F121A, which reduces the interaction between Beclin 1 and its endogenous inhibitor B-cell lymphoma 2 (Bcl2) <sup>[19]</sup>. By conducting a knock-in Beclin 1<sup>F121A</sup> in mice, constructive activities of autophagy in many tissues including the brain are observed <sup>[19]</sup>. In the mouse model, Becn1<sup>F121A</sup>-mediated autophagy hyperactivation significantly decreases  $\beta$ -amyloid accumulation and prevents the decline of cognition; thus, the survival rate is restored in the PDAPP mice model (another amyloid model) <sup>[19]</sup>. Interestingly, the induced autophagy in their knock-in model only targets  $\beta$ -amyloid oligomers accumulation rather than APP <sup>[19]</sup>. They also showed that Becn1<sup>F121A</sup> and ML246, which is a brain-penetrable autophagy-inducing small molecule decrease  $\beta$ -amyloid accumulation and improves cognitive function in the 5XFAD Alzheimer's mouse model with voluntary exercise <sup>[19]</sup>. With such a gene therapy approach, the target for restoring autophagy activity is more specific with fewer side-effect and cellular toxicity <sup>[17,19]</sup>.

### **3.3. Treatments-maturation of autophagy by CCZ1-MON1A complex**

A new study raised in 2022 used a complex known as CCZ1-MON1A to alleviate memory and neuropathology deficits via promoting autophagy maturation <sup>[20]</sup>. They found that a small GTPase required for both endosome and autophagosome maturation, RAB7, in its active form can be decreased in autophagosome fractions isolated from cells and tissues of AD mice model <sup>[20,21]</sup>. CCZ1-MON1A is a guanine nucleotide exchange factor (GFE), which relates to the active form of RAB7; decreasing RAB7 causes impaired activity in the CCZ1-MON1A complex <sup>[20]</sup>. Therefore, they overexpressed CCZ1-MON1A during autophagy, which enhances autophagosome maturation by inducing the active form of RAB7 <sup>[20]</sup>. This also positively regulates the degradation of APP-CTFs (C-terminal fragments),  $\beta$ -amyloid and tau protein in an autophagy-dependent manner in cells of N2S <sup>[20]</sup>. The researchers further proved this by using adeno-associated virus (AAV) to mediate CCZ1-MON1A overexpression in the hippocampus in 3xTg AD mice, which is demonstrated to alleviate autophagy impairment and AD-related behavioural and neuropathological change by evaluating the mice behaviors in the Morris water maze <sup>[20]</sup>. This novel finding provides a feasible approach to enhancing autophagosome maturation against AD by manipulating the CCZ1-MON1A-RAB7 complex <sup>[20]</sup>.

### **3.4. Treatments-promotion of autophagy by BMMSC transplantation**

A study has shown that transplantation of bone marrow-derived mesenchymal stem cells (BMMSCs) has a positive effect on AD treatment by alleviating neuropathology and improving cognitive and memorial deficits in an autophagy-dependent manner <sup>[22]</sup>. By quantifying the expression of autophagy-related signal molecules, such as Beclin-1, Atg5, LC3-II, and mTOR after BMMSC transplantation, induced autophagy is observed <sup>[23]</sup>. Behavioral test on AD-mice via the Morris water maze test, Y-maze alternation test, plus-maze discriminative avoidance task, social recognition test, and open-field evaluation demonstrates

improvements in symptoms [22,23]. BMMSC transplantation stimulates  $\beta$ -amyloid peptides clearance, causing decreased aberrant accumulating  $\beta$ -amyloid peptides, and dysfunctional tau aggregates by upregulating the expression of BECN1/Beclin 1 and increasing LC3-II-positive autophagosomes in the hippocampus [22,23]. Moreover, other researchers reveal the potential connection and synergistic relationship between autophagy and apoptosis under BMMSC transplantation on the preconditioning of stem cells, suggesting the better performance of BMMSC transplantation under autophagy/apoptosis modulation [23]. With that, the effects of BMMSC transplantation treatment and autophagy induction are complementary to each other.

### **3.5. Treatments-promotion of autophagy by TFEB**

Transcription factor EB (TFEB) is a master regulator of autophagy and lysosomal biogenesis [24]. Dr. Li's group found small autophagy enhancing molecule targeting TFEB, which is celastrol [24]. It is an active ingredient isolated from root extracts of *Tripterygium wilfordii*, and such a molecule has been shown to enhance enhanced TFEB-mediated autophagy and lysosomal biogenesis in vitro [24]. They built two common AD animal models, P301S Tau and 3xTg mice, observing activated TFEB activity via inhibiting mammalian target mTOR, and reduced phosphorylated Tau aggregates, which improved memory and cognition in the mice model [24]. Therefore, celastrol inducing TFEB provides a novel and promising approach to treating AD, AD-like neuropathology, and tauopathy [24].

## **4. Early diagnosis and assessments of AD**

### **4.1. Nursing-assessments of AD**

As more therapeutic approaches regarding AD rise, the investigation for AD treatment is steadily moving forward; however, besides the potential gene targets and small molecule treatment, early diagnosis and assessment is also important as many of the approaches work much better in the early stage of AD. For instance, nutrition, continence, and movement are aspects to pay attention to; as AD patients often being observed with malnutrition, gait, and continence disorders. Besides those home-based observing categories, there are assessments to examine AD progression more accurately [25]. With early diagnosis, a wider range of treatments can be chosen from, and better performance treatments can exhibit. Early intervention is more ideal than late remediation.

### **4.2. Cognitive assessment-SIB**

Severe Impairment Battery (SIB) is a global cognitive functional assessment measuring memory, language, visuospatial awareness, attention, orientation, and construction along with brief evaluations of social skills, praxis, and ability to respond to one's name and interpretation of external information on a scale ranging from 0 (greatest impairment) to 100 (least impairment) [26]. SIB relies on single-word or one-step commands to enhance compliance and minimize patient frustration, making SIB useful in both clinical and home-caring cases; clinical researchers stated that SIB is a more accurate and sensitive scale for cognitive function compared to prior mental examination [25, 26].

### **4.3. Functional assessment through Ferm's D-test**

Ferm's D-test measures patients' behavioral and functional disability in geriatric patients with dementia [27]. The advantage of this test is that Ferm's D is modified and extended to qualify severe patients' symptoms, which means it has a wider range of assessment [25]. The modified Ferm's D test contains 16 functional variables, covering important aspects of characteristics of AD patients, such as mobility, communication, and ability to keep personal hygiene [25]. This D-scale is not only applied successfully in the clinical field but also can be a user-friendly application in nursing home settings [25].

## 5. Conclusion

With progressing studies regarding the potential mechanisms of AD, more treatment approaches are developed, and better solutions are evolving, such as targeting gene beclin1 and protein RAB7 or BMMSC Transplantation [19-20,23]. Besides alleviating autophagy activity, there are other approaches (enhancing lysosomal activity or restoring endosomal trafficking for instance) to target the neurodegeneration problem, but autophagy plays an important role in the mechanism of AD and is the most promising entry point for AD treatment up to now [17]. There are still countless challenges remaining; for example, the rapamycin inhibiting mTOR (mentioned in **Section 3.1.**) to improve tau-pathology and autophagy. However, for long-term usage and treatment, such an approach may not be ideal, especially considering the strong inhibition for mTOR. The mTOR pathway itself is involved in other cellular functions; inhibiting the mTOR pathway can cause cellular toxicity and side effects rise due to disrupted cell growth and gene expression [17]. Moreover, many therapeutic approaches mentioned above exert a distinct impact on AD symptoms, but since most of them are novel treatments, effects are only observed and proved in an animal model; still, a long way to go in the clinical trial. Nonetheless, with more studies of the particular pathway and signaling cascade conducted, more target and treatment strategies arise. Hopefully, AD will no longer be mentioned as a “no existing treatment.”

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## References

- [1] Bhushan I, Kour M, Kour G, et al., 2018, Alzheimer's Disease: Causes & Treatment – A Review. *Ann Biotechnol*, 1(1): 1002.
- [2] Li Q, Liu Y, Sun M, 2017, Autophagy and Alzheimer's Disease. *Cellular and Molecular Neurobiology*, 37(3): 377–388.
- [3] Katzman R, 1986, Alzheimer's Disease. *The New England Journal of Medicine*, 314: 964–973.
- [4] Davis-Ajami ML, Lu ZK, Wu J, 2022, Exploring the Home Healthcare Workforce in Alzheimer's Disease and Related Dementias: Utilization and Cost Outcomes in US Community Dwelling Older Adults. *Archives of Gerontology and Geriatrics*, 98: 104536.
- [5] Citron M, 2010, Alzheimer's Disease: Strategies for Disease Modification. *Nature Reviews Drug Discovery*, 9(5): 387–398.
- [6] Cummings BJ, Head E, Afagh AJ, et al., 1996,  $\beta$ -amyloid Accumulation Correlates with Cognitive Dysfunction in the Aged Canine. *Neurobiology of Learning and Memory*, 66(1): 11–23.
- [7] Nixon RA, 2007, Autophagy, Amyloidogenesis and Alzheimer Disease. *Journal of Cell Science*, 120(23): 4081–4091.
- [8] Uddin MS, Stachowiak A, Mamun AA, et al., 2018, Autophagy and Alzheimer's Disease: From Molecular Mechanisms to Therapeutic Implications. *Frontiers in Aging Neuroscience*, 10: 4.

- [9] Lee JA, Beigneux A, Ahmad ST, et al., 2007, ESCRT-III Dysfunction Causes Autophagosome Accumulation and Neurodegeneration. *Current Biology*, 17(18): 1561–1567.
- [10] Nilsson P, Loganathan K, Sekiguchi M, et al., 2013, A  $\beta$  Secretion and Plaque Formation Depend on Autophagy. *Cell Reports*, 5(1): 61–69.
- [11] Ohsumi Y, 2014, Historical Landmarks of Autophagy Research. *Cell Research*, 24(1): 9–23.
- [12] Saido T, Leissring MA, 2012, Proteolytic Degradation of Amyloid  $\beta$ -Protein. *Cold Spring Harbor Perspectives in Medicine*, 2(6): 6379.
- [13] Hampel H, Hardy J, Blennow K, et al., 2021, The Amyloid- $\beta$  Pathway in Alzheimer's Disease. *Molecular Psychiatry*, 26(10): 5481–5503.
- [14] Sun YX, Ji X, Mao X, et al., 2014, Differential Activation of mTOR Complex 1 Signaling in Human Brain with Mild to severe Alzheimer's Disease. *Journal of Alzheimer's Disease*, 38(2): 437–444.
- [15] Caccamo A, De Pinto V, Messina A, et al., 2014, Genetic Reduction of Mammalian Target of Rapamycin Ameliorates Alzheimer's Disease-Like Cognitive and Pathological Deficits by Restoring Hippocampal Gene Expression Signature. *Journal of Neuroscience*, 34(23): 7988–7998.
- [16] François A, Rioux Bilan A, Quellard N, et al. 2014, Longitudinal Follow-Up of Autophagy and Inflammation in Brain of APPswePS1dE9 Transgenic Mice. *Journal of Neuroinflammation*, 11(1): 1–14.
- [17] Liu J, Li L, 2019, Targeting Autophagy for the Treatment of Alzheimer's Disease: Challenges and Opportunities. *Frontiers in Molecular Neuroscience*, 12: 203.
- [18] Majumder S, Richardson A, Strong R, et al., 2011, Inducing Autophagy by Rapamycin Before, but not After, the Formation of Plaques and Tangles Ameliorates Cognitive Deficits. *PloS One*, 6(9): 25416.
- [19] Rocchi A, Yamamoto S, Ting T, et al., 2017, A BECN1 Mutation Mediates Hyperactive Autophagic Sequestration of Amyloid Oligomers and Improved Cognition in Alzheimer's Disease. *PLoS Genetics*, 13(8): 1006962.
- [20] Cai CZ, Zhuang XX, Zhu Q, et al., 2022, Enhancing Autophagy Maturation with CCZ1-MON1A Complex Alleviates Neuropathology and Memory Defects in Alzheimer Disease Models. *Theranostics*, 12(4): 1738.
- [21] Hyttinen JM, Niittykoski M, Salminen A, et al., 2013, Maturation of Autophagosomes and Endosomes: a Key Role for Rab7. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*, 1833(3): 503–510.
- [22] Qin C, Lu Y, Wang K, et al., 2020, Transplantation of Bone Marrow Mesenchymal Stem Cells Improves Cognitive Deficits and Alleviates Neuropathology in Animal Models of Alzheimer's Disease: A Meta-Analytic Review on Potential Mechanisms. *Translational Neurodegeneration*, 9(1): 1–20.
- [23] Qin C, Bai L, Li Y, et al., 2022, The Functional Mechanism of Bone Marrow-Derived Mesenchymal Stem Cells in the Treatment of Animal Models with Alzheimer's Disease: Crosstalk Between Autophagy and Apoptosis. *Stem Cell Research & Therapy*, 13(1): 90.
- [24] Yang C, Su C, Iyaswamy A, et al., 2022, Celastrol Enhances Transcription Factor EB (TFEB)-Mediated Autophagy and Mitigates Tau Pathology: Implications for Alzheimer's Disease Therapy. *Acta Pharmaceutica Sinica B*, 12(4): 1707–1722.
- [25] Feldman H, and Woodward M, 2005, The Staging and Assessment of Moderate to Severe Alzheimer Disease. *Neurology*, 65(6 suppl 3): 10-17.
- [26] Saxton J, McGonigle-Gibson KL, Swihart AA, et al., 1990, Assessment of the Severely Impaired Patient: Description and Validation of a New Neuropsychological Test Battery. *Psychological*

Assessment: A Journal of Consulting and Clinical Psychology, 2(3): 298.

[27] Ferm L, 1974, Behavioural Activities in Demented Geriatric Patients. *Gerontologia Clinica*, 16(4): 185–194.

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