

Review

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Review

RhoA/ROCK/GSK3 β Signaling: A Keystone in Understanding Alzheimer's Disease

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Abstract: Alzheimer's disease (AD) is a neurodegenerative disease characterized by progressive cognitive decline and loss of neuronal integrity. Emerging evidence suggests that RhoA, Rho-associated coiled-coil kinase (ROCK), and their downstream effector molecule glycogen synthase 3 β (GSK3 β) interact within a complex signaling pathway (RhoA/ROCK/GSK3 β) that plays a crucial role in the pathogenesis of AD. RhoA, a small GTPase, along with its downstream effector, ROCK, regulates various cellular processes, including actin cytoskeleton dynamics, apoptosis, and synaptic plasticity. GSK3 β , a serine/threonine kinase plays a key role in neuronal function and AD pathology including regulation of tau phosphorylation, amyloid-beta cleavage. Overactive GSK3 β has been closely linked to tau hyperphosphorylation, neurodegeneration, and the progression of AD. Thus GSK3 β has been considered as a promising therapeutic target for treating AD and mitigating cognitive impairment. However, clinical trials of GSK3 β in AD have faced considerable challenges due to the complexity inhibition of GSK3 β and neuronal specific. In this review, we summarize the literature regarding the relationship of RhoA/ROCK and GSK3 β signaling pathways in AD pathogenesis. We further discuss recent findings of sTREM2 -transgelin-2 (TG2) axis as the potential mediators of this complex pathways and provide our review on a novel targeting strategy for AD.

Keywords: GSK3 β ; RhoA; ROCK; RhoA/ROCK; Alzheimer's Disease; sTREM2; Transgelin-2

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disease characterized by the accumulation of amyloid-beta (A β) peptides and neurofibrillary tangles (NFTs) induced by the hyperphosphorylation of tau proteins [1]. AD is the most common form of dementia and constitutes 60-70% of cases of senile dementia [2]. Prevalence of AD increases with age, and current treatment is mostly ineffective in preventing disease progression [3]. The etiology and pathogenesis of AD is complex and is dependent on interactions between cell types in the central nervous system (CNS) and multifaceted signaling pathways that lead to tau hyperphosphorylation [4–8]. Inhibiting GSK3 β to activate the Wnt/ β -catenin pathway has been proposed as promising therapeutic avenue and is currently under many active investigations; however, targeting the GSK3 β pathway for AD has presented challenges hitherto in clinic due to small-molecule inhibitor specificity [8]. In parallel, the Ras homolog family member A (RhoA)/Rho-associated coiled-coil containing protein kinase (ROCK) has emerged as a significant regulator of AD and been proposed, as well, as a promising therapeutic avenue with unclear mechanisms being defined [9]. In this review, we summarize the to-date investigations of each of these two major pathways and present our view on the potential complex cross-talk between RhoA/ROCK and GSK3 β pathways to regulate tau phosphorylation and current knowledge gap. Signaling by soluble Triggering Receptor Expressed on Myeloid Cells 2 (sTREM2) and its ligand transgelin-2 (TG2) is newly described pathway that plays a critical role in AD

pathogenesis [10]. We also presented our view on how STREM2/TG2 axis may modulate RhoA/ROCK and GSK3 β in regulating tau phosphorylation and serve as a viable therapeutic target.

RhoA/ROCK Pathway in Neuronal Regulation

RhoA is a small GTPase protein in the Rho family of GTPases composed of 3 isoforms, RhoA, RhoB, and RhoC [11]. RhoA transduces signals and mediates various cellular processes including cell migration, gene expression, and vesicle trafficking [12–14]. RhoA is abundant in smooth muscle cells, neurons, and immune cells [15–17]. RhoA regulates various cellular activities, including cytoskeleton modulation, cell death, mitochondrial homeostasis, autophagy, inflammation, and gene transcription [18]. In the brain, RhoA plays a role in regulating neuronal development, synaptic plasticity, and the progression of neurodegenerative diseases [16,18,19]. The RhoA/ROCK pathway specifically inhibits many of these processes [20,21]. Following brain injury, RhoA has been found to be upregulated and activated, resulting in growth cone collapse and failed axon regeneration [22,23].

RhoA activity is controlled by four main regulatory proteins: guanine nucleotide exchange factors (GEFs), GTPase activating proteins (GAPs), guanine nucleotide dissociation inhibitors (GDIs), and GDI dissociation factors (GDFs) [24]. Over 70 distinct GEFs for Rho GTPases have been identified in mammals [25]. GEFs act as positive regulators of RhoA by exchanging GDP for GTP and activate RhoA by dissociating GDP from the GDP-RhoA complex; this unbound RhoA is subsequently able to bind to GTP [25,26]. While around 80 distinct RhoGAPs have been found in mammals, only a small number of GAPs have been shown to be specific for certain GTPases within the Rho family [24]. RhoGAPs specific for RhoA, but not RhoB or RhoC, have not yet been discovered [24,25]. GAPs act as negative regulators of RhoA by increasing the intrinsic rate of GTP hydrolysis in GTP-bound RhoA [27]. The mechanism by which the intrinsic rate of GTP hydrolysis varies across the diverse pool of GAPs, but RhoGAPs specifically have been found to contain a characteristic RhoGAP domain capable of binding to Rho GTPases and promoting GTP hydrolysis [25,26].

Unlike RhoGEFs and RhoGAPs, only three isoforms of RhoGDIs have been found in mammals [28]. GDIs act as negative regulators of RhoA by inhibiting the dissociation of guanine nucleotides (i.e., GDP) and preventing the loading of GTP to the GDP-RhoA complex [29]. However, they do not inhibit the loading of GDP/GTP to nucleotide-free RhoA [30,31]. RhoGDIs were also found to inhibit RhoA activity, preventing intrinsic and GAP-stimulated GTP hydrolysis [32]. Another function of RhoGDIs is as a chaperone for inactive RhoA. GDIs keep GTPases inactive in the cytosol, where 90–95% of Rho proteins in the cell reside at any given time [33,34]. This chaperone function enables inactive RhoA to be rapidly deployed to any membrane in the cell for activation in response to cellular signals [28]. An additional class of positive regulatory proteins of RhoA are known as GDFs. GDFs have been proposed to catalyze the dissociation of RhoGTPase-GDI complexes, thus allowing for activation of Rho GTPases [28,35].

In addition to the various types of Rho GTPase regulatory proteins, various post-translational modifications (PTMs) can regulate RhoA activity, such as prenylation and phosphorylation [18]. Prenylation is required for RhoA activation, as RhoA must be bound to cellular membranes to be activated [36]. Prenylation of RhoA results in modifications that increase protein hydrophobicity and facilitate membrane association [37]. Moreover, numerous kinases phosphorylate RhoA, the most well documented being protein kinase A (PKA) and cyclic GMP-dependent protein kinase (PKG), both of which mediate the phosphorylation of Ser188, a residue close to the prenylated cysteine residue of the C-terminal essential for RhoA membrane binding [38–40]. The phosphorylation negatively regulates RhoA activity by preventing dissociation of GDP-bound RhoA from RhoGDIs [41]. Ser188 phosphorylation has also been implied to inhibit RhoA binding to ROCK but not to other effector proteins, effectively inhibiting the RhoA/ROCK pathway [42].

ROCK is a key regulator of the cytoskeleton and impacts various cellular functions, such as cell shape, motility, proliferation, and gene expression [43]. ROCK has 2 isoforms: ROCK-I and ROCK-II [44]. ROCK-I is prominently expressed in non-neuronal tissues such as the liver, testis, and kidney. ROCK-II is mostly expressed in the brain and skeletal muscle [44]. ROCK contains an N-terminally

located kinase domain, coiled-coil domain, and a Rho-binding domain (RBD). The switch regions of activated RhoA can bind to the RBD to activate ROCK [45]. At the C-terminus, there is a pleckstrin homology (PH) domain containing a cysteine-rich C1 domain [46]. The PH-C1 tandem has two functions. The tandem inhibits ROCK by sequestering its N-terminal kinase domain and reducing its kinase activity. The tandem also facilitates binding to membrane bilayers [47]. ROCK is the most widely studied downstream effector molecule of RhoA and is activated when bound to GTP-bound RhoA [48,49]. RhoA activates ROCK through a mechanism known as derepression, where the PH-C1 tandem-induced autoinhibition of the kinase domain is relieved, leading to an active kinase domain [50]. Other mechanisms of ROCK activation have been alleviated, such as the binding of arachidonic acid to the PH domain or cleavage of the carboxyl-terminus by granzyme B or caspase-2/3 [51,52] [Figure 1].

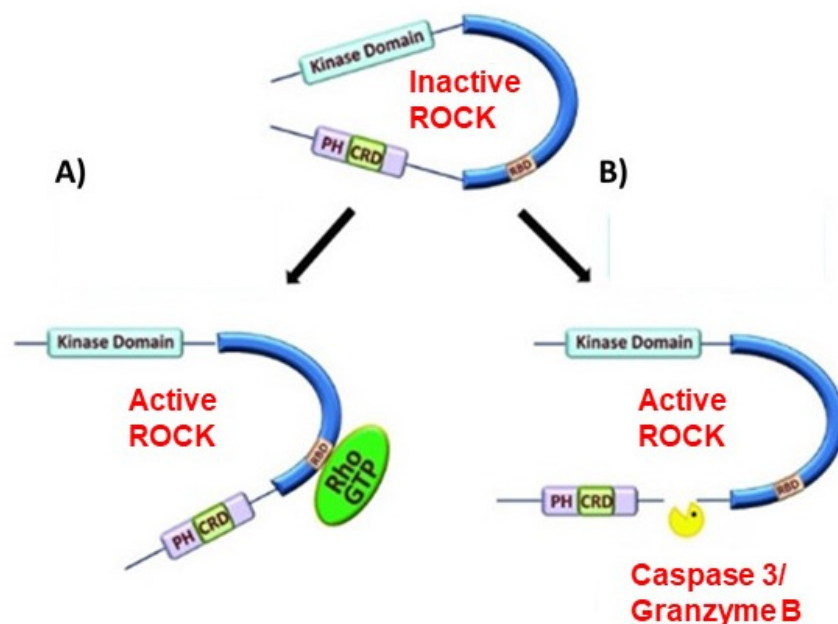


Figure 1. Activation pathways of ROCK. (A) Rho GTPase-dependent activation of ROCK through binding to the RBD. (B) Rho GTPase-independent activation of ROCK through Caspase 3 or Granzyme B cleavage of the carboxyl terminal region of ROCK. Images A and B were adapted from Julian, L., & Olson, M. F. (2014). Rho-associated coiled-coil containing kinases (ROCK): Structure, regulation, and functions. *Small GTPases*, 5(2). <https://doi.org/10.4161/sgtp.29846>.

RhoA/ROCK signaling has been linked to AD risk factors, mainly tau hyperphosphorylation, synaptic damage, A β aggregation, and neuroinflammation [49]. When activated, RhoA/ROCK signaling pathways phosphorylate and activate downstream effectors that are also involved in regulating neuronal processes such as axonal guidance and regeneration and dendritic spine formation [53]. RhoA/ROCK phosphorylates various substrates, including Lim kinase (LMK), myosin light chain (MLC), and collapsing response mediator protein-2 (CRMP-2), which inhibit axonal growth [54]. Activated ROCK also stimulates actomyosin contractility by decreasing myosin phosphatase activity, MLC2 phosphorylation and resulting in spine shortening and retraction [55]. ROCK-I and ROCK-II differentially regulate actomyosin organization in influencing synaptic polarity [56]. ROCK-I is thought to be responsible for actin cytoskeleton destabilizing through regulating MLC2 phosphorylation while ROCK-II is required for stabilizing the actin cytoskeleton by regulating cofilin phosphorylation [57]. The RhoA/ROCK pathway indirectly activates GSK3 β by deactivating GSK3 β -inhibitory kinases such as Protein Kinase B (PKB or AKT) [58]. Both ROCK-I and ROCK-II have been shown to regulate RhoA/ROCK signaling to indirectly activate GSK3 β . It was shown that inhibition of either ROCK-I or ROCK-II efficiently inactivated GSK3 β and prevented

subsequent tau phosphorylation [59,60]. The role of GSK3 β in AD will be discussed extensively in the following sections.

GSK3 β Regulation

GSK3 β activation is well described to impact AD pathogenesis by regulating tau phosphorylation [61]. GSK-3 has two isoforms in mammals, GSK3 α and GSK3 β , which share 95% homology in the kinase domain but differ at the N and C terminal regions [62]. GSK3 α has slightly longer N and C termini [62]. While both isoforms are ubiquitously expressed, GSK3 β has greater abundance in the brain and has specificity for a broad range of substrates including metabolic proteins, transcription and translation factors, and cytoskeletal proteins [63,64]. GSK3 β is abundantly expressed in neurons and plays essential roles in regulating neuronal development, synaptic pruning, and influencing the pathogenesis of neurodegenerative diseases, primarily AD [65]. It was observed that ventricular progenitors in the developing neocortex experience a higher expression of GSK3 β that gradually declines following neuronal differentiation [66,67]. Transcripts of GSK3 β have two alternative splicing forms, the short form GSK3 β 1 and the long form GSK3 β 2. The GSK3 β 2 is neuron-specific with a high expression during brain development that persists until adulthood. Interestingly, GSK3 β 2 shows less phosphorylation activity of tau at the AD-associated Ser396 epitope in comparison to GSK3 β 1 [68], suggesting that GSK3 β 2 may not be associated with AD. In addition to neurons, GSK3 β is also expressed in microglia, astrocytes, and oligodendrocytes [69–71].

The function of GSK3 β is largely mediated by its activation and inhibition through phosphorylation at the Tyr216 and Ser9 residues respectively [58,72]. Protein kinase B (PKB/Akt), located downstream of phosphatidylinositol 3-kinase (PI3K) has been shown to rapidly phosphorylate Ser9 of GSK3 β both *in vivo* and *in vitro* with growth factor stimulation [58,73]. cAMP-dependent protein kinase A (PKA) and PKC have also been suggested to phosphorylate Ser9 *in vivo* and *in vitro* [74,75]. Phosphorylation of GSK3 β at Tyr216 at the activating loop is thought to be by autophosphorylation, although phosphorylation by other kinases, such as the Src family kinases (SFKs) and Janus kinases (JAKs) has also been reported [76–78]. Further, GSK3 β can be activated through the dephosphorylation of Ser9 by phosphatases such as protein phosphatase 2 (PP2A) [79]. To date, how GSK3 β activity is regulated is not fully understood and remains an active area of investigation.

Dopamine and glutamate are neurotransmitters that have been associated with the hyperactive state of GSK3 β . A study by Beaulieu et al. observed increased dopaminergic neurotransmission in dopamine transporter null (DAT^{-/-}) mice that resulted in reduced PKB activation and subsequent GSK3 β hyperactivation [80]. In the presence of glutamate, the interaction between GSK3 β and glutamatergic *N*-methyl-D-aspartate (NMDA) receptors is believed to be dual-directional [81]. One study reported rapid dephosphorylation of the inhibitory Ser9 residue in GSK3 β and simultaneous neurotoxicity in cultured hippocampal neurons following increased NMDA signaling [82]. This suggests that NMDA receptor signaling activates GSK3 β . NMDA receptor antagonists such as phencyclidine and memantine have also been shown to increase Ser9 phosphorylation of GSK3 β in murine models [83,84]. It was later observed that NMDA signaling activates protein phosphatase 1 (PP1), which is capable of dephosphorylating the Ser9 residue [85]. Conversely, GSK3 β plays a role in the regulation of NMDA receptor surface localization and function [86]. One of the most well-known clinical inhibitors of GSK3 β is lithium [87]. Its mechanism of action encompasses both direct and indirect pathways. Directly, lithium competes with Mg [2]⁺ ions, cofactors of GSK3 β that stabilize the enzyme's active site, thereby inhibiting its function [88]. Indirectly, lithium enhances the phosphorylation of GSK3 β at the Ser9, although the exact reported mechanisms behind lithium-induced inhibition are conflicting and unclear [89–91]. Consistent with this notion, chronic lithium exposure has been shown to diminish NMDA receptor signaling and subsequent glutamate-induced excitotoxicity in murine cortical neurons [92,93]. These interactions highlight how GSK3 β plays a fundamental role in the body's response to the usage of psychoactive drugs.

The expression of GSK3 β may be regulated epigenetically. The promoter region of GSK3 β was shown to contain the *cis*-regulatory molecule (CRM) that binds to cell-type specific transcription factors, such as Sox2, Sox9, and Neurogenin2 (Ngn2) [94]. Using Histone ChIP-seq analysis of the GSK3 β genomic region, it was shown that two histone marks, H3K4me3 and H3K27ac, exhibited open chromatin around the promoter region and exon 1 of murine GSK3 β [94]. These findings are consistent with prior studies demonstrating that the GSK3 β promoter region contains CCAAT/enhancer-binding proteins (C/EBP) consensus sequences, the known binding sites for transcription factors Sox 2 and Sox9, Ngn2 [94,95]. It is noteworthy that the GSK3 β gene is highly conserved evolutionarily across cell types in all eukaryotic species and that GSK3 β deletion was shown to cause postnatal fatality with multiple developmental defects [96,97]. These studies underscore the essential role of GSK3 β in both physiological functions and disease pathogenesis.

GSK3 β in Neuronal Development

GSK3 β is broadly expressed throughout normal tissues and plays a role in numerous cellular functions including gene expression, stress responses, cell survival and death, cell structure, migration, metabolism, and differentiation [63,98,99]. When concentrated to the CNS, GSK3 β is essential in regulating neurogenesis, neuronal differentiation, neuronal polarization, and progenitor proliferation [100,101]. It was shown that silencing of GSK3 in mice resulted in hyperproliferation of progenitor cells but a suppression of neuronal differentiation with increased cortical surface area and a thinner cortex [100,102]. It is believed that the ability of GSK3 β to mediate neurogenesis, progenitor proliferation, and neuronal differentiation originates from its ability to interact with and regulate various signaling pathways including Wnt, Sonic Hedgehog (SHH), and Notch. These pathways, through interactions with GSK3 β , promote the proliferation of neuronal progenitors by either inhibiting GSK3 β activity or utilizing GSK3 β as a regulatory molecule [103–106]. In regulating the Wnt pathway, GSK3 β exhibits a dual role. Under canonical Wnt ligation, GSK3 β can be inhibited by Dishevelled (Dsh), a transducer in the signaling cascade that inhibits GSK3 β -mediated phosphorylation and degradation of β -catenin [107,108]. In the absence of Wnt signaling, GSK3 β is able to mark β -catenin for proteasomal degradation, thereby preventing progenitor proliferation and differentiation [109]. Similarly, it has been suggested that GSK3 β can function as either a positive or a negative regulator of Notch signaling [110–112], suggestion the cell type-specific role of GSK3 β . It is understood that GSK3 β primarily acts as a downstream effector in the SHH pathway, although its role as either a positive or a negative regulator remains similarly controversial [113,114]. Nonetheless, inactivation of GSK3 β by phosphorylation at Ser9 by PI3K/AKT has been shown to promote axon formation [115]. Further studies in hippocampal neurons revealed high concentrations of inactive GSK3 β at the tips of newly formed axons, in correlation with the proper localization and functioning of Partitioning-Defective 3 (PAR3), a molecule that facilitates neuronal polarity and axon specification [116–118]. The dual role of GSK3 β in neuronal regulation underscores the complexity of the molecule in neural development and a potential challenge in inhibiting GSK3 β for treating AD.

RhoA/ROCK/GSK3 β in Alzheimer's Disease

The RhoA/ROCK pathway, when dysregulated, is a recognized significant contributor to tau phosphorylation and subsequent AD development [9]. Emerging evidence suggests that RhoA/ROCK may indirectly activate GSK3 β , through reducing the activity of the inhibitory kinase PKB in the PI3K/AKT pathway [Figure 2]. RhoA/ROCK has been found to be able to directly phosphorylate and activate phosphatase and tensin homolog (PTEN) [119,120]. Active PTEN inhibits PI3K signaling by dephosphorylating the lipid signaling intermediate phosphatidylinositol (3,4,5)-triphosphate (PIP₃) into PIP₂, effectively impeding the activation of the downstream GSK3 β inhibitor PKB [121,122]. Corroboratively, studies have shown that the inhibition of RhoA/ROCK leads to rapid activation of the PI3K/PKB signaling pathway [123]. Whether RhoA/ROCK is able to indirectly inhibit other GSK3 β -inhibiting kinases, such as PKA and PKC, remains to be elucidated.

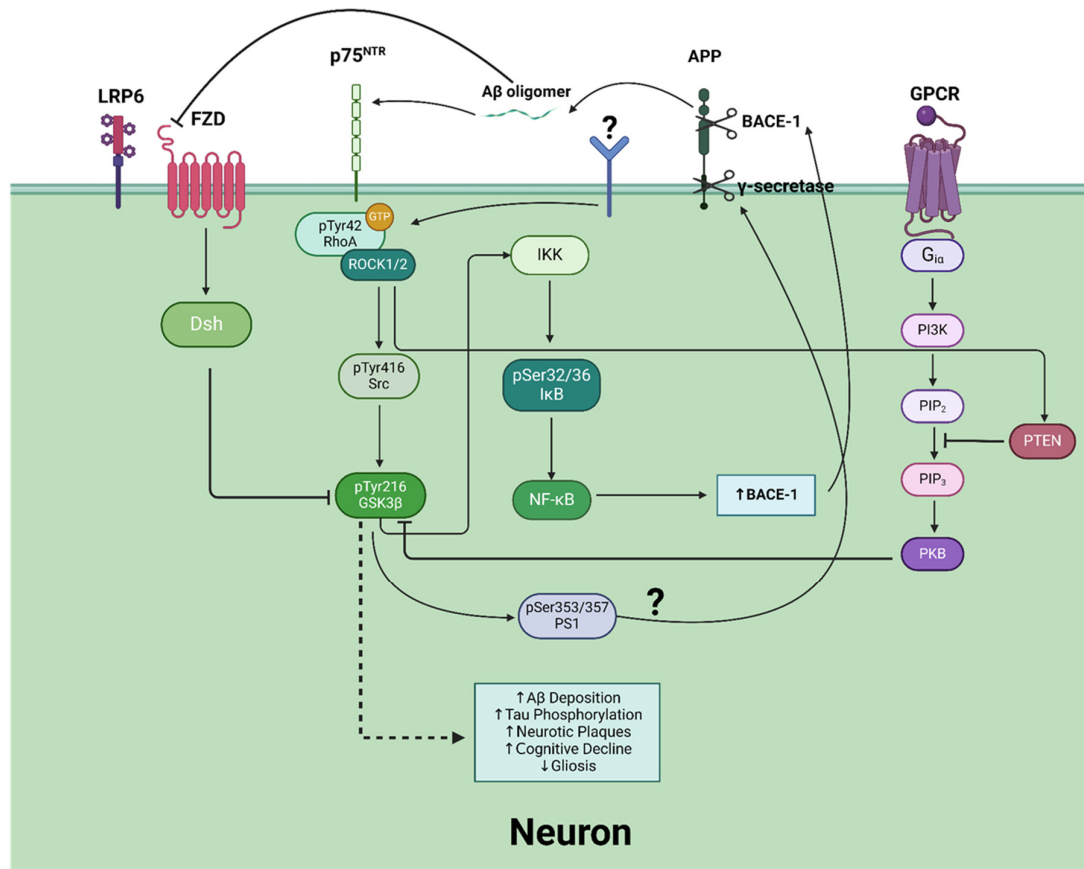


Figure 2. Signaling pathways directly and indirectly linked to RhoA/ROCK-mediated activation of GSK3β and positive feedback loop between Aβ deposition and GSK3β activation. (1) ROCK phosphorylates and activates PTEN, a phosphatase that inhibits the PI3K/AKT signaling pathway. PTEN dephosphorylates PIP₃ into PIP₂, preventing the activation of PKB (AKT). PKB inhibits GSK3β by Ser9 phosphorylation. Inhibition of PKB by RhoA/ROCK activates GSK3β; (2) Aβ ligation of p75^{NTR} activates RhoA by Tyr42 phosphorylation. Activated RhoA/ROCK phosphorylates and activates Src at the Tyr416 residue. Activated Src phosphorylates and activates GSK3β at the Tyr216 residue, subsequently activating IKK. IKK phosphorylates IκB at Ser32/36, marking it for proteasomal degradation and impeding NF-κB inhibition. NF-κB increases BACE-1 expression which increases the activity of amyloidogenic processing of APP into Aβ. Newly processed Aβ oligomers bind to p75^{NTR} and complete the positive feedback cycle; (3) Aβ binds to the extracellular domains of FZD and LRP6, preventing Wnt ligand interactions with the LRP6/FZD complex and successive inhibition of GSK3β. It is noteworthy that GSK3β is capable of phosphorylating PS1 and the Ser353 and 357 residues. However, the exact effect on Aβ processing is unclear; (4) Overactivation of GSK3β induces AD pathogenesis such as Aβ deposition, tau hyperphosphorylation, neurotic plaque formation, and cognitive decline.

Upon RhoA/ROCK dysregulation, the hyperactivation of GSK3β has been well demonstrated to be a major contributor to the pathogenesis of AD, largely by phosphorylating tubulin associated units (tau) [124–126]. Tau is an intrinsically disordered, microtubule-associated protein predominantly expressed in neurons [127,128]. In its normal state, tau binds to and stabilizes distal axonal microtubules (MTs) [129]. The stabilizing function of tau promotes the polymerization of axonal MTs, an essential regulatory mechanism for proper axonal transport [130,131]. Tau is the substrate of many Ser/Thr kinases including GSK3β, Cdk5, MARK, PKA, CamKII, MAPK, PKC, JNK, and ROCK [132]. Tau is also the substrate of the tyrosine kinases Fyn and Abl [133]. Phosphorylation of tau at specific residues, most notably Ser202, Ser396, Ser404, Thr181, and Thr231, is thought to induce conformational changes that create a positive feedback loop where tau can become sequentially

phosphorylated at multiple sites, leading to hyperphosphorylation, although the specific relationship between these residues and AD still needs to be better defined [134–137]. Hyperphosphorylated tau exhibits a reduced affinity for MTs, which results in axonal destabilization [138]. Hyperphosphorylated tau presents aggregation-prone properties and forms neurofibrillary tangles (NFTs) which are a hallmark of AD [139,140].

While RhoA/ROCK is a known activator of neuronal GSK3 β , other signaling pathways, such as Cdk5, ERK, and JNK are also key players in AD pathogenesis [132]. Cyclin-dependent kinase 5 (Cdk5) closely modulates A β deposition and, when hyperactivated by p25, induces aberrant A β cleavage and tau hyperphosphorylation [141,142]. Pharmacological inhibition of Cdk5 activation has been shown to reduce tau phosphorylation and A β processing and ameliorates neuronal death in p25 transgenic mice [143,144]. Mitogen-activated protein kinase (MAPK) pathways, including the extracellular signal-regulated kinase 1/2 (ERK), c-Jun N-terminal kinase (JNK), and P38 kinase (P38) pathways, have been shown influence AD development and progression [145,146]. In AD mouse models, ERK, JNK, and P38 are all found in the overactivated state in the nervous system, spine, and brain respectively [147–149]. While moderate ERK activation is necessary for synaptic plasticity, hyperactivated ERK has been associated with NFT formation and early-stage AD-related protein deposition, leading to impaired hippocampal function in AD patients and murine models [146,150–152]. Similarly, overactivated P38 has been shown to increase tau phosphorylation and favor amyloidogenic processing of the amyloid precursor protein (APP), while inhibition of JNK by D-JNKI1 is suggested to suppress synaptic shrinkage in AD patients [148,153,154]. Inhibitors of MAPK pathways can reduce A β deposition, neuronal apoptosis, memory impairment, and tau hyperphosphorylation, making them a favorable therapeutic target to be investigated [155–157].

Activated GSK3 β was shown to phosphorylate tau at most of the Ser/Thr residues, which associated with tau hyperphosphorylation and AD pathogenesis [158,159]. Corroboratively, GSK3 β inhibition in diverse mouse models has shown to reduce tau phosphorylation and improve cognitive impairments measured through behavioral assays [160,161]. By contrast, the overexpression of active GSK3 β in murine forebrains was associated with tau hyperphosphorylation and somatodendritic tau accumulation in hippocampal neurons [162]. Interestingly, tau-deficient mice overexpressing GSK3 β displayed reduced neurodegenerative symptoms and milder cognitive deficits, suggesting that the interaction between GSK3 β and tau is critical for tau hyperphosphorylation and the development of AD [163]. Furthermore, postmortem examinations of brains of AD patients showed elevated levels of GSK3 β in comparison to non-AD patients of the same age [164].

GSK3 β has been suggested to serve as a bridge connecting A β and tau in the pathogenesis of AD [165]. A β formation has been observed to result in the activation GSK3 β by activating RhoA/ROCK through the phosphorylation of the Tyr42 residue in RhoA [166]. Once activated, RhoA/ROCK is thought to activate Src, which then phosphorylates GSK3 β at the Tyr216 residue [166]. The linking pathway between the presence of A β and activated RhoA/ROCK is not fully understood, but it is thought that A β oligomers bind to the p75 neurotrophin receptor (p75^{NTR}) [167]. Paradoxically, GSK3 β is also shown to regulate the production of A β [168,169]. A β is formed from successive proteolytic cleavages of APP. APP is a transmembrane protein abundantly expressed in the brain and is subject to two distinct metabolic pathways regulated by secretases [170]. The nonamyloidogenic pathway is modulated by α -secretases that cleave APP into easily-degradable fragments [171]. Conversely, the amyloidogenic pathway is mediated by a β -secretase (BACE-1) and γ -secretase complex. The amyloidogenic cleaving of APP forms an A β peptide that aggregates in AD brains [172]. GSK3 β regulates A β production by phosphorylating and mediating the activation of presenilin-1 (PS1), a component of the γ -secretase complex [169]. Alternatively, GSK3 β , through NF- κ B overexpression, is thought to upregulate BACE-1 expression [173]. While the upregulation of BACE-1 by GSK3 β increases A β cleavage, the effects of GSK3 β phosphorylation of PS1 and the resulting modulation of γ -secretase activation on A β processing are unclear and are a necessary area for future investigation [161,174]. Finally, it is suggested that A β blocks Wnt signaling-induced GSK3 β deactivation, leading to increased GSK3 β activation. It is thought that A β can bind to the

extracellular domain of the Wnt ligand receptor Frizzled (FZD), disrupting the interactions between Wnt ligands and the Low-Density Lipoprotein Receptor-Related Protein 6 (LRP6)/Fz complex [175]. Increased GSK3 β activation results in an increase in A β cleavage and tau hyperphosphorylation, completing the positive feedback loop [175]. This positive feedback loop underscores the central role of GSK3 β as a critical mediator linking A β production, tau hyperphosphorylation, and the amplification of pathological signaling in AD [Figure 2].

GSK3 β -induced tau hyperphosphorylation impairs axonal transport, leading to further cognitive decline [176]. Axonal transport disturbances are an early hallmark of neurological disorders including AD [176]. While GSK3 β is required to phosphorylate kinesin-1 to induce axonal transport of tau, the hyperphosphorylated tau disrupts axonal trafficking by destabilizing the MT cytoskeleton, diminishing the binding between motor proteins and cargo [177]. It is suspected that hyperphosphorylated tau impairs the function of c-Jun N-terminal kinase-interacting protein 1 (JIP1), which is responsible for facilitating the binding of cargo to motor proteins, although the complete mechanism is not clear [178]. Loss of axonal transport leads to vesicular aggregation and subcellular mislocation [179,180]. Additionally, the overactivation of GSK3 β is thought to decrease cholinergic function, parallel to the reductions of cholinergic neurons exhibited in AD brains [181,182]. GSK3 β is able to inhibit the production of the cholinergic neurotransmitter acetylcholine (ACh) by phosphorylating and deactivating pyruvate dehydrogenase, an essential enzyme for the functioning of choline acetyltransferase (ChAT), which is responsible for synthesizing ACh [181,183]. The loss of ACh causes loss of function in cholinergic neurons, inducing further cognitive impairment [182].

GSK3 β also plays an essential role in modulating cognitive functions at the presynaptic and postsynaptic levels. Within presynaptic regions, the overactivation of GSK3 β hinders the exocytosis of synaptic vesicles by phosphorylating P/Q-type calcium channels and disrupting the soluble NSF attachment protein receptor (SNARE) complex formation [184]. Such inhibition significantly diminishes the presynaptic release of glutamate and the clustering of synapsin I, a protein crucial for the release of neurotransmitters, preventing synapse formation [185]. Contrastingly, at the postsynaptic level, GSK3 β regulates synaptic plasticity by mediating long-term potentiation (LTP) and long-term depression (LTD) [186–188]. It has been shown that LTP induction inhibits GSK3 β activation by an increase in phosphorylated Ser9 levels, while transgenic mice overexpressing active GSK3 β showed impaired LTP, suggesting that overactive GSK3 β may impair LTP in cognition-associated conditions [185,189,190]. Further, it is suggested that GSK3 β activation facilitates the induction of N-methyl-D-aspartate receptor (NMDAR)-mediated LTD through mechanisms involving PP1-mediated dephosphorylation and Akt inhibition downstream of NMDAR activation, strongly suggesting that overactivated GSK3 β supports the induction of abnormal levels of LTD observed in multiple neuropsychiatric disorders [190–195]. It is noteworthy that GSK3 β may be involved in the cross-communication between LTP and LTD [188]. Multiple studies have shown that inhibitors of GSK3 β may be able to help normalize abnormal levels of both LTP and LDP in the brain, as treatment with GSK3 β inhibitors were accompanied by increased cognitive abilities in mouse models of Fragile X syndrome, and Down syndrome, and AD [191–195].

sTREM2 as a Regulator of RhoA/ROCK/GSK3 β Signaling

Multiple studies have suggested that the solubilized form of triggering receptors expressed on myeloid cells 2 (sTREM2) can reduce A β accumulation and slow AD progression [9,196–199]. TREMs are a broadly expressed family of cell surface receptors. TREMs primarily act as modulators of the immune response that regulate the activation of myeloid cells, notably macrophages [200,201]. Within the family, TREM2 is the most widely studied molecule and is implicated in the pathogenesis of numerous macrophage-associated and inflammation related diseases such as AD [202]. When membrane-bound, TREM2 binds to lipids and elicits essential neuroprotective effects by downregulating the expression of pro-inflammatory cytokines, such as TNF- α , IL-1 β , and NOS2, and upregulating the transcription of anti-inflammatory cytokines including IL-4, IL-10 and IL-11 [203–205]. Additionally, TREM2 signaling has been shown to mediate the expression of the activating toll-

like receptors (TLRs) [206]. In the brain, TREM2 is highly expressed by microglia in the temporal cortex surrounding A β plaques [207]. The TREM2 signaling pathway is involved in the microglial response and clearance of amyloid plaques, and is critical for successful synaptic pruning in the brain [208,209]. It has been shown with microglial RNA-seq analysis that TREM2 is required to sustain the microglial response to clear or prevent A β plaque formation [210,211].

The ectodomain of TREM2 can be solubilized by the α -secretases disintegrin and metalloproteinase domain-containing protein 10 (ADAM10) and ADAM17 at the His157-Ser158 bond within the stalk region to form soluble TREM2 (sTREM2) [212]. Studies revealed that elevated concentrations of sTREM2 are released by microglia into cerebral spinal fluid (CSF) in AD patients, although the extracellular conditions that increase or decrease sTREM2 shedding are uncertain [213]. sTREM2 has been suggested to influence the pathogenesis of AD, but the mechanisms behind the effects are unclear. Putatively, it is believed that sTREM2 plays a protective role in AD development [214]. This notion was supported by studies that sTREM2 is able to block the aggregation and neurotoxicity of A β plaques in murine models [215–217]. Furthermore, it has been suggested that sTREM2 reduces cognitive impairments induced by tau pathologies, such as preventing the loss of hippocampal synapses in tau P301S mice [10,218]. However, one study demonstrated that the injection of sTREM2 into the brains of healthy mice increased levels of pro-inflammatory cytokine production and microglial activation and proliferation [218]. Another study has implicated that sTREM2 may act as a decoy receptor and have indirect pro-inflammatory effects by reducing the modulatory anti-inflammatory function of TREM2 signaling [219]. Although TREM2 is generally considered advantageous in mitigating AD pathogenesis, the underlying mechanisms behind its neuroprotective effects are not well understood and would be an interesting area for future investigations.

Transgelin-2 (TG2 or SM22 β), an actin-binding protein, has recently been suggested to ligate sTREM2 and deactivate the RhoA/ROCK/GSK3 β signaling pathway, which may explain the neuroprotective effects observed following the shedding of sTREM2 [10]. TG2 is encoded by the *TAGLN2* gene and is one of three transgelin isoforms alongside TG1 and TG3 [220]. Transgelins characteristically have transformation and conformation-sensitive properties, although the full implications of such are not fully known [221]. TG2 is highly expressed in smooth muscle cells [222]. It is also found in non-smooth muscle cells, including fibroblasts, epithelial cells, and immune cells throughout the body [223–225]. In the brain, TG2 is expressed primarily in neurons and microglia [10,226]. Broadly, TG2 is involved in regulating actin binding and stabilization, smooth muscle contraction, cell motility, and migration [227–229].

Recently, an interesting study by Zhang et al., 2023, proposed that TG2 expressed on neurons may serve as a receptor for sTREM2 [10]. Affinity purification pulldown and affinity purification mass spectrometry revealed that sTREM2 and TG2 colocalized on the cell surface of hippocampal neurons from AD patients and tau P301S transgenic mice, implying that TG2 serves as a sTREM2 receptor [10]. Supportively, TG2 has recently been shown to localize at the cellular membrane and function as a regulatory receptor of the myosin cytoskeleton and airway smooth muscle [222]. It has been shown that the TG2 agonist, TSG12, induced RhoA phosphorylation at the Ser188 residue, deactivating the molecule and, by extension, inhibiting RhoA/ROCK/GSK3 β signaling [166,222]. Furthermore, neuroblastoma cell lines treated with sTREM2 exhibited decreased levels of activated RhoA and elevated levels of inactive RhoA phosphorylated at the Ser188 residue [10,230,231]. Further treatment with RhoA and ROCK inhibitors, Tat-C3 and Y-27632 respectively, emulated the inhibitory effect of sTREM2 on GSK3 β activation and subsequent tau phosphorylation [10]. Additional transfection of phosphorylation-resistant RhoA (RhoA S188A) into an endogenous RhoA-null background silenced the inhibitory effect of sTREM2 [10]. It was observed that sTREM2 dramatically reduced the phosphorylation of tau at S202 and S396 residues, identical sites that are targets of GSK3 β phosphorylation [10,232]. Concentration-dependent sTREM2 was also observed to significantly decrease the phosphorylation of GSK3 β at the Tyr216 residue, and inhibition of sTREM2 with an anti-TREM2 antibody depleted this effect, suggesting that sTREM2 is able to deactivate GSK3 β through

TG2 ligation [10,72]. Finally, TG2 knockdown attenuated the neuroprotective function of sTREM2 on the inhibitory and excitatory synapses in the hippocampus of P301S mice [10]. This study strongly suggests that the ligation of TG2 by sTREM2 deactivates the RhoA/ROCK pathway through inhibiting RhoA, effectively preventing GSK3 β activation and successive tau hyperphosphorylation.

Although TG2 was suggested to act as a cell surface receptor, it is primarily a cytosolic protein and is not known to possess a transmembrane domain when localized at the cellular membrane [233,234]. Thus, TG2 is not capable of inducing or inhibiting a signaling cascade directly. Alternatively, it is possible that TG2 may be coupled in low affinity with an adaptor molecule in the transmembrane domain that would not be detected in an affinity pulldown assay, potentially explaining how the ligation of TG2 exhibits RhoA/ROCK inhibition [Figure 3]. Perhaps, TG2 exhibits transient, low-affinity interactions with lipid raft-resident adaptor proteins or actin-linked scaffolding molecules. Given the proximity of TG2 to cortical actin networks, it is possible that TG2 interacts with adaptor proteins via weak electrostatic forces or PTMs that mediate binding affinity [10]. Notwithstanding, the interaction between sTREM2, TG2, and the RhoA/ROCK pathway warrants further investigation.

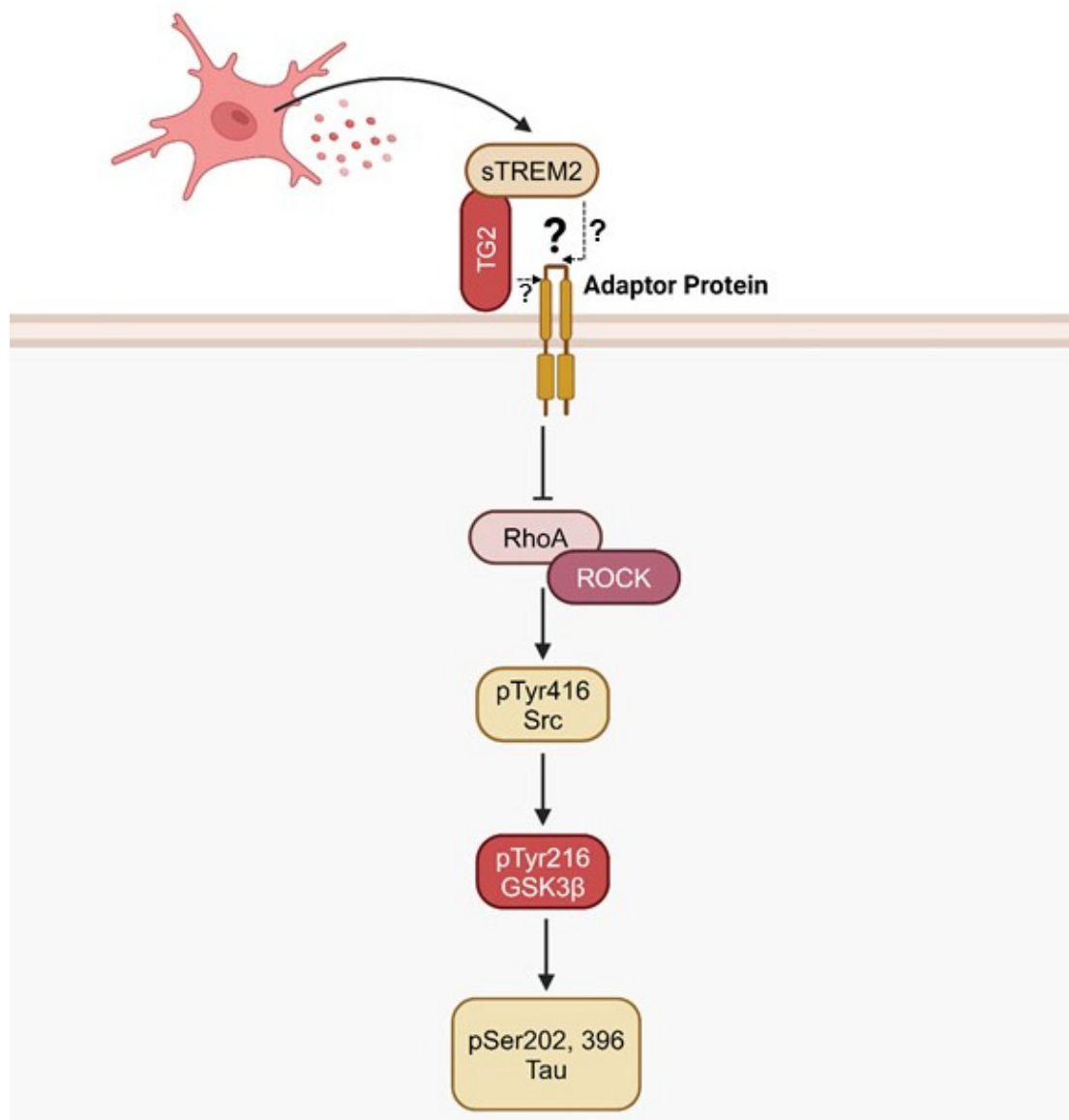


Figure 3. Signaling pathway depicting the potential for TG2 to serve as a sTREM2 receptor and inhibit the RhoA/ROCK/GSK3 β pathway. A potential adaptor molecule is shown, as TG2 lacks a transmembrane domain. However, what this adaptor protein is, and if there truly is an adaptor protein altogether, is still under investigation.

Emerging Perspectives and Challenges

Given the pivotal role of GSK3 β overactivation in tau hyperphosphorylation, many preclinical studies attempted to inhibit GSK3 β signaling as a therapeutic target for AD. Inhibitors including 6-bromoindirubin-3'-oxime (6BIO), Hymenialdisine (HD), CHIR98014, SB-216763, and Alsterpaullone have been tested in preclinical studies in mouse models, with decreased tau phosphorylation, A β deposition, inflammation, and spatial memory deficits [235–239]. Preclinical studies have also suggested potential offsite toxicity due to suboptimal GSK3 β specificity [236]. Targeting GSK3 β has advanced to clinical trials [Table 1], while some results are encouraging, they are mostly inconclusive and unsatisfactory at this moment.

Table 1. Clinical Trials targeting GSK3 β .

NCT/PMID	Drug Name	Outcome	Phase
NCT02129348	Lithium Carbonate	- Increased aggression - Moderate/marked improvement - Improvement on NPI delusions and irritability	Phase II
NCT00088387	Lithium Carbonate & Divalproex	No reported results	Phase II
NCT00948259	NP031112	No reported results	Phase I, Phase II
NCT01055392	Lithium Carbonate	- Improved performance on memory and attention tests for 2 years - Significant increase in CSF A β after 3 years - Increased adverse events	Phase II
25537011	Tideglusib	- No clinical benefit	Phase II

Although the therapeutic targeting of GSK3 β is promising, it is still in its infancy, pending the resolution of difficulties, most notably specificity. Due to the broad expression of GSK3 β and numerous physiological processes throughout the body, such as cell growth and survival, inhibition of GSK3 β in general will likely lead to adverse effects. Moreover, due to the broadly conserved ATP-binding sites across the kinase family, engineering a small molecule that can effectively target specific kinases is exceedingly challenging. Nevertheless, developing a selective inhibitor to specifically target hyperactive of GSK3 β in neurons may avoid such adverse effects. However, the difficulty in developing a GSK3 β -specific inhibitor is further solidified by blood-brain barrier (BBB) prevention, an inhibitor must be small enough to pass through BBB and a cargo such as a nanoparticle may be considered.

Given the aforementioned challenges of targeting GSK3 β in treating AD, targeting upstream pathways leading to GSK3 β activation in neurons may offer new treatment avenues. The intricate signaling network of RhoA/ROCK/GSK3 β establishes it as a significant and promising immunotherapeutic target for treating AD. The immensely wide evolutionary conservation of GSK3 β suggests that the GSK3 β gene is essential for regulating synaptic function. Dysregulation of this pathway has been shown to largely contribute to tau hyperphosphorylation, neuroinflammation, and synaptic dysfunction, leading to cognitive decline. While targeting the molecules of this pathway for therapeutic treatment of AD appears to be propitious, much remains to be understood about the complex functions and signaling cascades involved, especially in the context of disease progression. The RhoA/ROCK/GSK3 β pathway intersects with multiple cellular processes, and its modulatory

functions have been shown to be highly context-dependent. Future research is imperative to fully understand the mechanisms behind this pathway and its influence on AD.

While sTREM2 has been suggested to be a promising immunotherapeutic target, the participation of sTREM2 in AD pathogenesis seems highly complex based on many controversial findings. How sTREM2 plays a role in AD pathogenesis must be fully understood before therapeutic interventions. Given that TG2 has recently been demonstrated to have a positive role in reducing AD development [10], strategies to increase TG2 expression in neurons may be a viable therapeutic angle.

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