

# Rapamycin Responds to Alzheimer's Disease: A Potential Translational Therapy

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**Abstract:** Alzheimer's disease (AD) is a sporadic or familial neurodegenerative disease of insidious onset with progressive cognitive decline. Although numerous studies have been conducted or are underway on AD, there are still no effective drugs to reverse the pathological features and clinical manifestations of AD. Rapamycin is a macrolide antibiotic produced by *Streptomyces hygroscopicus*. As a classical mechanistic target of rapamycin (mTOR) inhibitor, rapamycin has been shown to be beneficial in a variety of AD mouse and cells models, both before the onset of disease symptoms and the early stage of disease. Although many basic studies have demonstrated the therapeutic effects of rapamycin in AD, many questions and controversies remain. This may be due to the variability of experimental models, different modes of administration, dose, timing, frequency, and the availability of drug-targeting vehicles. Rapamycin may delay the development of AD by reducing  $\beta$ -amyloid ( $A\beta$ ) deposition, inhibiting tau protein hyperphosphorylation, maintaining brain function in *APOE  $\epsilon 4$*  gene carriers, clearing chronic inflammation, and improving cognitive dysfunction. It is thus expected to be one of the candidates for the treatment of Alzheimer's disease.

**Keywords:**  $\beta$ -amyloid, chronic inflammation, therapeutic effect, macrolide antibiotic

## Introduction

Alzheimer's disease (AD) is a sporadic or familial neurodegenerative disease of insidious onset with progressive cognitive decline, accounting for 50–60% of all dementia patients.<sup>1</sup> As the global population ages, the prevalence of AD is increasing year by year. Currently, approximately 50 million people have dementia worldwide, and are expected to reach 152 million by 2050, which will put enormous pressure on society and the economy.<sup>2</sup> The main pathological features of AD are the presence of dense senile plaques (SPs) and neurofibrillary tangles (NFTs) in the brain, resulting in massive neuronal and synaptic loss and brain atrophy. Current hypotheses on the pathogenesis of AD include  $\beta$ -amyloid ( $A\beta$ ) deposition, microtubule-associated protein tau tangles, defective autophagy, insulin resistance, cerebrovascular dysfunction, inflammatory response, mitochondrial dysfunction, and oxidative stress. Although numerous studies have been done or are underway on AD, there have been no effective drugs to reverse the pathological features and clinical manifestations of AD.

Recent studies suggest rapamycin might be a new drug for AD.<sup>3</sup> Rapamycin is a macrolide antibiotic produced by *Streptomyces hygroscopicus*.<sup>4</sup> As a classical mechanistic target of rapamycin (mTOR) inhibitor, rapamycin has multiple functions in regulating cell metabolism, cytoskeleton formation, cell life span, and autophagy.<sup>5</sup> In addition, rapamycin prolonged the life span of mice,<sup>6</sup> and subsequent studies gradually confirmed the anti-aging effects of rapamycin.<sup>7,8</sup> In addition, increasing age is the greatest risk factor for the development of AD, and delaying aging may retard AD. Rapamycin has also been shown to be beneficial in a variety of AD mouse and cell models, both before the onset of disease symptoms and the early stage of disease, with improvements observed in AD mice and cells following rapamycin treatment.<sup>9–12</sup> Therefore, it is presumed that rapamycin may be a potential translational drug for AD.<sup>13</sup>

## Rapamycin Reduces A $\beta$ Deposition and Promotes A $\beta$ Clearance

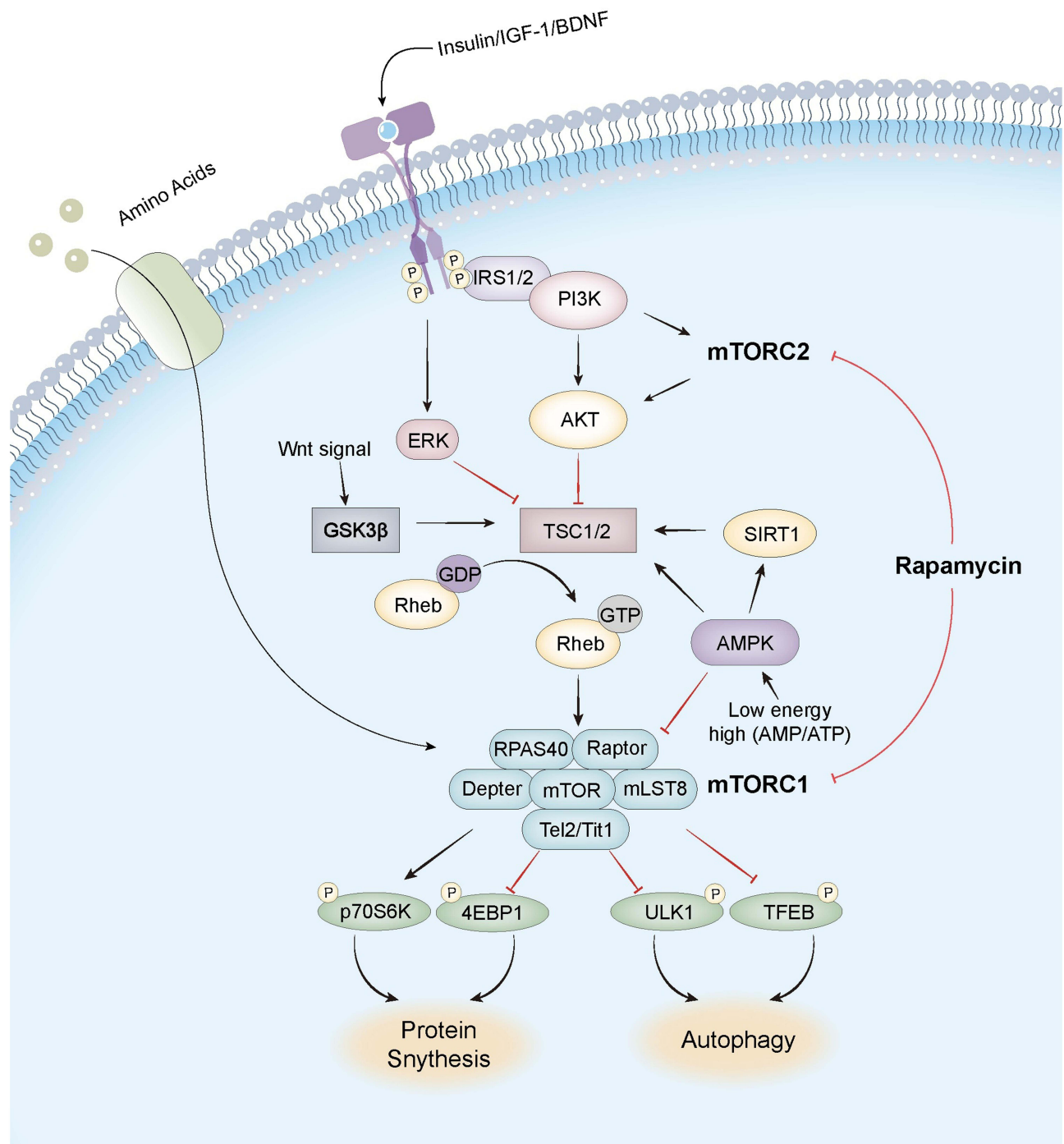
Soluble oligomeric A $\beta$  and insoluble  $\beta$ -amyloid are highly neurotoxic and can induce neuronal degeneration. The brain quickly clears A $\beta$  under normal circumstances. However, in the brain of a patient with AD, there is an imbalance between A $\beta$  generation and clearance. The abnormally activated mTOR affects A $\beta$  metabolism by inhibiting autophagy and increasing insulin resistance, thus hindering A $\beta$  clearance.<sup>14,15</sup> mTOR activity was closely related to A $\beta$ . AD cells and animal models found higher mTOR signaling pathway activity in cells and animal models with A $\beta$  accumulation.<sup>16</sup> The mechanism may be due to the over-activation of the mTOR pathway promoted by the aggregated A $\beta$ . Although whether the mTOR pathway has a direct effect on A $\beta$  production by  $\beta$  and  $\gamma$  secretases remains unelucidated, it has been shown that increasing sirtuin 1 (SIRT1) expression by inhibiting mTOR can inhibit A $\beta$  production.<sup>17</sup> SIRT1 is a key regulator of  $\alpha$ -secretase and therefore of the A $\beta$  production process.<sup>18</sup>

Rapamycin enhances autophagy by inhibiting mTOR signaling, regulates cellular metabolism, improves A $\beta$  clearance, and reduces A $\beta$  levels (Figure 1). For example, the treatment of an 8-month-old 3xTg-AD mice with rapamycin revealed a significant reduction in A $\beta$  immunoreactivity of neurons in the CA1 region of the hippocampus.<sup>19</sup> In the PDAPP transgenic mouse model, A $\beta$  plaque deposition was reduced in mice by feeding rapamycin.<sup>9</sup> Moreover, the addition of rapamycin to PC12 cells activated intracellular autophagy and upregulated A $\beta$ -induced Beclin-1 expression, thereby increasing cell viability, suggesting that rapamycin protects neuronal cells by reducing A $\beta$  levels through enhanced autophagy.<sup>20</sup> In addition, rapamycin increases SIRT1 expression by inhibiting mTOR, and SIRT1 can also negatively regulate mTOR.<sup>21</sup> The interaction between mTOR and SIRT1 continuously amplifies SIRT1 activity through a positive feedback loop, inhibits mTOR signaling, regulates A $\beta$  production, and clears A $\beta$  by increasing autophagy. Furthermore, rapamycin activates autophagy and inhibits A $\beta$  production by regulating signaling pathways, such as Wnt/GSK3 $\beta$ / $\beta$ -catenin,<sup>12</sup> insulin/insulin-like growth factor-1 (IGF-1)<sup>22,23</sup> and Calcium/calmodulin-dependent protein kinase  $\beta$ / AMP-activated protein kinase (CaMKK $\beta$ /AMPK)<sup>24</sup> to clear and reduce A $\beta$  deposition.

## Rapamycin Inhibits the Hyperphosphorylation of Tau Protein

Hyperphosphorylated tau protein aggregates form paired helical filaments in NFTs as one of the key hallmarks of AD.<sup>25</sup> Tau protein hyperphosphorylation directly promotes the formation of NFTs, which affect axoplasmic transport function by disintegrating the microtubule system, whereas NFTs do not require alteration of microtubule integrity to have a significant impact on fast axonal transport, leading to neurodegeneration and reduced memory and cognitive function. Overactivation of mTOR signaling may be an important factor in the formation of tau protein hyperphosphorylation.<sup>26</sup> Aberrantly activated mTOR drives excessive tau mRNA translation through activation of downstream targets p70 ribosomal S6 protein kinase (p70S6K) and eIF4E-binding protein 1 (4EBP1) (Figure 1). Autopsy of AD brain tissue reveals phosphorylated p70S6K associated with increased tau protein.<sup>27,28</sup> Phospho-eukaryotic translation initiation factor 4E (p-eIF4E), p-mTOR, and p-4EBP1 were significantly increased in AD brains and showed a significant positive correlation with total tau and phospho-tau (p-tau).<sup>29,30</sup> Over-activated mTOR signaling can also indirectly up-regulate tau protein phosphorylation by inhibiting protein phosphatase 2A (PP2A), which can regulate tau protein phosphorylation from multiple sites.<sup>31</sup> In addition, elevated mTOR activity inhibits autophagy and affects the degradation of abnormally phosphorylated tau proteins.<sup>32</sup>

Since the mTOR signaling pathway is involved in the translation, phosphorylation, and degradation of tau protein, rapamycin may improve cognitive dysfunction by inhibiting the mTOR signaling pathway, suppressing tau mRNA translation, enhancing autophagy, and reducing tau protein hyperphosphorylation. It was shown that rapamycin could reduce hyperphosphorylated tau protein in the 3xTg-AD mouse model, and the mechanism may be to enhance the clearance of hyperphosphorylated tau protein by enhancing autophagy and delaying the neurotoxic effect of NFTs.<sup>19</sup> In addition, rapamycin controls glycogen synthase kinase 3 $\beta$  (GSK3 $\beta$ ) function by affecting signaling pathways such as PI3K/Akt/mTOR and Wnt/GSK3 $\beta$ / $\beta$ -catenin, thereby regulating tau protein hyperphosphorylation.<sup>12,33</sup> In human neuroblastoma SH-SY5Y cells, rapamycin reduced the phosphorylation of tau protein ser214 by regulating cyclic adenosine monophosphate (cAMP) dependent protein kinase,<sup>34</sup> suggesting that rapamycin not only promotes the clearance of phosphorylated tau protein by enhancing autophagy but also inhibits the hyperphosphorylation of tau protein.



**Figure 1** The mechanism of mammalian target of rapamycin (mTOR) and signaling pathway of mTORC1. In the nervous system, brain-derived neurotrophic factor (BDNF), various neuropeptides, insulin, and insulin-like growth factor I (IGF-1) activate mTORC1 in neurons. BDNF first activates mTORC1 receptor tyrosine kinase, which further activates the phosphoinositide 3-kinase/protein kinase B (PI3K/Akt) pathway and the RAS extracellular signal-regulated protein kinase (ERK) pathway. Activated Akt and ERK activate Ras homolog enriched in brain protein (Rheb) to activate mTORC1 by inhibiting the mTORC1 inhibitor tuberous sclerosis protein-complex (TSC). AKT also acts as a signaling node between mTORC1 and mTORC2, which indirectly activates mTORC1 by inhibiting TSC through Akt phosphorylation. Elevated mTORC1 activity phosphorylates Unc-51-like kinase I (ULK1) and transcription factor EB (TFEB) to suppress autophagy. Furthermore, it also activates 4EBP1 and P70S6K, forming a transcription initiation complex to improve translation efficiency and accelerate protein synthesis. Rapamycin acts on the over-activated mTORC1 in the AD brain, restoring its normal activity and slowing down AD progression, but it also acts mTORC2, which may have some side effects.

## Rapamycin Maintains Brain Function in *APOE* $\epsilon 4$ Gene Carriers

The epsilon4 allele of the apolipoprotein E gene (*APOE*  $\epsilon 4$ ) is the strongest genetic risk factor for late-onset AD and plays an important role in the AD pathogenesis.<sup>35</sup> Several studies have shown that cerebral blood flow (CBF) decreases

over time in asymptomatic *APOE*  $\epsilon 4$  carriers prior to dementia onset. Inadequate cerebral perfusion leads to insufficient neuronal nutrition as well as oxidative stress, disruption of blood-brain barrier (BBB) integrity, and reduced cerebrovascular reactivity (CVR).<sup>36,37</sup> A magnetic resonance diffusion tensor imaging study finds that white matter (WM) integrity is compromised in healthy *APOE*  $\epsilon 4$  carriers.<sup>38</sup> These abnormalities can impair cognitive function in *APOE*  $\epsilon 4$  carriers.

Rapamycin has been shown to improve impaired brain function in *APOE*  $\epsilon 4$  transgenic mice, restore brain glucose uptake and BBB integrity, and may serve as an intervention to prevent vascular abnormalities and the progression of early cognitive dysfunction in *APOE*  $\epsilon 4$  carriers.<sup>39,40</sup> Cognitively normal *APOE*  $\epsilon 4$  carriers developed A $\beta$  plaques, and cerebrovascular metabolic and structural abnormalities decades before the onset of cognitive impairment.<sup>41</sup> Is rapamycin able to maintain normal brain function and prevent A $\beta$  deposition in *APOE*  $\epsilon 4$  carriers before A $\beta$  deposition occurs? Ai-Ling et al used the E4FAD mouse model, which expresses the human *APOE*  $\epsilon 4$  gene as well as overexpresses A $\beta$ .<sup>42</sup> It was found that rapamycin restored CBF and vascular function, enhanced A $\beta$  clearance by the BBB, reduced free fatty acid levels, maintained white matter structural integrity, restored brain function in young adult mice carrying the *APOE*  $\epsilon 4$  gene, and reduced the risk of AD development in E4FAD mice.<sup>43</sup>

## Rapamycin Clears Chronic Inflammation in AD

Chronic inflammation is a major factor in the development and progression of AD.<sup>44</sup> Neuroinflammation may be present for many years before the clinical onset of AD, and central inflammation is closely related to peripheral inflammation. Infections with various pathogens (viruses, bacteria, parasites, and many other microorganisms),<sup>45</sup> surgery, critical illness, and dysbiosis of the gut microbiota can elicit a systemic immune response, which in turn triggers a central immune response.<sup>46</sup> Pathogenic microorganisms, immune cells and inflammatory mediators directly or indirectly damage neuronal cells, inducing A $\beta$  deposition and tau protein hyperphosphorylation. Rapamycin has multiple anti-inflammatory mechanisms, including affecting inflammatory mediators, and immune cells, and restoring the BBB. Nuclear factor- $\kappa$ B (NF- $\kappa$ B) is the key mediator of the anti-inflammatory effect of rapamycin.<sup>47</sup> Rapamycin exerts anti-inflammatory effects by down-regulating p65, interleukin-1 $\beta$  (IL-1 $\beta$ ), tumor necrosis factor-alpha (TNF- $\alpha$ ), and other inflammatory factors of NF- $\kappa$ B in AD mice.<sup>48</sup> Rapamycin selectively enhances the activity of the inflammatory factor interleukin-6 (IL-6) signaling pathway.<sup>49</sup> IL-6 has a complex role in the central nervous system in AD, stimulating the synthesis of  $\beta$ -amyloid precursor protein,<sup>50</sup> but also exerts a neuroprotective effect by activating the phagocytic activity of microglia to degrade A $\beta$ .<sup>51</sup> Therefore, how rapamycin affects AD via IL-6 needs to be further explored.

In addition, rapamycin increases the expression of regulatory T cells (Treg).<sup>52,53</sup> Treg cells, as important immunomodulatory cells, play an important role in regulating tissue inflammation, avoiding over-activation of the immune system and maintaining lymphocyte homeostasis. In AD, impaired immune phenotype and function of Treg accelerates the development of inflammation and thus the development of AD.<sup>54</sup> Rapamycin can exert anti-inflammatory effects by enhancing Treg expression, thereby improving the development of AD. Rapamycin also inhibits mTOR activity and prevents BBB breakdown, limiting the entry of pro-inflammatory and neurotoxic factors from the blood into the brain parenchyma.<sup>55</sup> Towner et al found that rapamycin may play an important therapeutic role in inhibiting neuroinflammation by restoring cerebrovascular function and the blood-brain barrier in a rat model of lipopolysaccharide-induced brain inflammation.<sup>56</sup>

## Rapamycin Improves Cognitive Dysfunction in AD

Extensive experimental evidence that rapamycin improves AD-induced cognitive dysfunction.<sup>57–59</sup> Amyloid precursor protein/ Presenilin 1 (APP/PS1) mice treated with rapamycin showed significant improvement in memory.<sup>58</sup> Rapamycin enhances synaptic plasticity and synaptic protein expression by increasing mitochondrial autophagy, preventing cytochrome C-mediated apoptosis, and reducing oxygen species.<sup>60</sup> Synaptic plasticity and its associated proteins play an important role in the development and maturation of the nervous system, learning and memory, and influence cognitive function.<sup>61</sup>

Rapamycin breaks the negative feedback mechanism of mTOR on insulin signaling by modulating the mTOR signaling pathway (Figure 1), promotes glucose utilization by neurons, improves energy metabolism, enhances local

synaptic protein synthesis, maintains neuronal survival and increases synaptic plasticity, and improves learning and memory functions.<sup>62</sup> Although the PI3K/Akt/GSK3 $\beta$ /mTOR axis is a key mechanism for regulating synaptic plasticity, such as long term depression (LTD) and long term potentiation (LTP),<sup>63</sup> sustained over-activation of mTOR in AD leads to abnormal translation of synaptic proteins, which may impair higher mental functions severely, such as fragile X disorder and may also lead to over-translation of APP.<sup>64</sup> Upregulation of mTOR signaling leading to abnormal LTP in the CA1 region of the hippocampus was observed in an animal model of tuberous sclerosis, and short-term treatment with rapamycin rescued synaptic plasticity, memory, and behavioral deficits.<sup>65</sup> In addition to directly improving hippocampal synaptic plasticity, rapamycin improves synaptic function indirectly by reducing A $\beta$  deposition and tau protein hyperphosphorylation, thereby delaying the development of AD.<sup>57,66</sup> Rapamycin improved learning ability and spatial memory in PDAPP transgenic mice, and LC3 immunoreactive spots in the hippocampal CA1 region were increased and A $\beta$ 42 levels were reduced in the brains of transgenic mice after rapamycin administration.<sup>9</sup> Rapamycin increases neuronal autophagic activity through inhibition of mTOR may contribute to reduced A $\beta$  and improved cognitive function in the brain of AD mice.

## Controversy Over the Effect of Rapamycin on AD

Although many basic studies have demonstrated the therapeutic effects of rapamycin in AD, many questions and controversies remain, which may be due to the variability of experimental models, different modes of administration, dose, timing, frequency, and the availability of drug-targeting vehicles.

We searched the PubMed database for the last 5 years by using the search formula “rapamycin Alzheimer’s disease [Title/Abstract] not review”, suggesting that the main models are hippocampal injection of A $\beta$ -induced AD mice, intracerebroventricular injection of zinc sulfate-induced AD mice, hAPP (J20) mice, APP/PS1 mice, 3 $\times$ Tg-AD mice, SH-SY5Y cells, HEK293T cells, and neuroblastoma N2a cells (Table 1). Although the models were constructed based on the A $\beta$  deposition hypothesis and the microtubule-associated protein tau entanglement hypothesis, the whole-brain spatial distribution of A $\beta$  plaques<sup>67</sup> and the extent of A $\beta$ -induced tau protein phosphorylation differed between models.<sup>68</sup> In vivo AD models at different ages and in vitro AD models at different backgrounds represent different stages of AD pathophysiology, and rapamycin may then exhibit different effects. Subsequently, in the in vivo models, there are various modes of administration, such as intraperitoneal and tail vein injections, and gavage and feed administration; the dose ranges from 0.24–10 mg/kg/2d; the duration of administration can be 7 days or 8 months; the frequency can be continuous administration into the diet, once daily or one day apart. Other studies have combined rapamycin with transferrin decorated-nanostructured lipid carriers<sup>69</sup> and reactive oxygen species (ROS) responsive targeted micelles<sup>70</sup> to effectively deliver rapamycin to lesions in the central nervous system for targeted therapeutic effects. In in vitro experiments, factors such as cell source, cell type, gene transfection, incubation conditions, concentration and time of rapamycin treatment are all experimental variables, and in vitro experiments are more susceptible to human factors than in vivo experiments, resulting in differences in experimental results.

Thus, the above numerous factors influencing the experiment have led to controversy about the effect of rapamycin on AD. However, mTOR signaling has complex effects on the AD brain. The different stage of AD models, the effect of rapamycin on microglia in the brain, and the side effects of rapamycin remain unexplored. mTOR signaling pathway is not only involved in a variety of cellular functions in the brain,<sup>71</sup> but is also present in many types of brain cells, such as mature neurons, microglia,<sup>72</sup> astrocytes,<sup>73</sup> and neural stem cells.<sup>74</sup> Rapamycin lacks specific targeted therapeutic effects and potential side effects may occur with non-selective autophagy induction in vivo.<sup>75</sup> To address this issue, a rapamycin formulation carried by transferrin decorated-nanostructured lipid carriers (RAPA-Tf-NLCS) was utilized to target neurons in AD lesions. Both mTOR activity and autophagy were regulated to levels similar to control, and moderate mTOR activity was found to achieve the expectant therapeutic effects.<sup>69</sup> mTOR pathway is not necessarily overly upregulated in AD because previous studies have shown that mTOR signaling is unchanged or even downregulated in AD models and that activation of mTOR has a neuroprotective effect.<sup>76</sup> The Tg2576 model revealed that inhibited mTOR signaling was associated with impaired hippocampal synaptic plasticity, which was associated with reduced expression of the downstream targets p-p70S6K and p-4E-BP1, and that upregulation of mTOR signaling prevented A $\beta$ -induced synaptic damage.

**Table 1** Therapeutic Targets and Effects of Rapamycin on Different AD Models

PMID	Year	Model	Drug Administration and Dosage	Administration Time	Potential Targets	Conclusion
35929260	2022	Hippocampal injection of A $\beta$ (25–35) induced AD mice	Tail vein injection rapamycin transferrin decorated-nanostructured lipid carriers (3.5 mg/kg)	Once a day for a week	A $\beta$ , SOD, MDA, p-mTOR/mTOR, LC3II/LC3I, pro-caspase 3/cleaved caspase 3	Brain-targeted drugs reduce peripheral adverse effects, modulate autophagy, reduce apoptosis levels, reduce AD-like pathological changes, and improve memory and cognitive function
3672148	2022	5xFAD Tsc1 <sup>IKO</sup> mice	Microencapsulated rapamycin in the diet (14 ppm), 2.24 mg/kg/d	1 month 2 months 3 months	p4EBP1, A $\beta$ , Iba-1, TREM2	Long-term use of rapamycin diminishes the uptake and clearance of A $\beta$ by microglia and exacerbates AD-like pathological changes
35609863	2022	Intracerebroventricular injection of A $\beta$ (25–35) induced AD mice	Intraperitoneal injection of rapamycin 10 mg/kg	Every other day for 2 weeks	LC3II, Park2, A $\beta$ , Iba-1	Enhances autophagy, blocks A $\beta$ deposition and microglia activation, and improves learning function
35197970	2022	SH SY5Y cells incubated in 300 $\mu$ M zinc sulfate for 4 hours Intracerebroventricular injection of zinc sulfate -induced AD mice	20 ng/mL rapamycin Intraperitoneal injection, 1.5 mg/kg	1 hour Every other day for a total of 3 injections	p-mTOR/mTOR, p-P70S6K/P70S6K, Nrf2, HO-1, p-tau/t-tau, 4-HNE, 8-OHdG, TOMM20, SNAP25, synaptophysin, PSD-95	Inhibits tau protein hyperphosphorylation, reduces oxidative stress, protects synaptic and neuronal cells, and improves learning and memory deficits
33888602	2021	hAPP (J20) mice	Microencapsulated rapamycin in the diet (14 ppm), 1.65 mg/kg/d	2 months 8 months	nNOS, eNOS, non-NO-dependent components and A $\beta$ 42	Restoration of cerebrovascular function, improvement of neurovascular coupling and memory deficits, reduction of A $\beta$ deposition
3400967	2021	Mouse neuroblastoma N2a cells APP/PS1 mice	5.5 nm rapamycin Intraperitoneal injection of rapamycin solution, 1 mg/kg	24 hours Once a day for 2 weeks	nNOS, Hsp90 LC3II, Parkin, Beclin-1, SQSTM1/P62, TOMM20, LAMP2, SYP, PSD-95, Bax-xl, cleaved caspase-9, cleaved caspase-3	Enhances mitochondrial autophagy, improves synaptic plasticity and cognitive function, maintains mitochondrial function and prevents mitochondria-dependent apoptosis
34977433	2021	3 $\times$ Tg-AD mice	Intravenous administration, ROS-responsive targeted micelles (TT-NM/Rapa), 0.24 mg/kg/d	4 weeks	4E-BP1, SK61, Beclin-1, Parkin, Cathepsin D, p62/SQSTM1, LAMP2, soluble and insoluble A $\beta$ 1-40 and A $\beta$ 1-42, p-tau, SYP, Iba-1	Promotes autophagy, attenuates AD-like pathological changes, inhibits microglia proliferation, rescues neuronal cell damage, and improves cognitive deficits
31115485	2019	APP/PS1 mice SH-SY5Y cells transfected with the APP <sup>swe</sup> gene	Gavage administration of rapamycin solution, 2mg/kg 50 or 100 nM rapamycin	Once a day for 4 weeks 24 hours	BACE1, PS1, t-tau, PHF-1, LC3-II/I, Beclin-1, p62, Wnt/GSK3 $\beta$ / $\beta$ -linked protein Edu-positive cells, mRFP-positive cells, GFP-positive cells	Attenuates AD-like pathological changes, increases the number of autolysosomes and autophagosomes, induces autophagy and promotes cell proliferation

29962282	2019	Injection of A $\beta$ (1–40) -induced T2DM+AD model into the hippocampus of type 2 diabetic rats	Intraperitoneal injection of rapamycin solution, 5 mg/kg	Every other day for 4 weeks	APP, p-tau, AMPK, p-AMPK, mTOR, p-mTOR	Reduces AD-like pathological changes and improves learning and memory functions
35799293	2019	HEK293T cells	2.5 $\mu$ mol/L, 5 $\mu$ mol/L, 10 $\mu$ mol/L rapamycin	6 hours	p-4EBP1, 4EBP1, p-ULK1, ULK1,	Tau protein accumulation inhibits autophagy through activation of the mTORC1 signaling pathway
29351469	2018	hAPP (J20) mice and LDLR $-/-$ mice	50 $\mu$ mol/L vinblastine with rapamycin Microencapsulated rapamycin in the diet (14 ppm), 1.65 mg/kg/d	6 hours 4 months	p-p70S6K1, p70S6K1, p-4EBP1, 4EBP1, LC3 JAM-A	Maintaining the integrity of the BBB

**Abbreviations:** AD, Alzheimer's disease; A $\beta$ , amyloid  $\beta$ ; APP, amyloid precursor protein; AMPK, AMP-activated protein kinase; BBB, blood brain barrier; BACE1, beta-site amyloid precursor protein cleaving enzyme 1; eNOS, endothelial nitric oxide synthase; GSK3 $\beta$ , glycogen synthase kinase 3 $\beta$ ; HO-1, heme oxygenase-1; Iba-1, ionized calcium binding adapter molecule 1; Hsp90, heat shock protein 90; JAM-A, junctional adhesion molecule A; LAMP2, lysosome-associated membrane protein 2; LDLR, low-density lipoprotein receptor; LC3, light chain 3; MDA, malondialdehyde; mTOR, mammalian target of rapamycin; nNOS, neuronal nitric oxide synthase; Nrf2, nuclear factor erythroid 2-related factor-2; PSI, presenilin 1; PHF-1, paired helical filament 1; PSD-95, postsynaptic density protein-95; PMID, PubMed unique identifier; ROS, reactive oxygen species; SOD, superoxide dismutase; SYP, synaptophysin; SQSTM1, sequestosome 1; t-tau, total-tau; TOMM20, translocase of outer mitochondrial membrane 20; TREM2, triggering receptor expressed on myeloid cells 2; 4EBP1, eIF4E-binding protein 1.

Although many studies have shown that inhibition of the mTOR signaling pathway can significantly reduce AD-like neuropathological features and improve cognitive function in mouse models, these mouse studies have administered rapamycin before or early in the development of pathological features. Different states of the lysosomal system occur in mice of different ages and stages of AD disease. Few studies have been performed on aged AD mice aged  $\geq 18$  months, and most studies have used mice aged  $\leq 15$  months. Moreover, the use of rapamycin restored abnormally enlarged lysosomes in the cortex of AD mice to the size of controls and increased the number of lysosomes, enhanced lysosomal activity, promoted autophagosome-lysosome fusion, and cleared A $\beta$  and p-tau.<sup>70</sup> Rapamycin was found to increase the number of autophagosomes and autophagic lysosomes in SH-SY5Y cells stably transfected with the *APP<sup>swe</sup>* gene. Rapamycin has an autophagy-promoting effect on A $\beta$ -depositing cells and attenuates further cellular damage by A $\beta$ , which may be one of the mechanisms by which rapamycin can be used to treat patients who have already developed pathologic features.<sup>12</sup> However, the lysosomal system in the brain is less expressed and slower in the later stages of AD and with aging.<sup>77</sup> Rapamycin inhibits mTOR and activates autophagy to clear A $\beta$ . Decreased lysosomal clearance leads to autophagosomes accumulation, causing autophagic stress and promoting the amyloid plaques formation.<sup>78</sup> Thus rapamycin has a complex, even harmful effect on late AD. Clinical trials on the efficacy of rapamycin should be conducted in MCI patients at risk of progression to AD and in patients with a recent diagnosis of AD,<sup>3</sup> or that clinical trials should focus on exploring the efficacy of rapamycin in middle-aged populations with early amyloid plaque and accumulation of microtubule-associated protein tau (MAPT) tangles.<sup>77</sup> Therefore, rapamycin may have a more significant effect on preventing AD and delaying the progression of early AD. Currently, there is a Phase I clinical trial conducted in 2020 (NCT04200911) on the feasibility and safety of rapamycin in the treatment of MCI patients that have just been carried out ([www.clinicaltrials.gov](http://www.clinicaltrials.gov)). However, whether individualized treatment for patients with mid- to late-stage AD<sup>79</sup> can enter the therapeutic time window for rapamycin remains unclear.<sup>80</sup>

Furthermore, rapamycin inhibits microglia activation in the brain and reduces microglia proliferation, yet microglia activation may play a dual role in the AD pathogenesis.<sup>81</sup> However, acute microglia activation reduces A $\beta$  deposition by increasing phagocytosis or clearance, but it was found that the use of rapamycin for 2–3 months inhibited mTOR activity and reduced TREM2 expression in microglia of AD mice, weakening the uptake and clearance of A $\beta$  by microglia and aggravating AD-like pathological changes in the brains of AD mice.<sup>72</sup> Therefore, detailed longitudinal characterization of microglia activation in relation to AD pathogenesis should be explored immediately, and whether the inhibitory effects of rapamycin on various phenotypes of microglia in different dosage forms, as well as routes, frequency and doses of administration, are therapeutic for AD. In addition, the different side effects of rapamycin may have hindered its introduction as an anti-neurodegenerative agent. Although genetic and pharmacological inhibition of mTORC1 can slow the AD progression and other neurodegenerative diseases, side effects, such as glucose intolerance, diabetes, and immunosuppression resulting from long-term rapamycin inhibition of mTORC2 are of concern; thus, exploring additional rapamycin-targeted agents in the future may be an effective solution to reduce adverse effects.

## Conclusion

Rapamycin may delay the development of AD by reducing A $\beta$  deposition, inhibiting tau protein hyperphosphorylation, maintaining brain function in *APOE  $\epsilon 4$*  gene carriers, clearing chronic inflammation, and improving cognitive dysfunction, and is expected to be one of the candidates for treating Alzheimer's disease.

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## Disclosure

The authors report no conflicts of interest in this work.



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