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REVIEW

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GSK3: A potential target and pending issues for treatment of Alzheimer's disease

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Abstract

Glycogen synthase kinase-3 (GSK3), consisting of GSK3α and GSK3β subtypes, is a complex protein kinase that regulates numerous substrates. Research has observed increased GSK3 expression in the brains of Alzheimer's disease (AD) patients and models. AD is a neurodegenerative disorder with diverse pathogenesis and notable cognitive impairments, characterized by Aβ aggregation and excessive tau phosphorylation. This article provides an overview of GSK3's structure and regulation, extensively analyzing its relationship with AD factors. GSK3 overactivation disrupts neural growth, development, and function. It directly promotes tau phosphorylation, regulates amyloid precursor protein (APP) cleavage, leading to Aβ formation, and directly or indirectly triggers neuroinflammation and oxidative damage. We also summarize preclinical research highlighting the inhibition of GSK3 activity as a primary therapeutic approach for AD. Finally, pending issues like the lack of highly specific and affinity-driven GSK3 inhibitors, are raised and expected to be addressed in future research. In conclusion, GSK3 represents a target in AD treatment, filled with hope, challenges, opportunities, and obstacles.

KEYWORDS
Alzheimer's disease, glycogen synthase kinase-3, targeted drug, therapeutic target

1 | INTRODUCTION

Glycogen synthase kinase-3 (GSK3) was first identified in the 1980s as a protein kinase responsible for phosphorylating and deactivating glycogen synthase in rabbit skeletal muscle.[1,2] Since then, GSK3 has been recognized as an evolutionarily conserved Ser/Thr protein kinase with a multitude of substrates. To date, over 100 substrates of GSK3 have been identified, with an additional 500 awaiting confirmation.[3,4] This extensive range of downstream regulated effects renders GSK3 one of the most functionally complex kinases involved in various cellular processes, including motility, metabolism, differentiation, proliferation, and apoptosis.[3] The two isoforms of human GSK3, GSK3α, and GSK3β, consist of 483 amino acids (51 kDa) and 420 amino acids (47 kDa), respectively, as evidenced by research.[5,6]

The first three authors contributed equally to this work.

These isoforms are derived from chromosome 19 and chromosome 3, respectively.[6,7] Both GSK3α and GSK3β have been detected in almost all species, with over 90% similarity across different species.[8] In Homo sapiens, the amino acid sequences of GSK3α and GSK3β show 84%–85% similarity, while their kinase domains exhibit a high degree of homology, with 19 differential amino acids from the 285 amino acids that make up the kinase domains (Figure 1A), according to sequence alignment.[6,7] Though hitherto no accurate structural information of GSK3α is obtained. Predicted result in UniProt (https://www.uniprot.org/uniprot/P49840#structure) suggests that the glycine-rich region distinguish between these two isoforms does not form a stable secondary structure. Hence, the secondary structures of GSK3α and GSK3β (Figure 1B) are also thought to be similar especially in kinase domains, which arrive at a conclusion that GSK3α and GSK3β showed approximate biological activity. Nonetheless, research confirms that the glycine-rich region plays a role in localizing GSK3α inside the cell.[9] The wild-type GSK3α is typically situated in the cytoplasm rather than the nucleus, as stated in scholarly literature.[7] Intriguingly, research indicates that truncated GSK3α lacking the N-terminal region congregates in the nucleus, with calcium stimulation further amplifying this effect.[10] In contrast, GSK3β displays greater nuclear mobility, particularly during the S-phase of the cell cycle and in the context of apoptosis, as reported in studies.[11,12]

Alzheimer's disease (AD) is a neurological condition that is typified by cognitive impairment and memory loss. It is the foremost cause of dementia, and while it remains a growing concern, the ever-increasing number of patients coupled with slow drug development has resulted in AD becoming a condition that is among the most destructive, expensive, and burdensome.[13,14] For instance, in the United States alone, it has been estimated that the cost of AD was approximately $305 billion in 2020, with around 5.8 million Americans affected.[15] In view of latest statistics, it can be anticipated that the figure of affected individuals is likely to rise to 13.8 million by 2050.[15] While acetylcholinesterase inhibitors like donepezil and excitatory amino acid receptor antagonists such as memantine remain the preferred treatment options for AD, it is worth noting that they have been approved by the U.S. Food and Drug Administration (FDA) for multiple decades and offer

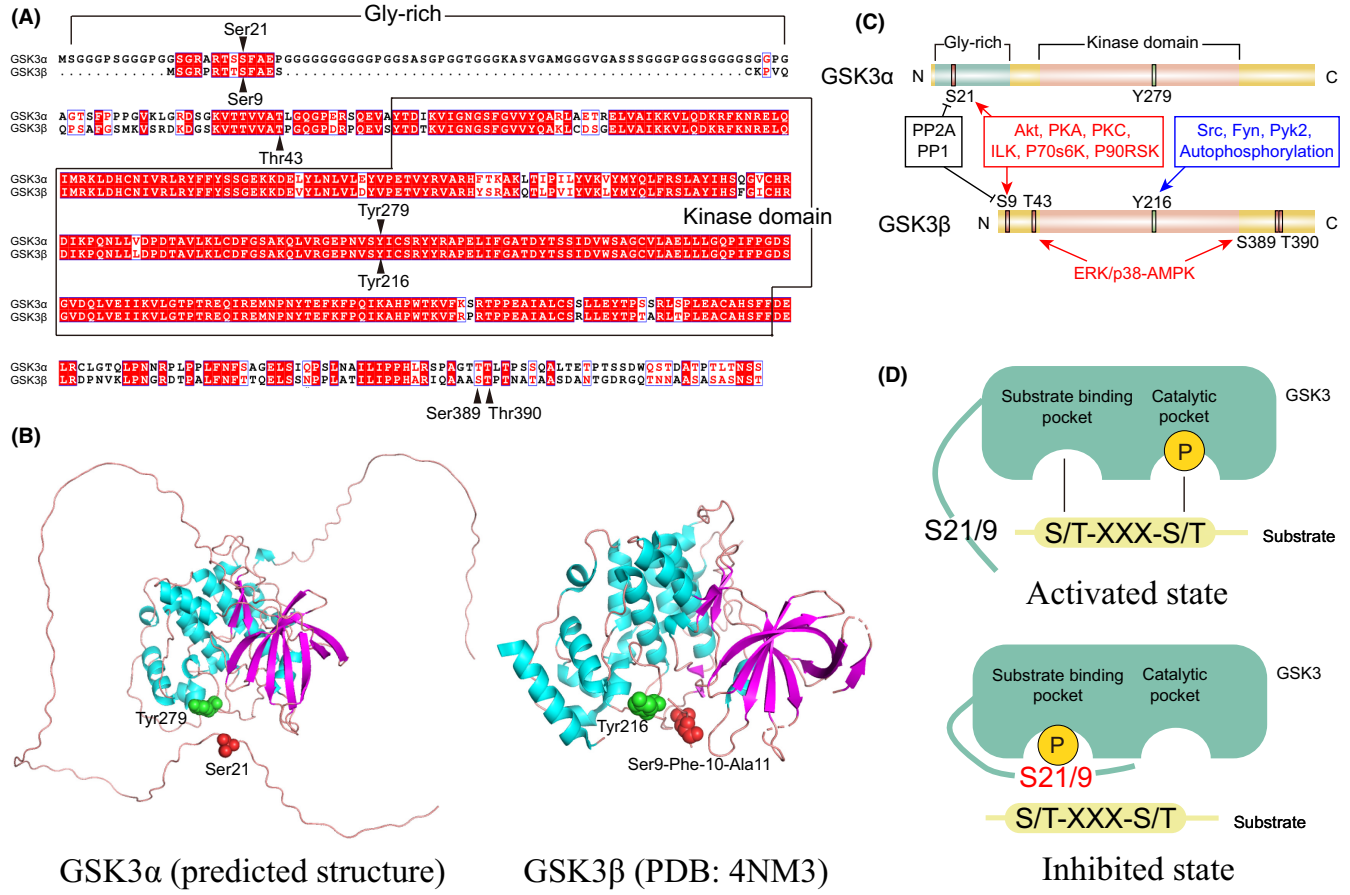


FIGURE 1 The structure and functional regulation of GSK3. (A) Amino acid sequence alignment of GSK3α and GSK3β. (B) Crystal structure of GSK3α (predicted) and GSK3β (PDB: 4NM3). The crucial phosphorylation sites, GSK3α (Ser21) and GSK3β (Ser9), are highlighted in red. GSK3α (Tyr279) and GSK3β (Tyr216) are marked in green. (C) Phosphorylation sites and upstream regulatory proteins of GSK3α and GSK3β. (D) The phosphorylation regulatory mechanism of GSK3. In the absence of phosphorylation at Ser21/9, GSK3 exists in an activated state. Its substrate binding pocket binds to substrates and facilitates the transfer of phosphate groups from the catalytic pocket. Conversely, when Ser21/9 gets phosphorylated, GSK3's substrate binding pocket tightly closes, inhibiting substrate binding and phosphorylation. Hence, GSK3 assumes an inhibited state.

only partial relief of symptoms.[16,17] Recently, another drug called Aduhelm (aducanumab), which is a monoclonal antibody that targets Aβ (amyloid beta), has received FDA approval for treating AD.[18] However, there is still ongoing debate regarding its curative effectiveness.[19] Despite exhaustive research, there exists an incomplete understanding of the fundamental mechanisms at play in AD. Supported by characteristic pathological markers including the aggregation of Aβ and the formation of neurofibrillary tangles (NFTs), these factors are believed to be the primary contributors to AD.[13] Furthermore, observations of inflammation,[20] mitochondrial malfunction, oxidative stress,[21] autophagy impairment,[22] gut microbiota deregulation[23] and occurrences of ferroptosis[24] have all been documented in relation to the onset and progression of AD. Above all, AD is a highly intricate neurodegenerative disorder that requires ongoing exploration of therapeutic targets and drug development efforts.[13,14]

High expression of GSK3 in the brain stands out as a significant phenomenon compared to its expression in other tissues.[6] This finding was first noticed in animal models at both mRNA and protein levels and subsequently mirrored in the human brain.[25,26] Given its considerable expression levels, the role of GSK3 in normal brain physiology and neurological conditions such as bipolar disorder, AD, Parkinson's disease, and psychiatric disorders has become increasingly intriguing to researchers.[7] Taking into account the multifunctional nature of GSK3 and its relevance to several pathological features of AD, notably the formation of NFTs,[27,28] significant attention has been devoted to GSK3 in the course of developing drugs for AD.[29] The purpose of this paper is to comprehensively explicate the link between GSK3 signaling and AD, and to summarize the latest advances in drug development for AD that specifically target GSK3. Ultimately, the intention is to supply relevant guidance on basic research on GSK3 signaling and to chart potential directions for creating AD drugs that specifically target GSK3.

2 | THE FUNCTION AND REGULATION MECHANISM OF GSK3

2.1 | The location and function of GSK3 in the brain

From a spatial perspective, the brain is the organ with the highest abundance of GSK3 in healthy adults.[6] However, from a temporal perspective, the content of GSK3 in the brain of mature rats is significantly higher than that of fetal rats and newborn rats.[30] This phenomenon is related to the important role of GSK3 in brain development, while in the mature brain, GSK3 may primarily play a role as a maintainer of function. Research involving the transfection of primary neurons with shGSK3 has demonstrated significant axon growth inhibition.[31] In cases where GSK3 is specifically eliminated in astrocytes, mice display excessive anxiety and anomalous social behaviors.[32] Additionally, neural progenitor cells from GSK3 KO mice are prone to massive proliferation instead of differentiating into intermediate neural progenitors and postmitotic neurons.[33] Meanwhile, cortical progenitor cells from GSK3 KO mice exhibit a disruption of radial migration and dendritic orientation.[34]

Noteworthily, GSK3α and GSK3β do not exhibit complete functional equivalence. GSK3α knockout (KO) mice, for example, are able to survive but have a shortened lifespan and male infertility.[35–37] These mice also experience considerable abnormalities in cerebellum structure and demonstrate various mental disorders and behavioral deficits, including increased emotionality, reduced depression-associated behaviors, decreased social interaction and aggression, altered information processing, and impaired long-term memory function.[38] In addition, GSK3α KO mice exhibit enhancements in glucose and insulin sensitivity, along with a reduction in fat mass.[35,38]

The GSK3β gene undergoes alternative splicing, resulting in the production of a neuron-specific long form called GSK3β2.[39] This isoform is enriched during brain development,[40,41] and plays a significant role (Figure 2A) in promoting neurodevelopment such as neurogenesis,[42,43] axon formation and growth,[30,31,44] synaptogenesis,[45] dendrite development, and neuronal survival.[28] Furthermore, GSK3β is crucial for both the formation and maintenance of neuronal polarity (Figure 2A).[46] Mice with complete KO of the GSK3β gene exhibit evident structural and functional abnormalities in the brain, but they succumb to liver and heart dysfunction in the early embryonic stage.[47,48] Heterozygous mice with GSK3β KO can survive into adulthood, yet they display reduced exploratory behavior,[49] decreased aggression,[50] and impaired memory.[51] Furthermore, silencing GSK3β solely in the dentate gyrus of the hippocampus leads to an antidepressant-like state in mice.[49,52,53]

There are also differences in the spatial distribution of GSK3β and GSK3α in the brain. The Allen Brain Atlas shows that GSK3β is expressed relatively evenly throughout the brain regions,[25,54] while GSK3α is only significantly expressed in the adult brain cortex, hippocampus, striatum, and cerebellum.[6] To sum up, although GSK3 plays a crucial role in the generation and development of the brain, the levels of GSK3α/GSK3β are tightly regulated both spatially and temporally. The distinct distribution of GSK3α and GSK3β in the brain is likely associated with their functional differences. An experiment using mutant mice with GSK3α and GSK3β mutations demonstrated that GSK3α plays a more critical role than GSK3β in hippocampal bidirectional synaptic plasticity.[55] The dysregulation of GSK3 is indeed a key contributing factor to various neurological disorders, making the maintenance or restoration of GSK3 homeostasis an important subject of research in the field of neurological diseases.

2.2 | Regulation of the GSK3 activity

As a protein kinase, GSK3 possesses the ability to phosphorylate almost all downstream proteins that bear the S/T-X-X-X-S/T(P) motif, as evidenced by numerous substrates.[4,56] To date, crystal structures of GSK3β have been acquired, mainly due to its shorter sequence. These structures serve as the foundation for GSK3β's phosphate-primed substrate specificity and autoinhibition.[57]

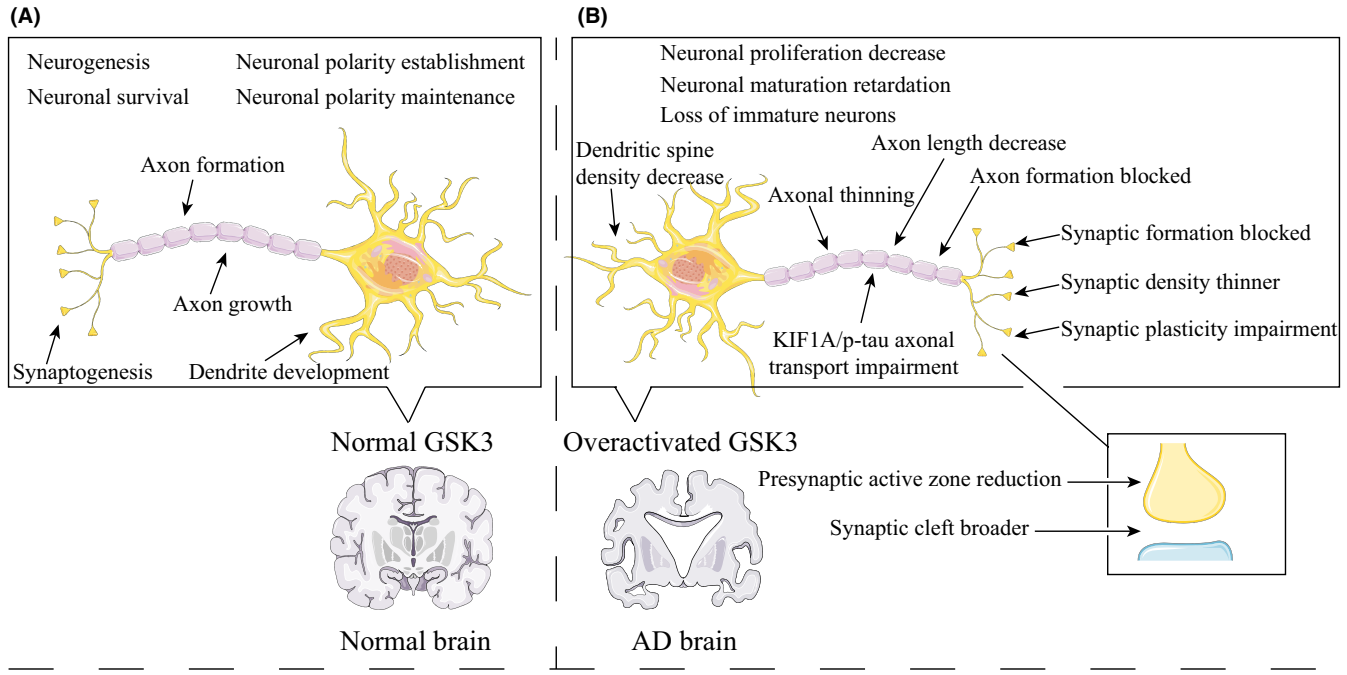


FIGURE 2 The role of GSK3 in neuronal growth, development, and function. (A) The conventional levels of GSK3 in normal brain and its positive impact on neurons. (B) The detrimental effects of GSK3 hyperactivation in the brains of AD patients on neuronal structure and function.

It is anticipated that GSK3α operates in a comparable manner.[4] As shown in Figure 1D, the specific motif leads to a functional conformational change in GSK3, allowing substrate binding and phosphorylation.[4,58,59] A groove adjacent to the catalytic pocket plays a crucial role in recognizing and accommodating S/T from the specific motif, and is therefore named the substrate binding pocket.[57,58] It is important to note that the groove discussed is significant for two exact opposite states of GSK3β, namely p-GSK3β^{Ser9} and p-GSK3β^{Tyr216}. This is illustrated in Figure 1D, where Ser9 phosphorylation results in an inhibited state of GSK3β by altering the position of the loop and occupying the substrate binding pocket with the phosphorylated Ser9, thereby preventing GSK3β and substrates from binding.[60] This phosphorylation state is crucial for maintaining normal cell homeostasis.[61] In contrast, phosphorylation of Tyr216 activates GSK3β as the side chain of Tyr216 occupies the substrate binding pocket prior to phosphorylation and releases it after phosphorylation.[58,62] The states of p-GSK3α^{Ser21} and p-GSK3α^{Tyr279} reflect the inhibited and activated state of GSK3α, respectively, and although no direct evidence from structural biology is available, the identical inhibited and activated state suggests a similar phosphorylation regulatory mechanism for these two isoenzymes.[62,63]

Not only catalyzing many substrates, GSK3 is under dynamical regulation of various of kinases as well (Figure 1C). Ser21/9 can be phosphorylated by protein kinase B (PKB), commonly known as AKT, as well as its highly homologous proteins protein kinase A (PKA) and protein kinase C (PKC), along with Integrin-linked kinase (ILK), p70S6K, and p90RSK.[7,64] Additionally, two subfamilies of mitogen-activated protein kinase (MAPK), extracellular signal-regulated kinases (ERK) and p38, play a role in the phosphorylation of Thr43, Ser389, and Thr390, which results in GSK3β Ser9 phosphorylation.[65,66] The activating kinases of GSK3β have been previously documented to perform the crucial function of phosphorylating GSK3β at Tyr216. Specifically, Src, Fyn, and Pyk2 have been identified as the kinases responsible for this activation process.[7] In addition, it should be highlighted that GSK3β is also capable of performing autophosphorylation at Tyr216.[67] Of note, Ser21/9 dephosphorylation is also reported for GSK3 activity regulation, protein phosphatase 2A (PP2A) and protein phosphatase 1 (PP1) have been identified as the key enzymes involved in this process.[68]

3 | THE ROLE OF GSK3 IN AD

There is a wealth of evidence that supports a strong correlation between GSK3 and AD. Studies have shown that inhibiting the state of GSK3β, specifically p-GSK3β^{Ser9}, is beneficial for long-term memory formation.[69] Through the identification of genes and cerebrospinal fluid biomarkers in hundreds of AD patients and healthy volunteers, it has been revealed that genetic variants of GSK3β are closely associated with Aβ and p-tau.[70] Moreover, high activity of GSK3β in the peripheral blood of AD patients has been found to be positively correlated with the severity of dementia.[71] Animal models have provided similar insights, as both the content and activity of GSK3β were observed to increase with age.[72–74] In a CamKIIα-tTA/GSK3β mouse model, where GSK3β was overexpressed starting at 6 months, neurodegeneration and other AD symptoms were observed at 12 months of age.[75] Further mechanistic studies have revealed that the excessive activity of GSK3 influences the onset and progression of AD through various pathways.

3.1 | Impairment of the neuronal structure and function

Given the complex biological functions of GSK3 and its precise regulation, the dysregulation of GSK3, especially the excessive activation in AD patients or animal models, can have multiple negative effects on neurons (Figure 2B). Researchers have activated GSK3 in model animals using different methods, and although the focus and specific indicators observed may vary, they have all discovered similar abnormalities in neuronal structural and functional processes. Abnormal neuronal proliferation and maturation processes, as well as resulting dendritic atrophy, have been found in CamKIIα-tTA/GSK3β mice.[76] Similarly, rats carrying the GSK3β mutant with Ser9 mutated to Ala experience sustained activation of GSK3β, leading to significant disruptions in neuronal oscillations in the prefrontal cortex and hippocampal ventral side.[77] Conversely, pharmacological inhibition of GSK3β can promote neurogenesis in APP/PS1 mice.[78]

Specifically, GSK3 overexpression or excessive activation also damages substructures within neurons, such as synapses, dendrites, and axons. GSK3β overexpression can inhibit synaptogenesis.[79] In rats with elevated GSK levels caused by exogenous or endogenous factors, the presynaptic active zone, postsynaptic density, and synaptic cleft are all negatively affected, accompanied by long-term potentiation (LTP) inhibition.[80] LTP inhibition and related synaptic damage are direct causes of memory impairment.[81–83] Excessive activation of GSK3β in STZ-induced sporadic AD rats ultimately leads to decreased dendritic spine density and axonal thinning in the hippocampal CA1 region.[84] In AD mouse models exposed to Aβ oligomers, excessive GSK3β activation is accompanied by dendritic spine loss.[85] Axonal transport function is also severely impaired by GSK3 overactivation, leading to excessive accumulation of p-tau in the distal axon and impaired axonal transport of KIF1A (an essential protein for the delivery of neurotrophic factors), both contributing to the development of AD.[86–89]

In terms of mechanisms, based on the existing reports, the interaction between presenilin 1 (PS1) and GSK3 appears to be a crucial factor. PS1 serves as a significant biomarker of AD and has been demonstrated to be associated with the incidence of the disease, being utilized for establishing AD animal models through genetic mutations.[90] Phosphorylation of PS1 by GSK3β weakens its interaction with N-cadherin/β-catenin, resulting in the formation of trimeric complexes at synapses and a decrease in synaptic plasticity and neuronal vitality.[91] Mice with PS1 deficiency or mutations display increased GSK3β activity, leading to notable axonal transport impairments. Mechanistically, GSK3β is involved in the augmented release of membrane-bound organelles driven by kinesin-I.[92] Furthermore, excessive activation of GSK3β increases the phosphorylation level of collapsin response mediator protein 2 (CRMP2), which hinders CRMP2 signal transduction and its regulation of axonogenesis and synaptic formation.[93,94]

GSK3 is a critical participant in the β-catenin-dependent Wnt signaling pathway.[95] The pathway is known to have a significant impact on synaptic plasticity as well as memory.[96] The Wnt family is comprised of 19 lipid-modified glycoproteins and plays a crucial role in a range of cellular processes including cell metabolism, cell fate determination, polarity, and cytoskeleton alterations. The activation of the β-catenin-dependent Wnt signaling pathway involves the binding of Wnt to various cell surface receptors such as Frizzled (Fz), low-density lipoprotein receptor-related protein 5 (LRP5), and LRP6, ultimately triggering β-catenin to exert its biological effects.[95] GSK3 is a vital regulator of β-catenin degradation, and it forms a complex with casein kinase Iα (CKIα), Axin, and adenomatosis polyposis coli (APC) to phosphorylate β-catenin. Subsequently, E3 polyubiquitin ligases recognize the phosphorylated β-catenin, and it is degraded by proteasome. Upon activation of Wnt signaling, the complex is eliminated, inhibition of GSK3 stabilized β-catenin, thus as a transcriptional coactivator that translocates to the nucleus to promote transcription of related genes.[95] Wnt-dependent protein degradation is a common occurrence in cells when GSK3 dysfunction manifests. GSK3's involvement in the Wnt/β-catenin pathway aggravates AD-associated neuron damage.[97]

Following an evaluation of gene expression in the prefrontal cortex of both normal brains and AD patients, it was observed that the protein levels and activities of β-catenin and GSK3β underwent a dynamic switch. These proteins are associated with Wnt signaling and suggest that such signaling is significantly impaired in AD patients.[98] Further research has indicated that overexpression of the Wnt antagonist Dickkopf-1 (Dkk-1) in the hippocampus can lead to a reduction in learning and memory ability in mice. This effect was linked to synapse loss, LTP impairment, and long-term depression enhancement. However, the negative consequences were eliminated through associative inhibition of GSK3 and RhoA-Rock. Repression of Dkk-1 expression displayed a similar effect.[99] Overall, GSK3β plays a negative role in Wnt signaling-based neural functional recovery. Notably, activating muscarinic receptors inhibited GSK3β and restarted the Wnt/β-catenin pathway in $A\beta_{1\text{-}40}$-managed hippocampal neurons and glutamate-managed PC12 cells. This process helped protect neurons from serious damage.[100,101]

3.2 | Tau pathology

Tau is an intrinsically disordered protein that contains 85 phosphorylation sites, with over 50 of these sites having been identified as being phosphorylated.[102] The protein is predominantly expressed in neurons, where it exists in six primary isoforms.[103] The process of phosphorylation, a posttranslational modification, is essential for the biological function of tau, which tracks axonal microtubules and enhances their stability, thereby contributing to axonal growth and transportation.[104] However, hyperphosphorylated tau has been implicated in the formation of intracellular NFTs, a typical pathological characteristic of the brains of AD patients.[105] Consequently, phosphorylated tau (p-tau) is recognized as a reliable biomarker for AD diagnosis and is also considered a promising target for AD treatment.[106]

GSK3 is a kinase of significant importance for p-tau, with approximately 30 serine or tyrosine residues from tau identified as potential phosphorylation sites for GSK3.[107] It has also been observed that GSK3's phosphorylation of tau is correlated with the formation of tangle-like filament morphology.[108] Importantly, recent studies illustrate that GSK3β can directly acetylate the K15 site of tau protein, which has the negative consequence of inhibiting the ubiquitination degradation process and reducing its activity-inhibiting state (p-GSK3β^{ser9}), ultimately causing GSK3β to become excessively activated.[109] In essence, a detrimental cycle appears to have developed between tau pathology and GSK3 over-activation in the brains of AD patients.

A meta-analysis conducted on studies related to AD involving the use of 3×Tg-AD mice found a positive correlation between Morris water maze (MWM) performance and p-tau levels. Additionally, p-GSK3β^{Ser9} was identified as the primary contributor.[110] Further research involving GSK3α deficiency mice suggested that GSK3α also played a role in tau hyperphosphorylation.[111] AChE inhibitors, such as donepezil, remain the frontline treatment for AD, scientific investigators have discovered that in AD, AChE is colocalized with p-tau in neurofibrillary tangles. They have also found that GSK3β-induced tau hyperphosphorylation suppressed the expression of AChE. Clinical studies involving the use of irreversible GSK3β inhibitors have reported a 35 ± 16% increase in AChE activity among patients.[112] Here we introduce the exacerbation of tau pathology by GSK3 signaling mediated by various biomacromolecules and micromolecules (Figure 3).

3.2.1 | PI3K/AKT-mediated GSK3 signaling in tau pathology

The AKT enzyme is responsible for transferring the phosphate group to Ser21/9 of GSK3, thereby inhibiting excessive activation of GSK3 and maintaining the stability of p-tau. It has been established that AKT is initially phosphorylated and activated by phosphoinositide 3-kinase (PI3K).[113] Therefore, it is widely believed that the PI3K/AKT/GSK3 signaling pathway plays a crucial role in tau phosphorylation.[6] Various upstream factors influencing PI3K or AKT may also partly affect tau phosphorylation through GSK3, which to some extent explains the interplay between neuroinflammation, neuronal apoptosis, and GSK3-mediated tau pathology.

C-reactive protein, an immunomodulatory protein upregulated during inflammatory responses, induces tau phosphorylation in conjunction with alterations in AKT/GSK3β.[114] The excessive phosphorylation of tau induced by the inflammatory cytokine IL-1β serves as a hallmark of the association between inflammation stimulation and tau pathology in AD. The phosphorylation capability of AKT on GSK3β is nullified by the sulfhydration of AKT at Cys77, which is contingent on elevated levels of intracellular hydrogen sulfide (H_2S) provoked by IL-1β. This phenomenon leads to tau hyperphosphorylation and synaptic dysfunction.[115] Interestingly, a separate study demonstrated that exogenous administration of H_2S can directly sulfhydrate GSK3β, resulting in decreased tau phosphorylation.[116] Thus, it is evident that high levels of H_2S have a dual impact on AKT/GSK3-mediated tau pathology. Therefore, in an inflammatory

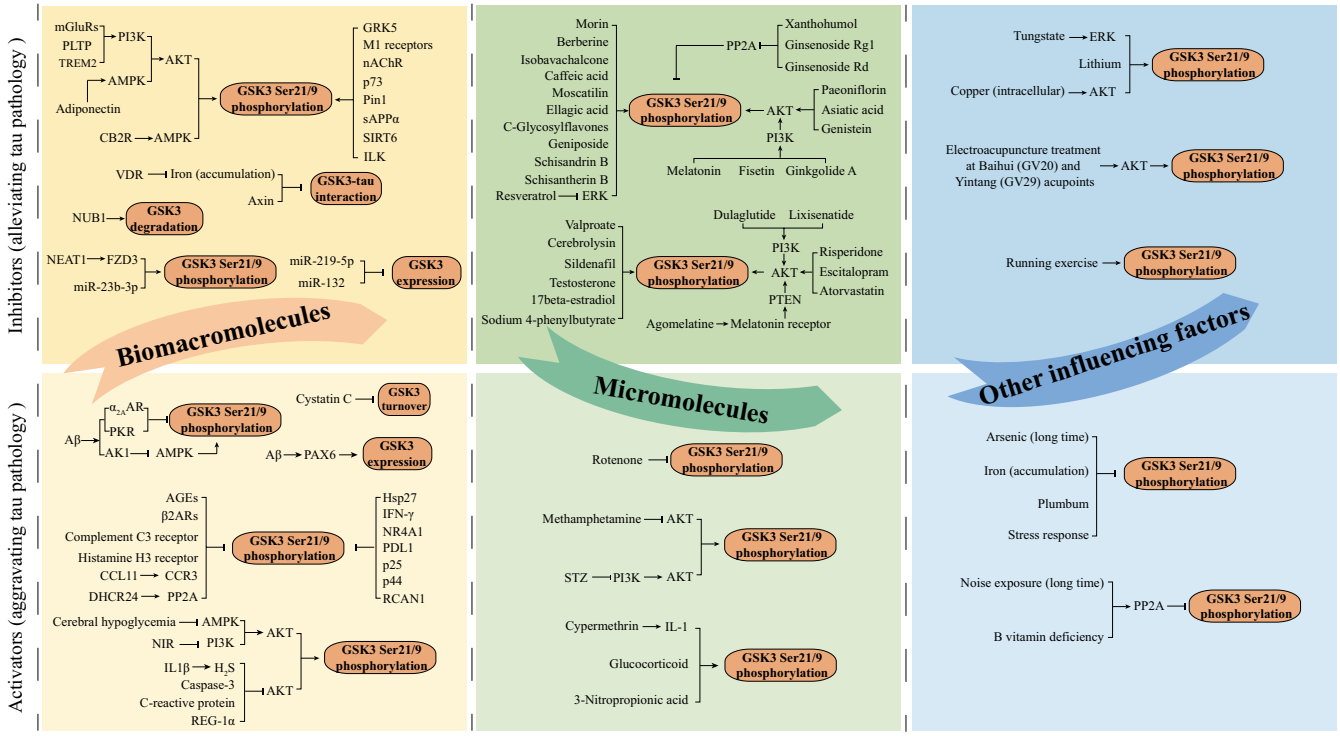


FIGURE 3 Factors influencing tau pathology through GSK3. Biomacromolecules influencing GSK3-tau are highlighted in yellow, micromolecules in green, and other influencing factors in blue. The darker shades in the upper half signify factors that inhibit GSK3 and alleviate tau pathology, while the lighter shades in the lower half represent factors that activate GSK3 and aggravate tau pathology.

environment, whether AKT is cysteinylated or GSK3 is cysteinylated by H_2S, which one takes precedence, is a thought-provoking question worthy of further exploration. The caspase family proteins, crucial proteases in cellular apoptosis, play essential roles in various biological processes in the nervous system.[117,118] Tau serves as a substrate for cysteine proteases, and caspase-cleaved tau has been detected in the AD brain but not in the normal brain. Importantly, caspase-cleaved tau exhibits higher fibrillogenicity compared to full-length tau. A combination of phosphorylation events and caspase activation contributes to the process of tau oligomerization in AD.[119] GSK3β-mediated tau phosphorylation precedes caspase cleavage. Additionally, caspase-3, a member of the caspase family, relieves the inhibition of GSK3β by cleaving AKT, thereby increasing the occurrence of phosphorylation events.[119,120]

Impairment of insulin signaling in the brain has been associated with AD. Overexpression of Regenerating islet-derived 1α (REG-1α) in the brains of AD patients triggers tau phosphorylation through the AKT/GSK3 signaling pathway.[121] Mice with neuron-specific insulin receptor (NIR) KO exhibit activation of the PI3K/AKT/GSK3β signaling pathway and significant tau pathology.[122] Overexpression of Phospholipid Transfer Protein (PLTP) in 3×Tg-AD mice alleviates various AD symptoms by limiting excessive activation of GSK3β and tau hyperphosphorylation through the PI3K/AKT pathway.[123,124] Metabotropic Group I glutamate receptors (mGluRs) can influence PI3K/AKT, resulting in reduced GSK3β-mediated tau hyperphosphorylation.[125] The aforementioned proteins involve cellular signal transduction and intracellular and extracellular substance exchange. While a few studies have observed changes in their levels associated with alterations in the PI3K/AKT/GSK3β signaling pathway, this phenomenon is likely the result of multiple factors influencing it, such as insulin signaling, lipid transport, and metabolism. The underlying mechanisms by which these factors ultimately impact tau pathology through GSK3β remain to be elucidated.

3.2.2 | Aβ-mediated GSK3 signaling in tau pathology

The investigation of the correlation between Aβ and tau has been a consistent area of focus in research on AD.[126] GSK3 serves as a crucial element in receiving stimulation from Aβ and promoting tau pathology.[127] The research on the interaction between Aβ and tau, as well as the role of GSK3 within it, primarily revolves around the conformation of Aβ. $Aβ_{1-42}$ monomers, as opposed to oligomers, induce a PHF-like conformation of tau, resulting in multiple effects on tau, including phosphorylation, conformational changes, and degradation, while simultaneously elevating GSK3 levels.[128] It is worth noting that $Aβ_{1-42}$ oligomers can also promote GSK3-mediated tau pathology, with solubility being a crucial prerequisite.[129] $Aβ_{1-42}$ oligomers can bind with tau to form soluble stable complexes, enhancing the possibility of tau receiving phosphoryl groups from GSK3β.[130] Additionally, $Aβ_{1-42}$ oligomers exhibit high affinity binding with GSK3α, thereby promoting GSK3α-catalyzed tau phosphorylation.[117] These findings confirm the interrelationship between Aβ, tau, and GSK3, although certain details still require further investigation. For instance, if Aβ can bind to both tau and GSK3α, does that imply the formation of a complex involving all three proteins? Does the binding of Aβ to tau facilitate GSK3β-mediated phosphorylation of tau, and does GSK3β exhibit a similar strong interaction with Aβ as GSK3α does?

In addition to directly binding to tau and promoting its phosphorylation by GSK3, the regulatory role of Aβ in GSK3 activity is equally significant. The indirect pathway of Aβ, mediated through norepinephrine α2A adrenergic receptor (α2AAR) signaling, surpasses direct GSK3β activation in terms of potency,[127] underscoring its crucial role in the pathogenesis of AD. The cerebral neurons exhibit widespread distribution of α2AAR, playing a pivotal role in transmitting norepinephrine signaling.[118] Aβ oligomers have the potential to bind to α2AAR and induce conformational changes, redirecting norepinephrine signaling towards GSK3β activation, thereby exacerbating tau hyperphosphorylation.[127] Moreover, Aβ also activates GSK3 and intensifies tau pathology through the promotion of adenylate kinase 1 (AK1) and double-stranded RNA-dependent protein kinase (PKR).[131,132]

Aβ also enhances the expression of E2F1 and c-Myb, which activate PAX6, a transcription factor linked to brain development and overexpressed in AD patients and animal models. PAX6 promotes GSK3β transcription and elevates the p-tau level at Ser356, Ser396, and Ser404. Besides, blocking PAX6 signaling partially mitigates Aβ-induced neuronal death.[133] Besides phosphorylation, Aβ also enhances tau accumulation via GSK3β/SC35 signaling, which activates tau exon 10 splicing and increases intranuclear distribution.[134,135]

The role of $Aβ_{1-42}$ in driving GSK3-mediated phosphorylation of p-tau has been extensively acknowledged. The non-amyloidogenic degradation pathway product of amyloid precursor protein (APP), known as sAPPα, exhibits a protective effect on AD pathophysiology by inhibiting GSK3β and reducing tau phosphorylation,[136,137] suggesting that other intermediate products of APP processing merit further attention in relation to their association with GSK3.

3.2.3 | AMPK-mediated GSK3 signaling in tau pathology

Recent studies have indicated that AMP-activated protein kinase (AMPK) serves as a crucial regulator of bioenergy metabolism and plays a significant role in AD.[138] In various animal models of AD, low AMPK activity has been detected, while artificially increasing AMPK expression or activity has been found to ease tau hyperphosphorylation. Notably, GSK3β agonists wortmannin have been found to limit the positive effects of AMPK on AD, emphasizing the pivotal role of GSK3β in AMPK-mediated suppression of p-tau.[139] Moreover, research has revealed that memory impairment and tau hyperphosphorylation were observed in type-2 cannabinoid receptor (CB2R) KO mice with lower activity of AMPK alongside higher activity of GSK3β.[139] In contrast, selective activation of CB2R was found to limit GSK3β activation and tau hyperphosphorylation; however, this

protective effect was lost when AMPK was simultaneously inhibited.[140] To summarize, the activation of AMPK inhibits GSK3β appears to be the primary mechanism by which CB2R mitigates tau pathology. Adiponectin, a hormone secreted by adipocytes, has been found to function as an euglycemic agent.[141] Long-term adiponectin deficiency has been associated with the induction of tau pathology and other AD pathologies.[142] Mechanistically, adiponectin triggers AMPK phosphorylation, leading to the up-regulation of p-AKT and p-GSK3β^{Ser9}.[142] Hence, adiponectin can effectively restrict tau hyperphosphorylation through the AMPK/AKT/GSK3β signaling pathway. However, it is noteworthy to mention that cerebral hypoglycemia has also been known to trigger tau pathology via the same signaling pathway, namely AMPK/AKT/GSK3β.[143]

3.2.4 | GSK3 signaling mediated by other proteins in tau pathology

In addition to the above few signaling pathways or stimuli, there are numerous proteins that have been reported to directly or indirectly affect GSK3-mediated tau pathology. Firstly, there is a class of proteins that have been found to be abnormally expressed in AD patients or AD animal models, including Deficiency in G protein-coupled receptor-5 (GRK5), Heat shock protein 27 (Hsp27), 3-β-Hydroxysteroid-Δ-24-reductase (DHCR24), Sirtuin 6 (SIRT6), regulator of calcineurin 1 (RCAN1), Cystatin C, and beta-2 adrenergic receptors (β2ARs). Researchers have discovered the abnormality of GSK and p-tau during the investigation of their roles in AD. However, there is currently a lack of evidence to directly support their involvement through the modulation of GSK3 expression or activity. Therefore, further mechanistic research is still required as substantiating evidence. Deficiency in G protein-coupled receptor-5 (GRK5) has been linked with mild cognitive impairment.[144] In GRK5 KO mice, overactivation of GSK3β and tau hyperphosphorylation were observed, thus providing additional evidence of GRK5's inhibitory effect on GSK3β.[145] Heat shock protein 27 (Hsp27) transgenic mice showed a striking pathological phosphorylation of tau, with upregulated p-GSK3β^{Tyr216} and downregulated p-GSK3β^{Ser9} indicating that GSK3β was the immediate cause of p-tau.[146] Increased expression of p44, a short isoform of p53, has been found to elevate the risk of tau pathology.[147] Moreover, haploinsufficiency for p73, a member of the p53 family, has demonstrated a similar phenomenon.[148] Several kinases, among them GSK3β, have been implicated in p44/p73-related tau pathology. 3-β-Hydroxysteroid-Δ-24-reductase (DHCR24), an enzyme that converts desmosterol to cholesterol, has been found to be down-regulated in individuals with AD.[149] Its levels are closely associated with caveolin-1, an intrinsic membrane protein that is responsible for structuring caveolae leading to a key role in PP2A phosphorylation site localization. DHCR24 has been shown to promote the activity of PP2A, resulting in increased levels of p-GSK3β^{Tyr216} and p-tau.[150] Sirtuin 6 (SIRT6), a "longevity gene" that optimizes energy homeostasis, is underexpressed in aging-related diseases such as AD.[151] SIRT6 KO mice have exhibited significant AD-like symptoms and tau hyperphosphorylation caused by GSK3 activation.[152] In patients with AD, the continuous overexpression of the regulator of calcineurin 1 (RCAN1) has been observed to result in the downregulation of Calcineurin, an essential phosphatase that catalyzes tau dephosphorylation. Furthermore, RCAN1 has been found to induce GSK3β-catalyzed tau hyperphosphorylation.[153] Cystatin C has also been observed to be upregulated in the brains of both AD patients and model animals, and its overexpression in neurons has been shown to accelerate tau phosphorylation by impeding the turnover of GSK3β.[154] A similar increase in beta-2 adrenergic receptors (β2ARs) has also been observed, and their stimulation has been found to influence GSK3β and Cyclin-dependent kinase 5 (CDK5) in contributing to tau pathology.[155,156]

Another major category of proteins, known as receptors, have the ability to perceive incoming signals and regulate cellular functions in response to signal stimulation. Several types of receptors have been reported, and their activation or inhibition ultimately influences GSK3-mediated tau hyperphosphorylation. The observation was made that the activation of M1 receptors in 3×Tg-AD mice led to a decrease in GSK3β activity and p-tau levels. It was also noted that administration of an M1 antagonist noticeably aggravated the pathologic phosphorylation of tau.[157] Based on these findings, M1 receptors were believed to play a critical role in the regulation of GSK3β and p-tau. Furthermore, the Alpha7 nicotinic acetylcholine receptor (nAChR) was found to be involved in the regulation of calcium channels, which, when activated, could mitigate tau hyperphosphorylation via GSK3β,[158] while the inhibition of the histamine H3 receptor was shown to relieve GSK3β-related pathologic phosphorylation of tau.[159] Complement C3 receptor has been reported to promote GSK3β activity, while its inhibition has been proven to alleviate tau hyperphosphorylation catalyzed by GSK3β.[160] The nuclear receptor subfamily 4 group A member 1 (NR4A1) is recognized for its positive impact on synaptic plasticity and memory formation.[161] Nonetheless, excessive expression of NR4A1 may activate GSK3β which can subsequently result in tau hyperphosphorylation.[162] Chemokine ligand 11 (CCL11) is a well-established inflammation marker that undergoes upregulation, and CC chemokine receptor 3 (CCR3) is the receptor for CCL11 found in hippocampal neurons.[163,164] CCR3/CCL11 has been associated with elevated p-tau levels, and GSK3β plays a partial role in this mechanism.[164]

Other proteins that influence GSK3-mediated tau pathology exert their effects through mechanisms that primarily involve direct binding to GSK3 or affecting the interaction between GSK3 and tau, as well as directly impacting the phosphorylation activation of GSK3, and so on. Studies have suggested that both GSK3β and tau bind to the same region of PS1, thereby promoting the enzymatic effects of GSK3β in phosphorylating its substrate, tau.[165] Furthermore, the key tau kinase, CDK5, is generally activated by the protein p35. However, the p35 calpain cleavage product, p25, has a higher preference for binding to and activating GSK3β compared to CDK5, which leads to the phenomenon of p-tau enhancement.[156,166] Nedd8 ultimate buster 1 (NUB1) is a protein that is induced by interferon and is known to accelerate the clearance of proteins eliminated by the

ubiquitin-proteasome system.[167] Research indicates that NUB1 has multiple functions in regulating tau pathology, including the aggregation and phosphorylation of tau. Importantly, NUB1 promotes the degradation of GSK3β and also impedes the interaction between GSK3β and tau.[168] Axin restricts the interaction between GSK3β and tau, which prevents GSK3β from pathologically phosphorylating tau.[169] The programmed cell death protein 1/programmed cell death 1 ligand 1 (PD1/PDL1) has established prominence in cancer therapy.[170] Recent studies unearthed a PDL1-GSK3β immune complex within the brains of APP/PS1 and 5×FAD mice, an entity that heightens GSK3β activity and p-tau levels.[171]

The preceding paragraph has indicated the potential of ILK to augment the inhibitory effects on GSK3.[7] In N1E-115 neuroblastoma cells, inactivation of ILK led to the loss of its inhibitory effect on GSK3β, which subsequently caused an elevation in p-tau levels.[172] The previous paragraph has also explained the role of GSK3 in the Wnt/β-catenin pathway. In aged rats, inhibited Wnt signaling by Dkk-1 decreased PP2A activity and increased GSK3β activity, which resulted in excessive tau phosphorylation at multiple sites in the hippocampus.[173] Pin 1 is a promising target for cancer therapy.[174] However, the inhibition of Pin1 in AD amplifies GSK3β activation and tau hyperphosphorylation.[175] It has also been reported that GSK3β affects the proportions of different isoforms of tau mRNA through alternative splicing. Prion protein PrPC serves as an upstream regulator that inhibits GSK3β activation.[176] Furthermore, interferon-gamma (IFN-γ) initiates cellular immunity and triggers tau hyperphosphorylation via GSK3β signaling.[177]

3.2.5 | MiRNA-mediated GSK3 signaling in tau pathology

In brain tissues of AD patients, there is a reduced content of miR-219-5p, which has a negative correlation with both GSK3β and tau-tubulin kinase 1 (TTBK1).[178] The 3′ untranslated region of GSK3β mRNA and TTBK1 mRNA share a base sequence of 5′-GACAAUC-3′, which can bind with miR-219-5p, thereby inhibiting the expressions of GSK3β (instead of the phosphorylation of GSK3β) and TTBK1, as well as the hyperphosphorylation of tau.[178] The most significant miRNA decrease observed in AD patients' neurons is miR-132,[179,180] whose down-regulation is closely linked to the pathological processes of AD, such as the formation of NFTs.[181] Mechanistically, miR-132 can regulate the expression and various post-translational modifications, including phosphorylation and acetylation, and limit the phosphorylation of tau through its negative role in both the transcription and translation of GSK3β.[179] The phenomenon of decreased miR-23b-3p has been observed in the plasma of AD patients, cortex of APP/PS1 mice, brain of SAMP8 mice, and SH-SY5Y/APPswe cells. Delivery of miR-23b-3p into the ventricles of APP/PS1 mouse brains can improve their cognitive deficits, alleviate AD pathology, and especially reduce tau phosphorylation at multiple sites. Mechanistic studies indicate that miR-23b-3p mainly exerts its effects by reducing the levels and activity of GSK3β.[182] Moreover, the LncRNA nuclear paraspeckles assembly transcript 1 (NEAT1) facilitates the transcription of frizzled class receptor 3 (FCR3), thus limiting the phosphorylation of tau by GSK3β.[183]

3.2.6 | Micromolecules and other stimulus mediated GSK3 signaling in tau pathology

Inducers of disease models, environmental pollutants, and certain physical factors like noise pollution are all significant contributors to tau pathology that cannot be overlooked. The link between AD and diabetes is widely recognized, with GSK3 playing a crucial role.[7,184] Glucocorticoids, the key hormone responsible for raising blood glucose levels, have also been reported to activate GSK3β, elevate p-tau levels, and impair memory in db/db mice.[185] As an inducer of sporadic AD models, STZ is frequently used in the modeling of both AD and diabetes mellitus.[186] Experiments have shown that STZ can stimulate tau hyperphosphorylation through the PI3K/AKT/GSK3 signaling pathway.[186,187] Rotenone, a renowned compound extracted from Derris known for its ability to induce apoptosis, is also frequently employed in the establishment of PD models, has been demonstrated to elevate p-tau levels through the activation of GSK3β.[188]

Methamphetamine abuse often leads to dementia, and experimental evidence has shown that methamphetamine contributes to tau hyperphosphorylation through the AKT/GSK3β signaling pathway.[189] Cypermethrin, a pesticide, can up-regulate GSK3β. Initially, cypermethrin blocks the heparin-binding epidermal growth factor (HB-EGF) signaling pathway, which results in neuroinflammation. The excessive generation of IL-1 ultimately increases the levels of GSK3β and p-tau.[190] Furthermore, 3-Nitropropionic acid triggers the mitochondrial origin of oxidative stress, while excessive activation of GSK3β and tau hyperphosphorylation were also observed in the hippocampus and cortex of mice exposed to 3-nitropropionic acid.[191]

Prolonged exposure to arsenic can elevate GSK3β activity and exacerbate tau hyperphosphorylation in SH-SY5Y cells, while multiple GSK3β inhibitors can alleviate the effects of arsenic.[192] Plumbum exposure can also lead to tau hyperphosphorylation, with the activation of GSK3β and CDK5 being the declared pathogenesis.[193] Furthermore, iron accumulation can increase the activities of GSK3β and CDK5, thereby worsening tau pathology.[194] Paricalcitol can alleviate iron accumulation by activating the vitamin D receptor (VDR), thus down-regulating p-GSK3β^{Tyr216} and ultimately preventing hyperphosphorylation of tau at Ser396 and Thr181 sites.[195] Intranasal administration of deferoxamine produces similar effects on GSK3β and tau.[194] Chronic noise exposure has also been linked to tau pathology, with PP2A/GSK3β being under-regulated.[196] Interestingly, B-vitamin deficiency has been found to exhibit a similar function as noise.[197] Moreover, stress responses such as electric shocks and ether anesthesia can exacerbate the pathologic phosphorylation of tau via GSK3β.[198,199]

These studies suggest the crucial significance of maintaining a healthy lifestyle in preventing the occurrence and progression of AD

symptoms. It is also advisable for AD patients to eliminate or minimize negative neurostimulants such as environmental pollutants and drugs while undergoing treatment. Nevertheless, further research is still required to substantiate the direct association between these stimulating factors and GSK3-mediated tau pathology. This is because, based on existing research, their impact on the nervous system is intricate and potentially irreversible, with GSK3-mediated tau pathology likely representing just one of the most overt pathological outcomes.

3.3 | Amyloid pathology

Just like GSK3 is associated with other typical pathological features, amyloid pathology is also known to be linked with it. This pathology occurs due to an accumulation of Aβ, which is cleaved from its precursor protein APP. APP is an integral membrane protein that is abundantly expressed in the synapses of neurons and plays vital roles in synapse formation and repair, anterograde neuronal transport, and iron export.[200] There are three isoforms of APP that mainly concentrate in different tissues or cells. These isoforms are named APP_{695}, APP_{751}, and APP_{770}, based on the number of amino acid residues. The shortest isoform, APP_{695}, lacks a Kunitz-type protease inhibitor sequence in the ectodomain and is the most abundant in brains and neurons.[200,201] However, APP_{751} and APP_{770} are expressed more abundantly in platelets and peripheral cells.[200]

It should be noted that not all cleavage processes of APP lead to the production of Aβ. Depending on whether or not Aβ is produced, cleavage processes can be classified into non-amyloidogenic pathways and amyloidogenic pathways. Figure 4 shows that in the non-amyloidogenic pathway, APP is firstly cleaved by α-secretase to form sAPPα and α-C terminal fragment (CTFα).[200] Unlike Aβ, sAPPα plays crucial roles in neuroprotection, synaptic plasticity related learning and memory,[136] and it is produced more along with increased neuronal activity.[202] CTFα can be further cleaved to P3 and the APP intracellular domain (AICD), catalyzed by γ-secretase.[200] The amyloidogenic pathway, on the other hand, involves β-secretase instead of α-secretase. In this pathway, APP is initially cleaved by α-secretase to form soluble amyloid precursor protein beta (sAPPβ) and β-C terminal fragment (CTFβ). The γ-secretase also catalyzes CTFβ cleavage, leading to the generation of AICD. However, under further processing by γ-secretase at multiple sites, 40 amino acid $Aβ_{1-40}$ and the 42 amino-acid $Aβ_{1-42}$ are ultimately generated.[200] These highly neurotoxic peptide fragments are the inducers of amyloid pathology. Therefore, it is significant to reduce Aβ generation by suppressing β-secretase and γ-secretase activities. Additionally, activating α-secretase could be helpful.

GSK3β is involved in various stages of Aβ formation, both directly and indirectly. These stages include its impact on APP expression, phosphorylation, lysosomal degradation, as well as its influence on α-secretase, β-secretase, and γ-secretase (Figure 4). The association between GSK3 activation and Aβ pathology has been observed in numerous cellular or animal models. CDK5 knockdown in 3×Tg-AD

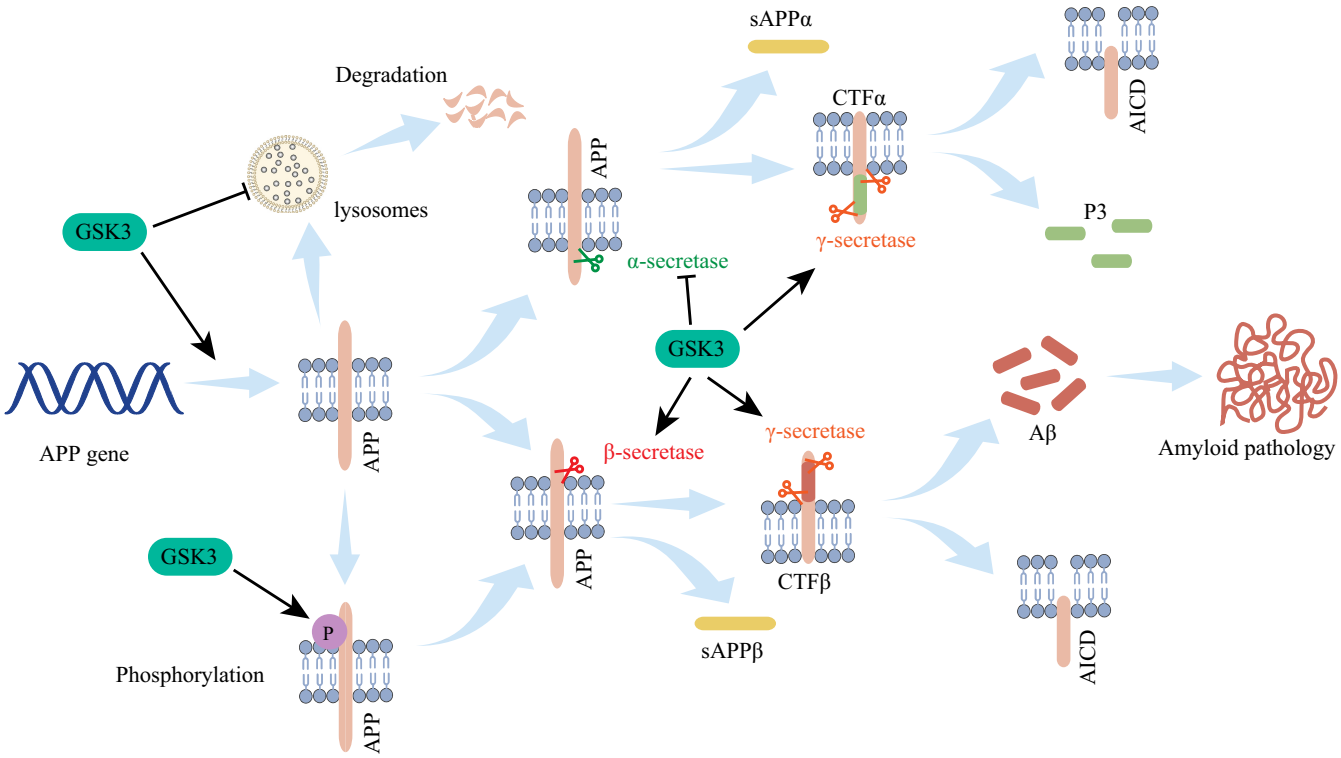


FIGURE 4 The impact of GSK3 on the process of APP cleavage. GSK3 modulates the cleavage of APP by regulating α-secretase, β-secretase, and γ-secretase activities. It also exerts influence over the expression, phosphorylation, and degradation of APP.

mice executed at the age of 15 months, showed significant reduction in amyloid plaques. The activation of GSK3β and PP2A was declared to be the primary reason.[203] The author of this report primarily focuses on the association between CDK5 and GSK3β. Unfortunately, the direct cause of GSK3β activation leading to the formation of amyloid plaques in this one of the most classic AD animal models has not been thoroughly investigated. Nevertheless, GSK3β siRNA was employed to knockdown GSK3β in STZ-induced, in vitro and in vivo models of AD. The remarkable outcomes were overcome down-regulation of Aβ in cell models and mitigation of Aβ accumulation in the cortex and hippocampus of animal models. Inhibition of APP and β-secretase 1 (BACE1) by GSK3β siRNA was found to be the crucial factor contributing to this phenomenon.[204] Conversely, inhibition of β-secretase was observed to stimulate GSK3β and intensify tau hyperphosphorylation.[205] Senescence-accelerated prone mouse strain 8 (SAMP8) mouse model is a form of rapid aging animal models associated with cognitive impairment. In SAMP8 mice, the modification of the PI3K/AKT pathway gradually activated GSK3β with age. Ultimately, both β-secretase and γ-secretase were activated, leading to an increase in the levels of $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ in platelet and hippocampi.[206]

Hence, the impact on β-secretase and γ-secretase appears to be crucial in linking GSK3 with Aβ pathology, and several literature reports provide strong evidence supporting this inference. As a kinase of glycogen synthase, the over-activation of GSK3 is one of the causes of glucose metabolism disorder leading to diabetes. Advanced glycation end products (AGEs) are toxic substances generated in the body after long-term hyperglycemia.[7] Receptor for AGEs (RAGE) had been established to contribute to amyloid pathology. GSK3β and p38 were identified as the connecting links between RAGE signal and Aβ. Gene silencing of RAGE curtailed GSK3β and ameliorated Aβ accumulation, accompanied by attenuated activities of β-secretase and γ-secretase.[207] Through the association of T-cell factor-4 with the BACE1 promoter, the Wnt/β-catenin pathway could impede BACE1 transcription. It must be noted that the GSK3 inhibitor propelled Wnt signaling and concurrently repressed BACE1 transcription.[208]

The transcriptional expression, phosphorylation, and degradation processes of APP also exert an influence on the ultimate accumulation of Aβ, wherein GSK plays a significant role. When comparing gene expression between normal people, patients with mild cognitive impairment, and AD patients, GSK3β expression was positively linked to the expression of Amyloid beta precursor protein binding family B member 2,[209] a transcription activator of APP.[210] In an inflammatory milieu, astrocytes have the potential to transmit CK1 to neurons via extracellular vesicles, which finally exacerbates the formation of amyloid plaques, where GSK3 has a definitive function. More specifically, in response to the stimulation of IL-1β, astrocytes secrete extracellular vesicles that envelop CK1. Then, after the transfer of CK1 to neurons, it engages in a complex with APC and GSK3, which obstructs the degradation of β-catenin. Consequently, this escalates the level of APP mRNA and in turn, intensifies the co-localization of BACE1 and APP. In essence, GSK3 is instrumental in regulating APP expression and amyloid processing through this pathway.[211] Suppressing GSK3β also diminished APP phosphorylation,[212] which might change the APP cleavage process and stop Aβ production.[213] Besides, restraining GSK3 fostered the nuclear translocation of the transcription factor EB, thereby enhancing the number of lysosomes, which ultimately improved APP autophagic degradation, consequently curtailing Aβ level.[214] Apart from curbing Aβ accumulation, GSK3β also influenced the sensitivity of the hippocampus towards Aβ. It was found that the voluntary running of mice prevented their hippocampal network from disturbance caused by Aβ, and this defense was dependent on the inhibition of GSK3β.[215]

3.4 | Oxidative stress and neuroinflammation

Efforts have been made in recent decades to alleviate amyloid pathology and tau pathology in AD, but drug development progress has been slow.[216] Therefore, researchers have also shown great interest in other pathogenesis, such as oxidative stress and neuroinflammation in AD. Elevated reactive oxygen species (ROS), mitochondrial dysfunction, lipid peroxidation, and ferroptosis are common phenomena observed in AD patients or animal models, thus characterizing oxidative stress.[21] Inflammation, as indicated by the high levels of inflammatory cytokines TNF-α, IL-6, and others found in AD patients' serum and brain, has also been linked to AD. The alteration of immune cells, such as reactive astrocytes, T cells, and particularly activated microglia in the brain of AD patients, explains the occurrence of inflammation at the cellular level.[217]

The role of GSK3 in oxidative stress and neuroinflammation has been implicated in its known substrate nuclear factor erythroid 2-related factor 2 (Nrf2), a significant transcription factor that responds to environmental stimuli, particularly elevated ROS.[218] Nrf2 transfers from the cytoplasm into the nucleus to perform its biological functions. The dimerization of the transcriptional co-activator sMaf stabilizes Nrf2 in the nucleus, allowing it to interact with Antioxidant Response Element (ARE) to enhance transcription of various genes (Figure 5), such as heme oxygenase-1 (HO-1), NAD(P)H:quinone oxidoreductase 1 (NQO1), superoxide dismutase (SOD), etc.[219]

As a sophisticated regulator, the content of Nrf2, particularly the content in the nucleus, is tightly controlled by the ubiquitination system. Kelch-like ECH-associated protein 1 (Keap1) functions to concatenate Nrf2 and Cul3-Rbx1, a ubiquitin ligase complex, ultimately leading to ubiquitination and degradation of Nrf2.[220] However, Keap1 is not the only ubiquitination process involved in Nrf2 degradation. As shown in the Figure 5, another ubiquitin ligase complex, β-TrCP-Cul1, is also responsible for Nrf2 ubiquitination and degradation. Crucially, when p-GSK3β^{Y216} phosphorylates Nrf2, it greatly promotes the negative regulatory process of Nrf2.[218] Therefore, suppressing GSK3β is considered a promising therapeutic strategy for AD due to its Nrf2 activation effect. For example, an antisense oligonucleotide of GSK3β that reduced the level of GSK3β in the cortex of SAMP8 mice also exhibited Nrf2 activation and improved learning and memory abilities.[221]

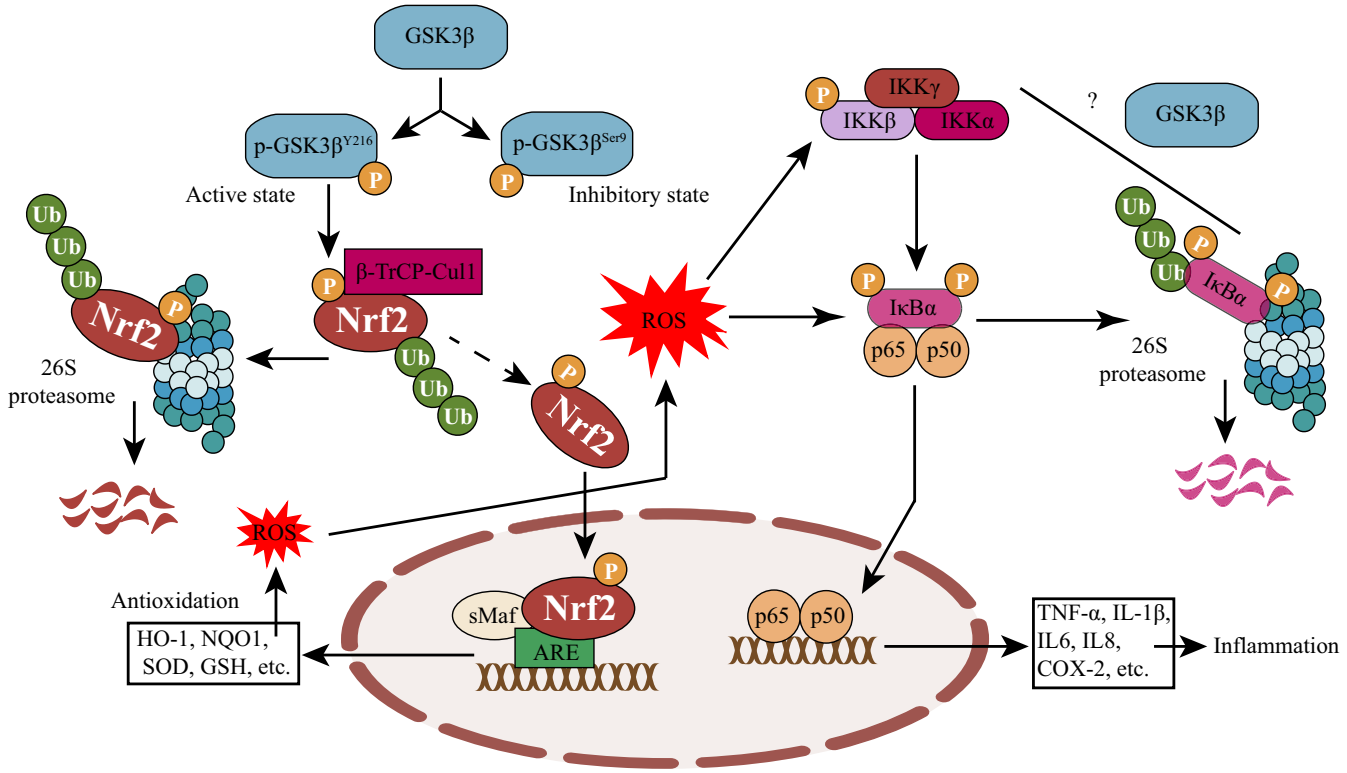


FIGURE 5 Effects of GSK3β on cellular oxidative stress and inflammation via Nrf2 and NF-κB. Activated GSK3β phosphorylates Nrf2, ultimately leading to its ubiquitination and degradation. Consequently, the reduced levels of Nrf2 weaken the transcriptional expression of cellular antioxidant enzymes, resulting in excessive accumulation of ROS. Excessive ROS triggers the degradation of IκBα, leading to the release of NF-κB (p65 and p50) into the nucleus, thereby upregulating the transcriptional expression of pro-inflammatory factors and exacerbating inflammation. However, the direct impact of GSK3β on IKK and IκBα remains uncertain.

GSK3 has also been found to be involved in the muscarinic M1 receptor-induced Nrf2 accumulation in the nucleus. Overexpression of the M1 receptor in PC12 cells enhanced the level of Nrf2, activated the antioxidant response element (ARE), and facilitated the transcription and translation of HO-1. GSK3β(Δ9) nullified the Nrf2-regulated oxidation while GSK3β(Y216F) had little to no effect on it.[222]

The activity of Nrf2 is closely linked with inflammation, largely due to its crosstalk with NF-κB, a well-known regulator of inflammation.[223] As shown in Figure 5, excessive production of ROS would facilitate multiple steps of NF-κB activation, which also relies on upstream regulatory factor IKKα ubiquitination and degradation.[223] Although there is currently no evidence of GSK3 involvement in IKKα ubiquitination, the collective suppression of GSK3β and NF-κB has been observed in some cells and animal models.[224,225] For instance, soluble epoxide hydrolase (sEH) is a promising target for inflammatory and AD treatment.[226] Inhibiting sEH in $A\beta_{1\text{-}42}$ induced AD mice was found to stabilize epoxyeicosatrienoic acids, a series of compounds with anti-inflammatory properties. Interestingly, a high level of p-GSK3β^{Ser9}, accompanied by Nrf2 activation and NF-κB inhibition, was also observed.[227]

From the perspective of signaling pathways, the Nrf2 and NF-κB signaling pathways are crucial for the neuroinflammation caused by excessive activation of GSK3. Meanwhile, from the perspective of neural cells, both microglia and astrocyte activation have been found to be associated with GSK3. Microglia, which are resident immune cells in the brain, release a large amount of inflammatory factors after M1 polarization in pathological conditions. Similarly, activated astrocytes can also release inflammatory factors and chemokines to attract microglia into the damaged area. $A\beta_{1\text{-}42}$ induces neuronal apoptosis and releases phosphatidylserine, thereby activating microglia and promoting neuroinflammation. The GSK3/Wnt signaling pathway plays a crucial role in this process, inhibiting PI3K/AKT to activate GSK3β and facilitate microglial activation.[228] A low level of DHCR24 was accompanied by GSK3β activation and hyperphosphorylation of tau.[149,150] In addition to tau pathology, overexpression of DHCR24 in BV-2 cells reversed the microglia polarization trend induced by $A\beta_{25\text{-}35}$. Increased levels of arginase-1, IL-4, and TGF-β, and decreased levels of iNOS, IL-1β, and TNF-α reflected that DHCR24 promoted M2 polarization of microglia, playing an anti-inflammatory role. The key mechanism is the activation of AKT and thereby the inhibition of GSK3β.[229] GSK3β is also involved in microglial activation via phosphorylation of astrocyte CCAAT enhancer-binding protein delta (CEBPD), a well-known participant in inflammation-related diseases.[230] In astrocytes of AD patients, excessive activation of the calcium-activated potassium channel KCa3.1 was observed. In LPS-induced neuroinflammation mice, the KO of KCa3.1 protected neurons and alleviated cognitive impairment. KCa3.1 deficiency mice showed activation of the PI3K/AKT pathway, suppression of GSK3β, and inhibition of NF-κB signaling.[231] Moreover, in vitro cultures of various glial cells extracted from rat cortex released various inflammatory factors including IL-1β,

TNF-α, and IL-10, which were significantly decreased by different GSK3 inhibitors.[232]

3.5 | Mitochondrial dysfunction

Mitochondria serve as the primary sites for cellular energy metabolism. The brain, being the most metabolically active organ, relies on the abundant ATP produced through mitochondrial oxidative phosphorylation. In the brain of AD patients, noticeable alterations occur in both the structure and function of mitochondria, including changes in glucose and oxygen metabolism due to respiratory chain dysfunction, as well as accumulation of ROS and mutations in mitochondrial DNA.[233]

Under oxidative stress conditions, GSK3β translocates from the cytoplasm to the mitochondria in a kinase activity and voltage-dependent anion channel 2 (VDAC2)-dependent manner. This process is associated with increased cell death and can be blocked by GSK3β inhibitors.[234] Studies have demonstrated that GSK3β inhibition can enhance mitochondrial respiration and membrane potential, while altering NAD(P)H metabolism. These metabolic effects are associated with the increased stability of PGC-1α protein, enhanced nuclear localization, and increased co-transcriptional activation. This suggests that the GSK3β/PGC-1α axis may play an important role in maintaining neuronal metabolic integrity.[235] Dynamin-related protein 1 (Drp1) is a key protein involved in mitochondrial division and is highly expressed in neuronal cells. Research has shown that GSK3β can induce the phosphorylation of Drp1, resulting in mitochondrial fragmentation and increased neuronal sensitivity to Aβ toxicity. In vivo and in vitro models have shown that limiting the phosphorylation of Drp1 induced by GSK3β is an effective way to protect the nervous system from Aβ damage.[236] Axonal transport defects caused by Aβ result in impaired mitochondrial transport, leading to acute impairments in mitochondrial trafficking in Aβ-stimulated hippocampal neurons. Researchers have found that the activation of GSK3β by Aβ is a significant contributor to mitochondrial transport dysfunction.[237] Another study has revealed that GSK3β can co-localize with and regulate HDAC6 phosphorylation in hippocampal neurons. HDAC6 regulates tubulin acetylation and affects the function of the motor protein-1, playing a crucial role in the anterograde transport of mitochondria within axons. Serotonin, a neuromodulator, can enhance mitochondrial transport through AKT-mediated inhibition of GSK3β, indicating that GSK3β may interfere with mitochondrial transport by affecting HDAC6 activity.[238]

3.6 | Autophagy impairment

Autophagy is a cellular catabolic process responsible for breaking down damaged organelles and protein aggregates through the lysosomal pathway. It helps to clear out Aβ and p-tau.[22] The mTOR, which is regulated by GSK3, plays a crucial role in promoting autophagy, thereby mitigating the pathological effects of Aβ and p-tau.[239] In the previous chapter, the relationship between the low expression of DHCR24 and the pathogenesis of AD, specifically tau hyperphosphorylation and microglia M1 polarization was presented. DHCR24 knockdown resulted in overactivation of GSK3β, which is a significant molecular mechanism. Additionally, DHCR24 knockdown SH-SY5Y cells showed autophagy inhibition characterized by decreased autophagosome. Mechanistically, the activated GSK3β phosphorylates mTOR at Ser2448, stimulating it to act as an autophagy inhibitor.[240] PS1 KO human neural stem cells also displayed GSK3β related autophagy suppression.[241]

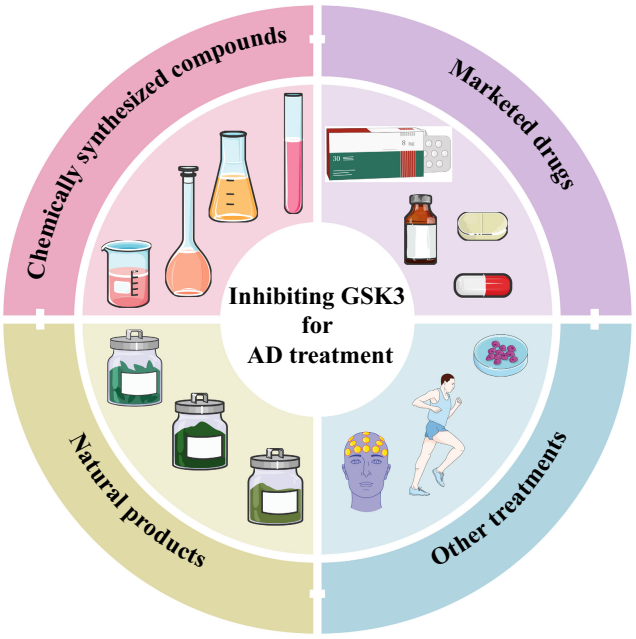


FIGURE 6 Pharmacotherapy and additional interventions for AD employing GSK3 inhibition as a strategic approach.

4 | THERAPEUTIC STRATEGIES FOR AD TARGETING GSK3

As previously stated, the excessive activation of GSK3 in AD patients is closely related to tau phosphorylation, Aβ pathology, neuroinflammation, oxidative stress damage, and other factors contributing to AD. Therefore, targeting GSK3 has been a major focus of AD treatment strategies. In this section, we will systematically summarize preclinical experiments that have exhibited GSK3 inhibition and AD therapeutic effects, including newly developed GSK3-targeting compounds, natural products discovered from traditional herbal medicine, and clinically approved drugs (Figure 6). Meanwhile, we also present an overview of other therapeutic approaches that can impinge upon the activity of GSK3 (Figure 6).

4.1 | Chemically synthesized targeted therapeutics

Using chemical synthesis to obtain compounds targeting the desired protein such as GSK3 has always been a direct and effective

method.[242,243] This article summarizes more than 10 new GSK3-targeting compounds, which have shown certain efficacy in AD model animals. Their chemical structures and biological functions are summarized in Table 1. Compound 47 is an imidazo[1,2-b]pyridazine that exhibits remarkable GSK3β and p-tau inhibition activities, with an IC_{50} of 0.37 nM and 58 nM for GSK3β and p-tau inhibition, respectively.[245] As a designed GSK3β inhibitor, compound 10a has shown comprehensive anti-AD effects in $AlCl_3$ combined with D-galactose induced AD mice, including improved cognitive ability, reduced tau hyperphosphorylation, and lowered hippocampal neuronal damage, among others.[246] In addition to the aforementioned compound designs that target GSK3β alone, many pharmaceutical chemists have shifted their focus towards the design of AD drugs targeting multiple targets, including GSK3β. Compound 11c is a dual inhibitor designed based on GSK3β and AchE, which has shown objective recovery of learning and memory ability in scopolamine- or $Aβ_{1-42}$-induced AD mice. The neuroprotective effects of Compound 11c include inhibiting brain tau phosphorylation and increasing acetylcholine and serotonin levels in the brain.[247] Another GSK3β, AchE dual inhibitor Compound GT15 can reduce tau phosphorylation induced by okadaic acid and ROS induced by LPS in vitro. In in vivo experiments, GT15 can also enhance the learning and memory ability of AD mice.[250] Compound LDC8 is a dual inhibitor of GSK3β and CDK5, which can alleviate neuronal inflammation damage. In the zebrafish model induced by $Aβ_{1-42}$, LDC8 therapy increased synaptic density by 67.6%.[249] Compound 2 is a synthetic BACE1/GSK3β dual inhibitor, which exhibits good inhibitory effects on Aβ formation, alleviating inflammation damage, and promoting neurogenesis.[251]

Regrettably, there is currently limited research on PROTAC targeting GSK3, as well as scarce reports on chemical drug designs focusing on GSKα. PROTAC, which enables targeted compound ubiquitination degradation, represents a novel and promising strategy in drug design. And compounds targeting GSKα provide an alternative option for inhibiting GSK3 in the treatment of AD. Compound PT-65 is a PROTAC, which has strong affinity for both GSK3α and GSK3β. By degradation of GSK3, PT-65 can inhibit tau hyperphosphorylation and protect against cognitive impairments.[253] Compound 1 is a recently reported rare inhibitor specifically designed for GSK3α. This compound and its derivatives designed based on it exhibit a selectivity for GSK3α that is significantly higher than that for GSK3β, up to 37 times higher. In addition, Compound 1 has also demonstrated good activity in inhibiting tau phosphorylation both in vitro and in vivo.[244]

4.2 | Natural products

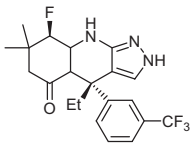
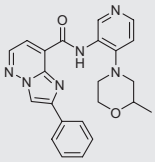
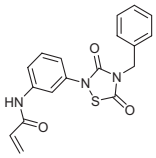
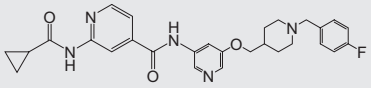
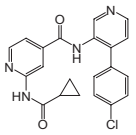
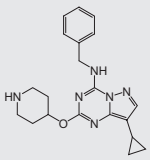
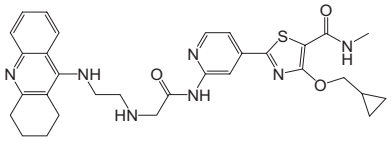
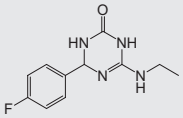
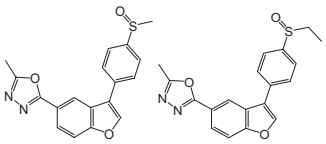
Searching for drugs or lead compounds from natural products has always been an important avenue for drug development. Many researchers have also attempted to identify natural products with therapeutic potential for AD and elucidate their mechanisms, based on the long-term experience of using traditional Chinese medicine or herbal remedies. The involvement of GSK3 in various pathological aspects of AD is evident. It is apparent that these natural compounds exert their effects on GSK3, thereby protecting neurons, alleviating tau pathology, reducing neuroinflammation, and mitigating oxidative stress damage.

By stimulating PI3K/AKT, selenomethionine effectively suppressed GSK3β and triggered Wnt signaling in the hippocampus. This resulted in a significant increase in the number of differentiated neurons from neural stem cells, coupled with an obvious decrease in astrocytes.[255] In separate studies, 20(S)-protopanaxadiol and oleanolic acid improved the learning and memory abilities of APP/PS1 mice by inhibiting GSK3β and activating Wnt/β-catenin in the hippocampus, ultimately enhancing neurogenesis.[256] Similarly, icarisid II exerted a similar molecular mechanism and promoted the proliferation of neural stem cells and neuronal differentiation, leading to considerable neurogenic benefits.[257,258]

Morin is a natural flavonoid that impacts tau hyperphosphorylation by inhibiting GSK3β, as suggested by both the crystal structure of complex (PDB: 1H8F) and surface plasmon resonance result.[259] Moreover, it has been observed that morin binds to the ATP binding pocket of GSK3β with a 67 μM dissociation constant value.[260] Ginsenoside Rg1 has shown significant efficacy in various animal models of dementia, such as OKA-induced AD rats, aluminum chloride-induced AD mice and Squirrel monkeys.[261–263] Although the detailed mechanisms revealed in different reports are not completely the same, Ginsenoside Rg1 is believed to inhibit GSK3 activity in all cases. For instance, in the AD mouse model, the inhibition of GSK3 by Ginsenoside Rg1 is accompanied by a decrease in the level of PP2A. In the Squirrel monkey model, the author suggests that the Wnt/GSK-3β/β-catenin pathway is the main signal pathway under regulation, and that neurocyte apoptosis, oxidative stress damage and inflammatory reactions are all inhibited.[261–263] Along similar molecular mechanism, ginsenoside Rd significantly attenuated tau pathology.[264] Lignans obtained from Schisandra chinensis Turcz. (Baill.) have a novel dibenzocyclooctadiene skeletal structure and considerable biological activities.[223] Among them, schisandrin B and schisantherin B exhibited substantial potential in alleviating tau pathology, and their roles in inhibiting GSK3β were also identified.[265,266] Furthermore, several other natural products including paeoniflorin,[267] genistein,[231] berberine,[268] isobavachalcone,[269] moscatilin,[270] xanthohumol,[271] resveratrol,[272] fisetin,[273] C-glycosylflavones,[274] geniposide,[275–277] ginkgolide A,[278] caffeic acid,[279] asiatic acid,[280] ellagic acid,[281] etc., also played crucial roles in inhibiting GSK3 and mitigating tau hyperphosphorylation, and the specific molecular mechanisms were illustrated in Figure 3.

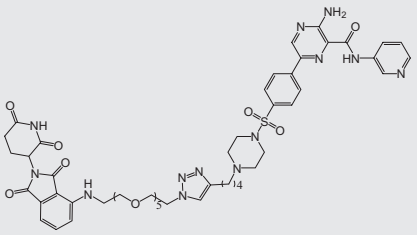
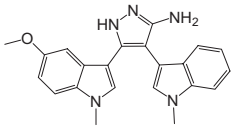
Certain natural compounds have demonstrated favorable anti-inflammatory and antioxidant activities in both in vivo and in vitro models of AD. Oxyphylla A effectively suppressed high ROS levels in N2a/APP cells, enhanced the learning and memory abilities of SAMP8 mice, and mitigated oxidative stress damage. Its effect on the GSK3β/Nrf2 pathway was identified as the key underlying molecular mechanism, whereby oxyphylla A triggered AKT phosphorylation, leading to the inactivation of GSK3β and ultimately activating Nrf2.[282] Similarly, thymol and artemether, two other

TABLE 1 Information on chemically synthesized GSK3 inhibitors.

Compounds	Models	Biological activities	Ref
 Compound 1	Rats at post-natal day 10	Alleviate tau hyperphosphorylation	244
 Compound 47	3×Tg-AD mice	Alleviate tau hyperphosphorylation	245
 Compound 10a	$AlCl_3$ combined with D-galactose induced AD mice	Improve learning and memory Alleviate tau hyperphosphorylation Reduce hippocampal neurons damage	246
 Compound 11c	Scopolamine induced ICR mice $A\beta_{1\text{-}42}$ induced ICR mice $A\beta_{1\text{-}42}$ induced SD rats	Improve learning and memory Inhibit AChE activity in brain	247
 Compound 33	3×Tg-AD mice	Alleviate tau hyperphosphorylation	248
 Compound LDC8	$A\beta_{1\text{-}42}$ induced zebrafish	Reduce synaptic degeneration	249
 Compound GT15	Scopolamine induced ICR mice	Improve learning and memory Alleviate tau hyperphosphorylation	250
 Compound 2	H4-APPswe cell line LPS induced primary rat microglia and astrocyte cells	Reduce inflammation of nerve cells	251
 Compound (S)-9b and (S)-9c	Cold water stress mice	Alleviate tau hyperphosphorylation	252

(Continues)

TABLE 1 (Continued)

Compounds	Models	Biological activities	Ref
 Compound PT-65	$A\beta_{1-42}$ induced SH-SY5Y cells okadaic acid induced SD rats	Relieve cell damage Alleviate tau hyperphosphorylation Improve learning and memory	253
 Compound 6h	LPS induced C57/B6J mice	Reduce microglial activation and astrocyte proliferation	254

natural products also activated Nrf2, albeit through different pathways. Thymol achieved GSK3β/Nrf2 activation via AKT, while artemether acted via AMPK.[283,284] Eupatin is a natural substance that has the ability to bind with GSK3β and hinder its activity. In mouse microglia cells and macrophages that were treated with lipopolysaccharides (LPS), eupatin was observed to diminish both the overall content as well as the level of p65 phosphorylation.[224] Anthocyanins were also investigated for their effects on LPS-induced neuroinflammation mice. It was discovered that, besides regulating AKT, GSK3β, and NF-κB, anthocyanins were able to decrease ROS in the hippocampus of these mice.[285] Additionally, another study reported that in APP/PS1 mice, anthocyanins exhibited antioxidant abilities through the GSK3β/Nrf2 pathway.[286] Neuroprotectin D1 is a pro-resolving mediator produced from docosahexaenoic acid in neuronal cells. In N2a/APPswe cells, neuroprotectin D1 inhibited GSK3β and led to enhanced autophagy with decreased expression of beclin 1.[287] Amentoflavone has been found to inhibit $A\beta_{1-42}$-induced neuronal apoptosis both in vitro and in vivo. In vivo studies have shown that Amentoflavone can increase neuronal activity in the hippocampal region of AD rats and alleviate cognitive impairment at an epigenetic level. In vitro experiments using SH-SY5Y cells with AMPKα knockdown demonstrated the importance of AMPK in the biological functions of Amentoflavone. Mechanistically, amentoflavone acting via the AMPK pathway inhibits GSK3β and exerts anti-apoptotic and anti-AD effects.[288] Meridianins are indole alkaloids isolated from marine ascidians, which display remarkable cognitive improvement in 5×FAD mice by inhibiting GSK3β and rescuing synaptic loss as well as suppressing neuroinflammation.[289]

As mentioned in paragraph 4 of Section 3.3, GSK3β is also involved in glucose metabolism, and AGEs are formed after chronic hyperglycemia.[7,290] AGEs increased GSK3β activity and tau phosphorylation in female APP/PS1 mice. Treatment with trehalose mitigated the negative effect of AGEs through down-regulation of GSK3β and triggered nuclear translocation of transcription factor EB (TFEB).[291] Similarly, Calycosin exhibits a neuroprotective effect against AGEs-induced neurotoxicity by inhibiting GSK3β. In a rat model of diabetes complicated with AD, Calycosin improves learning and memory, and reduces AD-related pathologies such as neurofibrillary tangles and amyloid deposits, as well as mitochondrial dysfunction.[292]

Finally, other natural products such as galangin,[293] naringenin,[294] wogonin,[295] curcumin,[296] and linarin[297] have exhibited certain GSK3 inhibitory activity and neuroprotective effects related to AD in vitro. However, these effects have not yet been validated by in vivo experiments.

4.3 | Marketed drugs

The secondary development of clinical drugs is an important aspect that cannot be ignored, with aspirin being the classic success story in this regard. Hence, many drugs already on the market have been investigated for their potential to relieve AD pathology related to GSK3β. The antidepressant fluoxetine inhibited GSK3β activity through PP2A, thereby activating the Wnt/β-catenin pathway. As a result, 3×Tg-AD mice treated with fluoxetine exhibited reduced neuronal apoptosis, synaptic damage, and Aβ accumulation.[298] Sodium selenate also activated the Wnt/β-catenin pathway via PP2A and GSK3β and effectively alleviated the symptoms of 3×Tg-AD mice.[299] Rapamycin is a well-known mTOR inhibitor and autophagy inducer with proven efficacy in clearing toxic proteins such as Aβ and p-tau. Researchers have found that Rapamycin can increase Wnt3a expression, resulting in GSK3β inhibition and increased β-catenin, thereby improving AD pathology.[300]

Lithium is a recognized inhibitor of GSK3 and is one of the most widely used mood stabilizers for the treatment of bipolar affective disorder. It has also been attempted to treat various GSK3-related diseases.[2,301] The relationship between lithium and GSK3, as well as its role in AD, have also been reported. For instance, lithium can dose-dependently reduce the mRNA levels of GSK3 in the cerebral cortex and hippocampal neurons of rats, and its inhibition of

GSK3β can promote AchE degradation by proteasomes and activate cholineacetyltransferase. In animal models of AD, Lithium has also been demonstrated to alleviate scopolamine-induced memory impairment.[302,303] LiCl also improved the level of Nrf2 in APP/PS1 mice, upregulated the levels of antioxidant enzymes such as SOD and GSH-Px, downregulated the level of lipid peroxidation product MDA in both brains and serum, further indicating the antioxidative effects of LiCl via the GSK3β/Nrf2 pathway.[304] A retrospective cohort study identified a correlation between the usage of lithium and a reduced risk of developing dementia. This clinical evidence validates the role of lithium and further supports the potential of GSK3 as a therapeutic target for AD.[305]

Melatonin, an amine hormone produced by the pineal gland, has gained recognition for its hypnotic effects, although it is currently only approved as a drug in some countries. Melatonin can inhibit GSK3β through the PI3K/AKT signaling pathway and exert a series of anti-AD effects such as inhibiting p-tau.[306] The GSK3β inhibition effect of melatonin can also lead to the downregulation of ADAM10 through the NF-κB signal and the upregulation of BACE1 and PS1.[307] In N2a cells induced by okadaic acid, the addition of melatonin can decrease GSK3β mRNA levels, increase nuclear localization of Nrf2, and reduce the expression of many anti-inflammatory factors.[308] Overall, melatonin may play a significant role in inhibiting both the expression and phosphorylation activation of GSK3β.

Moreover, certain drugs have exhibited promising results in alleviating GSK3-induced tau hyperphosphorylation. Figure 3 compiles these drugs, primarily medications for neurological disorders such as agomelatine,[309] risperidone,[310] escitalopram,[311,312] valproate,[313] and cerebrolysin.[314] It also includes other classes of drugs such as dulaglutide,[315] lixisenatide,[316] atorvastatin,[317] sildenafil,[318] 17beta-estradiol,[319] allantoin,[320] and sodium 4-phenylbutyrate.[321]

4.4 | Other treatments

Mesenchymal stem cell transplantation is a clinically objective and low-side-effect approach for treating diseases. In attempts to treat AD with mesenchymal stem cell transplantation, researchers have discovered numerous effects of the approach, including promoting lysosomal-autophagic clearance of Aβ and p-tau aggregates, inducing microglial M2 polarization, reducing MDA levels, enhancing neuronal dendrite growth, and reducing synaptic losses, among others. Mechanistically, mesenchymal stem cells regulate miR-134 levels by activating SIRT1, thereby inhibiting GSK3β.[322] In clinical-grade human umbilical cord mesenchymal stem cells (hUC-MSCs), significant restoration of cognitive function was observed in SAMP8 mice. In an AD cellular model, hUC-MSCs secreted hepatocyte growth factor (HGF) that targeted the cMet-AKT-GSK3β signaling pathway, downregulated hyperphosphorylated tau protein, reversed spine loss, and increased synaptic plasticity.[323] In another study using human fetal neuroepithelial cells (hNSCs) obtained from the endbrain of human fetuses aborted at 13 weeks and implanted in AD mice, TrkA/B phosphorylation in AD mice was significantly increased, which led to increased phosphorylation levels of AKT and GSK3β(Ser9). The inhibition of GSK3β was accompanied by decreases in p-tau, BACE1, Aβ, deactivation of microglia, and reduced inflammatory cytokine secretion.[324]

Exogenous administration of BDNF or neurotrophic peptidergic compounds is another emerging therapeutic approach for AD. Injection of BDNF into the hippocampus can enhance learning and memory in Okadaic acid-induced AD rats. Mechanistically, BDNF is thought to act on the PI3K/AKT pathway, inhibiting GSK3β activity and down-regulating the phosphorylation of tau protein at multiple sites, including p-tau (Thr231), and p-tau (Ser396/404).[325] A neurotrophic peptidergic compound, known as P021, has been reported to promote BDNF expression and exhibit a significant inhibitory effect on GSK3β in 3×Tg-AD mice. At the epigenetic level, P021 improves cognitive impairment, reduces tau hyperphosphorylation and amyloid plaque deposition, and promotes neurogenesis and synaptic plasticity in AD mice.[326] (D-Ser2)Oxm is a peptide hormone and growth factor similar to Oxyntomodulin that activates glucagon-like peptide 1 and glucagon receptors. In APP/PS1 mice, the administration of (D-Ser2)Oxm significantly increased p-PI3K and p-AKT expression in the hippocampus while reducing p-GSK3β-Y216 levels. Based on this potential mechanism, (D-Ser2)Oxm not only alleviated working memory and long-term spatial memory impairments, but also reduced the number of Aβ plaques in the hippocampus and reversed inhibition of LTP at the hippocampal synapses.[327]

Electroacupuncture has a long history in traditional Chinese medicine and recent years have witnessed an increasing focus on its role in regulating nerve signal transduction and related diseases.[328] In experiments involving APP/PS1 mice, electroacupuncture treatment at Baihui (GV20) and Yintang (GV29) acupoints has been shown to improve cognition while observably increasing p-AKT^{Ser437}, p-GSK3β^{Ser9}, and p-tau, emphasizing the importance of electroacupuncture on AKT/GSK3β/tau signaling.[329] Additionally, high-frequency (50 Hz) electroacupuncture at GV20 and BL23 was found significantly enhancing learning and memory ability in AD rats with $A\beta_{1\text{-}42}$ induction. This treatment also inhibited GSK3β, leading to increased synaptic curvature, reduced synaptic cleft width, increased postsynaptic density, and apparent neuroprotective effects against neuronal damage.[330] Moreover, for D-galactose-induced AD rats, preventive electroacupuncture at GV20-BL23 acupoints resulted in GSK3β inhibition, down-regulation of p-mTOR, and ultimately enhanced autophagy.[331] Regular physical exercise is beneficial for human health, and running is considered an effective way to combat the AD process. In APP/PS1 mice, treadmill exercise for 5 months has been shown to inhibit GSK3 and p-tau but have no effect on CDK5.[332]

5 | DISCUSSION AND PERSPECTIVES

GSK3 is a serine/threonine kinase that comprises two isoforms, GSK3α and GSK3β, and serves as a fundamental regulatory factor in many vital life processes owing to its ability to phosphorylate

numerous substrate proteins. Clinical evidence, AD animal models, and some AD related cell models all demonstrate varying degrees of GSK3 abnormalities. On one hand, the expression levels of GSK3 in the brains of AD patients and AD model animals are significantly higher than those in normal individuals and animals. On the other hand, these highly expressed GSK3 also exhibit relatively higher levels of activation, most notably reflected in the reduced proportion of inhibitory states (p-GSK3αSer21 and p-GSK3βSer9).

The abundant expression of GSK3 and the reduced proportion of its inhibitory states play various significant negative roles in AD. Firstly, neuronal damage (Figure 2) includes neuronal proliferation decrease, neuronal maturation retardation and loss of immature neurons, as well as abnormalities in synaptic, axonal, dendritic spine structures, and functions. Importantly, as a kinase, GSK3 can catalyze phosphorylation at approximately 30 sites in tau. The relationship between GSK3 and p-tau is most widely reported, with various AD-related pathologies such as Aβ or signaling pathways such as PI3K/AKT signaling and AMPK signaling ultimately leading to GSK3-mediated tau hyperphosphorylation (Figure 3). Additionally, GSK3 is involved in various processes of APP formation of Aβ (Figure 4), promoting the expression of the APP, inhibiting lysosomal degradation of APP, leading to an increased potential for the formation of Aβ precursor protein. Phosphorylation of APP, inhibition of α-secretase, activation of β-secretase and γ-secretase directly advance the process from APP to Aβ. GSK3 is also involved in oxidative stress and neuroinflammation in AD mainly through Nrf2 signaling and NF-κB signaling (Figure 5). GSK3 primarily increases the ubiquitination and degradation of Nrf2 through phosphorylation it, while its effects on NF-κB are reported to be indirect, such as through the impact of increased ROS. Other AD-related pathological processes such as mitochondrial dysfunction and autophagy impairment also involve the participation of GSK3.

There have been many studies on GSK3 inhibitors, but most of them are preclinical studies. Even the well-known GSK3 inhibitor lithium lacks sufficient clinical evidence to prove its effectiveness in treating AD. Therefore, many synthetic compounds not only target GSK3 as a single target, such as GSK3β/AchE inhibitors and BACE1/GSK3β inhibitors, have shown certain anti-AD effects in vivo and in vitro. Many natural products can inhibit GSK3 activity, but most lack sufficient experimental evidence to prove their direct inhibitory effects. Morin is the only one reported to bind to GSK3β and inhibit its activity, thus showing anti-AD effects. In terms of marketed drugs, besides lithium, melatonin, escitalopram, and others have been reported to have some impact on GSK3 activity in AD. In addition to drug treatments, alternative therapies such as electroacupuncture and emerging therapeutic approaches like mesenchymal stem cell transplantation may also have positive effects on AD, possibly related to GSK3.

Considering the lack of clinically proven effective GSK3-targeted drugs for AD, we present some perspectives and recommendations herein with the hope of facilitating future research related to GSK3 and AD.

1. Differences in functionality between GSK3α and GSK3β. The cellular localization and brain distribution of GSK3α and GSK3β differ, hence their roles in AD may also vary. Drug development research has primarily concentrated on the functions and inhibition of GSK3β, as the crystal structure of GSK3α remains unclear. Nevertheless, recently designed compounds targeting GSK3α have shown significant therapeutic effects on AD. The aforementioned evidence suggests that researchers now need to give equal attention to GSK3α as they do to GSK3β.
2. The relationship between conformational changes in GSK3 and its substrate selectivity. The conformational transitions of GSK3 play a decisive role in the execution of its functions. However, current research has only focused on the activation or inhibition of GSK3 activity. Clarifying the relationship between conformational changes in GSK3 and its substrate selectivity can aid in the more targeted design of therapeutic drugs targeting GSK3 and help avoid unnecessary side effects.
3. The role of GSK3 in the crosstalk among various pathologies in AD. It is widely acknowledged that complex crosstalk exists between different pathologies in AD. While GSK3 has been shown to exacerbate multiple AD pathologies, its role in the crosstalk among these pathologies remains partially understood. For instance, while it is known that Aβ activates GSK3β indirectly by activating α2AAR, the feedback regulatory effect of p-tau on GSK3 remains unclear. The role of GSK3 in the crosstalk between oxidative damage and neuroinflammation has been partially elucidated (Figure 5). However, it is yet to be explored whether GSK3 has a direct activating effect on NF-κB or if it exerts an indirect influence solely through the regulation of ROS.
4. The role of GSK3 in other neuronal cells apart from neurons. Existing research has found that GSK3 is overexpressed or hyperactivated in the entire brain region of AD patients or animal models. However, current studies primarily focus on GSK3 in neurons. As research progresses, increasing evidence suggests that other types of neural cells, such as microglia, astrocytes, and oligodendrocytes, also play a crucial role in the occurrence and development of AD. Therefore, it remains to be explored whether there are significant changes in the expression levels or activity of GSK3 in these neural cells during AD, as well as the impact of GSK3 on their respective unique functions.
5. Novel GSK3 inhibitors designed using updated methodologies. Present chemically synthesized GSK3 inhibitors mainly focus on inhibiting target proteins by combining them. PROTAC technology that prioritizes ubiquitination and degradation of target proteins is becoming more widely used as drug chemistry advances. Currently, only one study has been reported on GSK3β-treated AD using PROTACs. However, we believe this is an excellent attempt, and we anticipate that novel structured GSK3 inhibitors will exhibit potential in anti-AD medication.
6. Combination therapy of GSK3 inhibitors with other drugs. Considering that existing GSK3 inhibitors are still in the preclinical stage, there may be limitations in developing therapeutic drugs solely targeting GSK3. Furthermore, certain compounds targeting

both GSK3 and other proteins such as AchE, CDK5, and BACE1 have shown potential for AD treatment. These findings provide some insights into combination therapy for AD, exploring whether the strategy of using GSK3 inhibitors in conjunction with existing AD drugs like donepezil and lecanemab can better control the progression of AD. This represents a meaningful endeavor.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this review article as no new data were created or analyzed in this review manuscript.

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