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REVIEW

Deciphering the roles of glycogen synthase kinase 3 (GSK3) in the treatment of autism spectrum disorder and related syndromes

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Abstract

Autism spectrum disorder (ASD) is a complex and multifactorial neurodevelopmental disorder characterized by the presence of restricted interests and repetitive behaviors besides deficits in social communication. Syndromic ASD is a subset of ASD caused by underlying genetic disorders, most commonly Fragile X Syndrome (FXS) and Rett Syndrome (RTT). Various mutations and consequent malfunctions in core signaling pathways have been identified in ASD, including glycogen synthase kinase 3 (GSK3). A growing body of evidence suggests a key role of GSK3 dysregulation in the pathogenesis of ASD and its related disorders. Here, we provide a synopsis of the implication of GSK3 in ASD, FXS, and RTT as a promising therapeutic target for the treatment of ASD.

Keywords Autism spectrum disorder · Fragile X syndrome · Rett syndrome · Glycogen synthase kinase 3

Introduction

Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder that affects an average of one in 160 children worldwide [1]. It is diagnosed based on the clinical observation of behavioral features that primarily comprise impaired social communication in addition to restricted interests and repetitive behaviors [2]. Although it is believed that ASD is caused by a combination of environmental factors and genetic predisposition, our knowledge about the etiology of ASD is still incomplete. However, in about 10% of ASD cases, termed "syndromic", ASD is caused by an underlying genetic disorder, such as Fragile X Syndrome (FXS) and Rett Syndrome (RTT), which are caused by mutations in the *Fmr1* and *MECP2* genes, respectively [3]. Moreover, the efficacy of the behavioral interventions in treating ASD is variable, and the use of drugs has not proven efficient in improving social communication and is limited by various adverse effects [2, 4].

Glycogen synthase kinase 3 (GSK3) is a serine/threonine-protein kinase that is involved in a wide range of biological processes and participates in numerous signaling pathways. It regulates diverse neurodevelopmental processes, including neuronal proliferation, differentiation, migration, polarization, axon formation, neuronal survival, and apoptosis [5, 6]. Recently, GSK3 has gained much attention due to its implication in various neurodegenerative and neuropsychiatric diseases, making it a potential therapeutic target [7]. Moreover, dysregulation of GSK3 activity has been linked to the pathophysiology of ASD and ASD-related syndromes such as FXS and RTT. Studying the role of GSK3 in ASD and its related syndromes could pave the road to targeting it as a potentially effective therapeutic intervention. The purpose of the present study is to perform a review of the literature to assess the role of GSK3 in ASD and ASD-related syndromes as a promising therapeutic target for such disorders.

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Autism spectrum disorder

Autism spectrum disorder (ASD) is a complex and multifactorial neurodevelopmental disorder characterized by deficits in social communication and the presence of restricted interests and repetitive behaviors [2]. On average, one in 160 children worldwide has an ASD [1] with a male-to-female ratio lying between 3:1 and 4:1 [8]. The diagnosis is mainly made by clinical observation of aberrant behavior based on the diagnostic criteria determined by the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) [2, 4]. Such behavioral symptoms start appearing on children by the age of 18 to 24 months, as social requirements start surpassing their limited capabilities [9]. According to DSM-5, ASD includes Autism, Pervasive Developmental Disorder not other-wise specified (PPD-NOS), and Asperger Syndrome [2]. However, in DSM-4, ASD was referred to as pervasive developmental disorder (PDD), which included autistic disorder, Asperger's disorder, childhood disintegrative disorder, PDD-NOS, and Rett syndrome (RTT) [4, 10]. Typically, no single definite cause of ASD has been established yet; however, it is believed that a complex interaction between genetic, epigenetic, and environmental factors contribute to its development [11]. ASD cases are classified as "idiopathic", i.e., without a known cause, or "syndromic", where ASD is caused by an underlying genetic disorder, such as Fragile X Syndrome (FXS) and RTT. In addition, hundreds of genetic variants have been shown to be associated with ASD, including single nucleotide variants (SNVs) and copy number variants (CNVs) [12]. Nevertheless, 70% of ASD cases are idiopathic with an unknown genetic etiology [3, 13].

Molecular pathways implicated in ASD

Wnt/β-catenin signaling pathway

The Wnt protein family comprises several molecules that are implicated in a wide range of functions. This pathway plays a crucial role in neurodevelopmental processes, being implicated in neural progenitor cells (NPC) differentiation, neuronal migration, axon guidance, synaptogenesis, dendrite development, and neuronal plasticity [14, 15].

Wnt proteins are involved in three major signaling pathways: the planar cell polarity (PCP), Wnt/Ca^{+2}, and the canonical Wnt/β-catenin signaling pathway [16]. The latter is the most studied pathway in association with ASD. Wnt ligand binds to Frizzled (FZD) receptor and LRP5/6 coreceptors (Fig. 1). This leads to the dissociation of β-catenin from the degradation complex consisting of Axin, adenomatous polyposis coli (APC), casein kinase 1 (CK1), and glycogen synthase kinase 3β (GSK3β). Ultimately, β-catenin translocates into the nucleus and interacts with T-cell factor/lymphoid enhancing factor (TCF/LEF) transcription factors, leading to the activation of gene expression [17]. Several components and modulators of the Wnt signaling have been identified as high-risk genes in ASD. For instance, the product of chromodomain helicase DNA binding protein 8 (*CHD8*) gene is a negative Wnt regulator [52]. It is an ATP-dependent chromatin remodeler protein—the gene of which is frequently mutated in ASD [19, 20, 22]. Mutations in the *β-catenin* gene, as well as the Frizzled-9 receptor (*FZD9*) gene have also been identified in ASD patients [48–51].

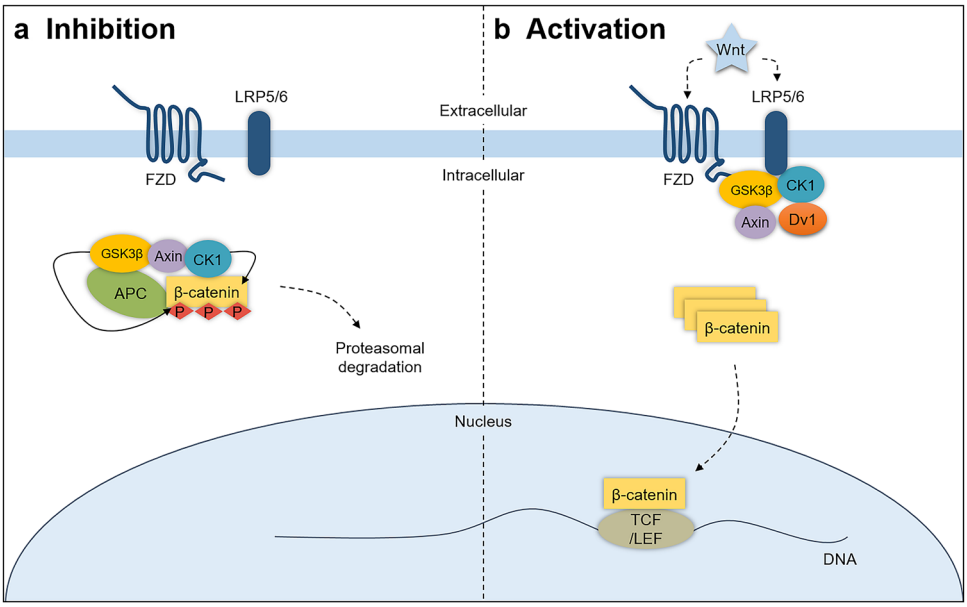

Fig. 1 Canonical Wnt signaling pathway. **(a)** In the absence of Wnt, the Wnt signaling pathway is inhibited. The complex made up of APC, GSK3β, axin, and CK1 phosphorylates β-catenin targeting it to proteasomal degradation. **(b)** In the presence of Wnt, the pathway is activated by Wnt via binding to FZD receptor and LRP5/6 co-receptors leading to the dissociation of β-catenin from the destruction complex and its translocation into the nucleus, where it activates gene expression by interacting with TCF/LEF transcription factors

PI3K/Akt/mTOR signaling pathway

PI3K signaling pathway (Fig. 2) starts by the activation of a receptor tyrosine kinase or a metabotropic glutamate receptor (mGluR) via a subset of growth factors. This leads to the activation of phosphoinositide 3-kinase (PI3K), which phosphorylates phosphatidylinositol-3, 4-bisphosphate (PIP2) into phosphatidylinositol-3, 4, 5-triphosphate (PIP3). This action is counteracted by PTEN (phosphatase and tensin homolog deleted on chromosome ten), which converts PIP3 into PIP2 [23, 24]. Subsequently, PIP3 triggers the phosphorylation and activation of Akt, which inhibits the formation of the inhibitory TSC1-TSC2 complex, activating the mammalian target of rapamycin (mTOR), which is involved in activating protein translation [23, 24]. mTOR pathway is involved in the generation and differentiation of NPCs, neuronal migration, axon formation and regeneration, dendritic growth, and synaptic plasticity [24–26].

Mutations in components of this pathway, including *PTEN*, *TSC1* and *TSC2*, have been associated with ASD [27, 28]. Deletion of *PTEN* in mice was shown to induce autism-like behavioral deficits, in addition to neuronal and synaptic aberrations [29, 30]. Mice with mutations in *TSC1* and *TSC2* genes are used as animal models of ASD [31]. Moreover, evidence supports the involvement of the PI3K/Akt/mTOR pathway in the pathophysiology of VPA-induced ASD in animal models of idiopathic ASD [32]. Also, mTOR inhibitor, rapamycin, was found to ameliorate social interaction deficits in VPA-exposed mice [33].

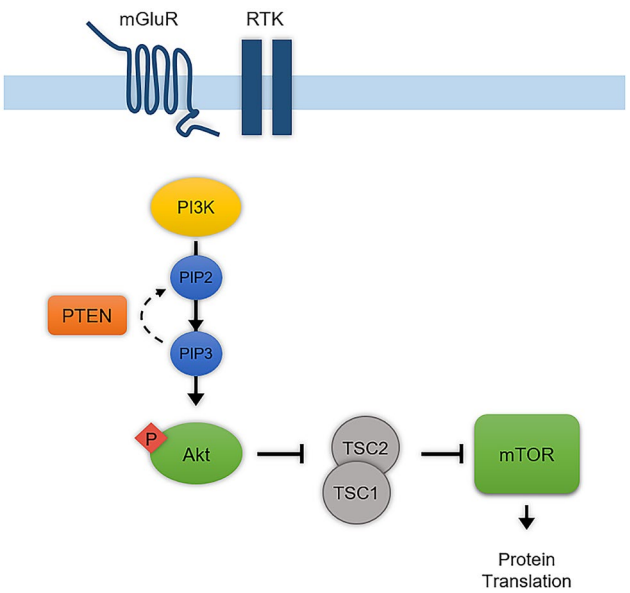

Fig. 2 PI3K/Akt/mTOR signaling pathway. PI3K signaling starts by the activation of either a receptor tyrosine kinase or a mGluR, which leads to the activation of PI3K that phosphorylates PIP2 into PIP3. Subsequently, PIP3 triggers the phosphorylation and activation of Akt, which inhibits the formation of the inhibitory TSC1-TSC2 complex, activating the mammalian target of rapamycin (mTOR), which is involved in activating protein translation. The action of PI3K is counteracted by PTEN (phosphatase and tensin homolog deleted on chromosome ten), which converts PIP3 into PIP2

Other signaling pathways

Several other signaling pathways have been also found to be dysregulated in ASD and might be involved in the pathophysiology of the disorder. These include hedgehog (Hh), Retinoic acid, and ERK/MAPK signaling pathways [34–36].

Glycogen synthase kinase 3 (GSK3)

Glycogen synthase kinase 3 (GSK3) belongs to protein kinases family [37]. It was initially discovered in the late 1970s as a metabolic regulatory protein involved in glycogen metabolism [38]. GSK3 was originally characterized by its ability to phosphorylate and inactivate glycogen synthase, the rate-limiting enzyme in glycogen biosynthesis [39]. The name "glycogen synthase kinase" describes the diverse targets and functions attributed to GSK3. It is a multi-tasking kinase involved in a wide range of biological processes including glucose metabolism [40], cell signaling [41, 42], gene transcription [43], cellular transport [44], proliferation [45], cell motility [46] and apoptosis [47, 48].

Isoforms of GSK3

GSK3 is a serine/threonine protein kinase ubiquitously expressed in all mammalian tissues and subcellular organelles with particularly high levels in the brain [49], both in neurons and glial cells [50]. Molecular cloning of GSK3 retrieved two isoforms, GSK3α (51 kDa) and GSK3β (47 kDa) encoded by two different genes and share a nearly identical amino acid sequence of the kinase catalytic domain. However, considerable structural differences do exist in their N- and C-termini sequences in which GSK3α possesses an extended N-terminal glycine-rich tail that is absent in GSK3β [51]. Despite the high homology within their catalytic domain, the functions and regulations of the two isoforms are not always the same [52, 53]. GSK3α is highly expressed in the cerebral cortex, striatum, hippocampus and cerebellum [54, 55], whereas GSK3β is highly expressed in almost all brain regions [56]. Two splice variants of GSK3β have been discovered in human, GSK3β1 and GSK3β2, the latest carrying a 13-amino acid insert near the kinase domain [57]. GSK3β1 is expressed in neuronal cell bodies and their processes whereas GSK3β2 occurs predominantly in cell bodies [58]. They show distinct substrate preferences [59] and different phosphorylation activity [60].

Regulation of GSK3 activity

As GSK3 is involved in a large number of cellular processes, its activity is highly regulated. To achieve this, multiple levels of regulation contribute to controlling the activity of GSK3 including phosphorylation, subcellular localization, protein–protein interaction, substrate priming, and proteolytic cleavage.

Regulation by phosphorylation

GSK3 is differentially regulated by phosphorylation. Its activity is significantly inhibited upon phosphorylation of an N-terminal serine residue, Ser21 in GSK3α and Ser9 in GSK3β [61]. Many kinases, including protein kinase A (PKA), protein kinase C (PKC), Akt and p90Rsk can phosphorylate Ser21/9 in response to extracellular cues [62]. On the other hand, GSK3 is activated by phosphorylation of Tyr279 and Tyr216 in the kinase domain of GSK3α and GSK3β, respectively [63, 64]. This phosphorylation is mediated by autophosphorylation [65] and by certain tyrosine kinases [63].

Regulation by subcellular localization

GSK3 is considered a cytoplasmic protein; however, it can be detected in the nuclei and mitochondria where it is highly active compared to cytosolic GSK3 [66, 67]. Different GSK3 isoforms show distinct patterns of subcellular localization [68]. Subcellular localization of GSK3 is critical for its function. For example, the nuclear level of GSK3 is elevated during early apoptosis enabling GSK3 to modulate gene expression through regulation of transcription factors [69]. In the mitochondria, GSK3β contributes to regulatory mechanisms of mitochondrial biogenesis, permeability, and apoptosis [70]. In growth cone it phosphorylates microtubule associated proteins such as (MAP1B), enhancing axon growth; however, its inactivation abolishes axon formation [71].

Regulation by protein–protein interaction

An important mechanism for regulating the activity of GSK3 is achieved by protein complexes that contain GSK3 [62]. The classical example is the destruction complex in the canonical Wnt signaling pathway where GSK3 binds to the scaffold protein axin enabling GSK3 to phosphorylate β-catenin which targets the latter for proteasomal degradation [72].

Regulation by priming/substrate specificity

The crystal structure of GSK3 provides insight into its preference for primed, pre-phosphorylated substrates [73, 74]. The preferred substrates of GSK3 usually contain a previously phosphorylated Ser or Thr that is located at the fourth amino acid C-terminal to the Ser or Thr residue (Ser/Thr-X-X-X-primed Ser/Thr) [73, 75, 76]. These substrates are pre-phosphorylated by several priming kinases such as CDK-5 [77], PAR-1 [78], CK1 [79] and PKA [80]. Although the pre-phosphorylated substrates are the common targets for GSK3, non-primed substrates may define a different group for GSK3 function [81]. Moreover, different GSK3 isoforms show distinct substrate preferences in the brain where they appear to differentially phosphorylate their targets [59].

Regulation by proteolytic cleavage

N-terminal proteolysis has been proposed as a new regulatory mechanism of GSK3 activity. A fragment from the N-terminal of GSK3α/β including the regulatory serines 21/9 is removed by the calcium-activated protease called calpain [82]. Both, GSK3α and GSK3β, become active after truncation although they have different susceptibility to cleavage by calpain in cortical mouse brain extracts [82]. Interestingly, GSK3β was reportedly truncated at its N-terminal domain by the matrix metalloproteinase (MMP)-2 in cardiomyoblasts in response to oxidative stress, enhancing GSK3β kinase activity [83].

GSK3 in different signaling pathways

GSK3 is considered one of the few molecules that plays a fundamental role in many signaling cascades including Wnt, PI3K/Akt, Notch, and hedgehog (Hh) among others.

Wnt/β-catenin signaling and GSK3

GSK3 is a core component that negatively regulates the Wnt/β-catenin pathway.

PI3K/Akt signaling and GSK3

It has been shown that Akt inhibits GSK3, and inhibition of PI3K blocks the activation of Akt and the subsequent inhibition of GSK3 [84]. However, GSK3 activity is differentially regulated between PI3K and Wnt pathways. While Wnt signaling regulates GSK3 by protein complexing, the PI3K pathway mediates its regulatory action through Akt-dependent serine phosphorylation [85]. Furthermore, GSK3 has been shown to phosphorylate components of the PI3K signaling, including PTEN [86], TSC1 and TSC2 [87, 88], and Rictor [89]. In addition, one study showed that GSK3α

could phosphorylate and suppress Akt activation in Th17 primary T cells, demonstrating a possible regulatory feedback mechanism [90].

Notch signaling and GSK3

The Notch pathway is an evolutionarily conserved signaling pathway that plays a crucial role in neurodevelopment by regulating neural stem cell proliferation, survival, self-renewal and differentiation [91]. Evidence supports the involvement of GSK3β in the regulation of Notch signaling. GSK3β has been shown to interact and phosphorylate the intracellular domain of Notch1 (Noch1ICD), which protects it from proteasomal degradation [92]. Another study revealed that GSK3β phosphorylated Notch2 in vitro and in vivo and inhibited the transcription of Notch-targeted genes. Overexpression of Wnt-1 or treatment with GSK3 inhibitor reversed the inhibitory effect of GSK3 resulting in increased Notch activity and enhanced gene transcription [93].

Hedgehog (Hh) signaling and GSK3

Hh cascade is an important signaling pathway for cellular growth and differentiation [94]. It is characterized by a number of negative regulators including GSK3 [95]. GSK3β can phosphorylate and stabilize the suppressor of fused protein (Sufu) and thus indirectly provoke degradation of the transcription factor Gli protein that in turn represses the transcription of a subset of Hh target genes [96].

Role of GSK3 in the nervous system

In recent years, GSK3 has emerged as an essential kinase in the nervous system that controls diverse neurodevelopmental processes. These processes include neuronal proliferation, differentiation, migration, polarization, axon formation, neuronal survival, and apoptosis [5, 6].

GSK3 and neural proliferation and differentiation

GSK3 signaling is critical for coordinating neural proliferation and differentiation [97, 98]. GSK3 is abundantly expressed and highly active in post-mitotic and differentiated neurons [49], which suggests that GSK3 may reduce neural proliferation. Selective deletion of both GSK3α and GSK3β in an animal model markedly enhanced neural progenitor proliferation and arrested neural differentiation at the progenitor state [97]. This indicates that the regulation of GSK3 is required for an appropriate transition from the proliferative state to the neurogenic phase during brain development [5].

GSK3 and neural polarization and axon formation

A large number of molecules are involved in neural polarization including GSK3 [99, 100]. In a hippocampal neuronal culture, constitutively active GSK3β inhibited axon formation and significantly abolished neural polarity. In addition, using pharmacological inhibitors of GSK3 has markedly increased the formation of multiple axons in hippocampal neurons [101]. Another in vitro study showed that selective inhibition of GSK3β enhanced neurites outgrowth and its activation causes neurite retraction [97].

GSK3 and neural migration

Under tightly controlled conditions during central nervous system (CNS) development, neuronal cells undergo directional migration to reach their final destination [102]. Several studies reported that GSK3 is an important signaling molecule in neural migration. Morgan-Smith et al. demonstrated that GSK3 is a critical enzyme for the migration of neurons and dendritic orientation in all areas of the cortex and in the hippocampus [103]. Active GSK3 was also reported to hinder centrosomal forward movement and neuronal migration in the developing neocortex whereas the inactivation of GSK3 influences microtubule stabilization at the leading process tip of migrating neurons thus favoring neuronal migration [104].

GSK3 and neuronal apoptosis and survival

Apoptosis is an essential process during CNS development, where it plays a critical role in defining the number of neurons and building neural circuits [105, 106]. GSK3 was proposed as an important modulator of neuronal apoptosis. An in vitro study suggested that the activation of GSK3β promotes apoptotic signaling in cultured NPCs derived from embryonic mouse brains subjected to apoptotic conditions, trophic factor withdrawal and genotoxic stress [107]. In another study using transgenic mice, the overexpression of GSK3β in specific regions of the brain resulted in apoptotic neuronal death [108]. This is supported by another in vitro study which showed that overexpression of GSK3β induced neuronal death, while its inhibition protected cortical neurons from apoptosis [109].

GSK3 and ASD

Dysregulation of GSK3 is implicated in the pathophysiology of a number of CNS diseases including Alzheimer's disease [50], Parkinson's disease [110], Schizophrenia [111], and Bipolar disorder [104, 112]. It is believed that the dysregulation of GSK3 contributes to the pathophysiology of ASD

as well (Fig. 3). T-lymphocytes isolated from children with ASD displayed decreased activity of GSK3α and GSK3β along with increased activity of the PI3K/Akt/mTOR signaling pathway, as compared to typically developing controls [113]. These data are consistent with a previous study showing that inhibitory phosphorylation of GSK3β is increased in VPA-induced rat model of ASD, and the inhibition of PI3K upstream of GSK3β reversed the impairment of social behavior via reversing the increased GSK3β phosphorylation [114].

GSK3 and VPA teratogenicity

The VPA rat model is one of the most commonly used animal models in the ASD research, in which prenatal exposure to VPA induces a range of behavioral impairments that resemble those displayed by human autistic patients [115]. On the molecular level, it has been demonstrated that VPA causes overactivation of Wnt and mTOR signaling pathways in several regions of the rat brain, and inhibition of either pathway relieves the autistic-like behaviors [32]. GSK3β resembles the key junction between these two pathways; in addition to its role as a Wnt signaling inhibitor, GSK3β enhances the activity of the mTOR inhibitor TSC2 [87]. It was found that inhibition of Wnt signaling relieved autistic behaviors in part by suppressing mTOR signaling in the VPA rat model of ASD [32]. In another study [116], VPA caused macrocephaly in rats, a feature commonly observed in ASD patients [117]. In addition, VPA-exposed rats displayed increased proliferation of NPCs and a subsequent increase in the neuronal density of the cortical region, which is caused, at least partly, by the inhibitory phosphorylation of GSK-3β and the subsequent accumulation of β-catenin, which regulates several downstream proteins implicated in cell proliferation [116]. These data are consistent with a postmortem study that reported a significantly higher number of neurons in the PFC of autistic children compared to unaffected controls [118]. An in vitro study using reprogrammed neurons derived from human embryonic stem cells showed that VPA exposure elevated β-catenin levels, and that inhibition of GSK-3β and histone deacetylase (HDAC) caused neurite impairments similar to those caused by VPA exposure [119]. Other studies linked VPA teratogenicity to an imbalance in oxidative homeostasis, mediated at least in part by the upregulation of the Wnt/β-catenin signaling pathway [120, 121]. Together, this evidence supports a key role of GSK3β in the increased ASD susceptibility caused by prenatal VPA exposure.

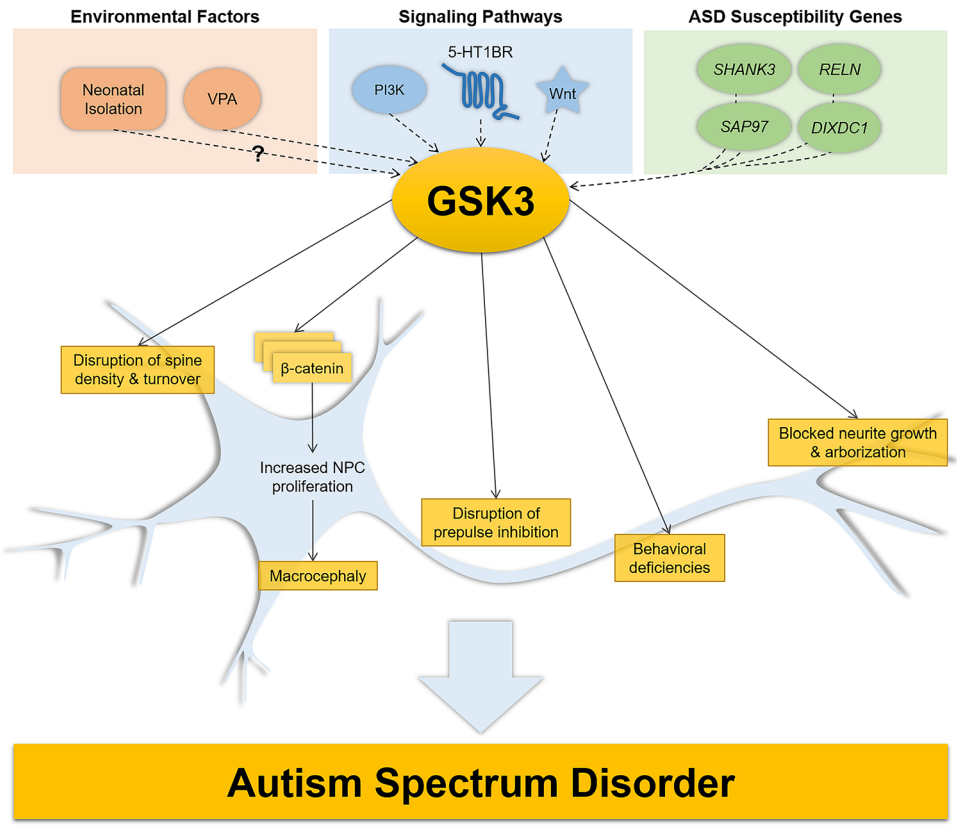

Fig. 3 Role of GSK3 in Autism Spectrum Disorder. Several ASD susceptibility genes, PI3K and Wnt signaling pathways, serotonin 1B receptor, prenatal exposure to valproic acid, and possibly neonatal isolation contribute to the pathophysiology of ASD at least in part by influencing the activity of GSK3. Disruption of GSK3 activity is associated with synaptic and behavioral deficiencies, in addition to disruption in prepulse inhibition and macrocephaly caused by the accumulation of β-catenin and the consequent increase in NPC proliferation

GSK3 and synaptic aberrations

Synaptic abnormalities are commonly observed in ASD patients, and various ASD susceptibility genes are involved in synaptic development and maturation [122–124]. Moreover, evidence support the implication of GSK3 suppression in the synaptic impairments observed in ASD. An in vitro study showed that three GSK3 inhibitors blocked neurite growth and arborization in cortical neurons derived from human pluripotent stem cells [125]. In another study, the knockout of GSK3β in a subset of cortical and hippocampal neurons caused a strong reduction in spine density and an increase in spine turnover [126].

GSK3 and ASD susceptibility genes

GSK3 was found to be regulated by many proteins known to be associated with ASD. Synapse-associated protein of 97 kDa molecular weight (SAP97) is a post-synaptic density (PSD) protein associated with ASD. In a study using HEK293 cells, it was found that SAP97 modified the phosphorylation state of GSK3β, resulting in the negative regulation of the Wnt/β-catenin signaling pathway. This might contribute to the mechanism by which SAP97 is implicated in ASD [127]. DIX Domain Containing 1 (*DIXDC1*) is another gene having higher numbers of rare sequence-disrupting single-nucleotide variants (SNVs) in ASD patients compared to controls. The systemic administration of lithium, which inhibits GSK3, or a selective small molecule GSK3 inhibitor, rescued the synaptic and behavioral impairments observed in Dixdc1KO mouse model, suggesting the implication of GSK3 in DIXDC1 contribution to ASD [128]. *SHANK3* is another ASD candidate gene the mutation of which is used to mimic ASD in mice [129, 130]. The *Shank3b*$^{-/-}$ model showed reductions in the spine density and stubby spines, impairments of the post-synaptic density, and reductions in c-Fos expression in response to social stimulus in the Anterior Cingulate Cortex (ACC). Interestingly, these results were associated with a decrease in the levels of phosphorylated GSK3β, and the expression of a constitutively active GSK3β in the ACC rescued the synaptic and social deficiencies [131].

Finally, several studies implicated Reelin, an upstream regulator of GSK3β, in the pathogenesis of ASD [132]. In a postmortem study to investigate the involvement of Reelin and its downstream proteins in ASD, no significant differences were observed between the levels of GSK3β in the cerebellum or the superior frontal cortex of autistic individuals compared to matched controls. However, this study did not measure the levels of GSK3β phosphorylation, which might be disrupted as observed in other studies [133]. Moreover, another study showed that multiple commonly used drugs in the treatment of neuropsychiatric diseases, including ASD, affected the levels of GSK3β in the context of Reelin signaling [134].

GSK3 and serotonergic transmission

Abnormalities in serotonergic transmission have been linked to autism [135]. One study investigated the role of GSK3β in the behavioral abnormalities associated with serotonin-1B receptors (5-HT1BRs). It showed that the pharmacological inhibition of GSK3β partially blocked prepulse inhibition (PPI) deficits induced by a 5-HT1BR agonist [136]. Another study showed that the genetic and pharmacological inhibition of GSK3β rescued the behavioral abnormalities induced by serotonin deficiency, including aggression, as well as depression- and anxiety-like behaviors [137].

GSK3 and neonatal isolation

One study showed that lithium ameliorates autistic-like features observed in rats exposed to neonatal isolation. However, this study did not evaluate the effect of lithium on GSK3 activity, and whether the observed effects are attributed to the known inhibitory effects of lithium on GSK3 [138].

GSK3 and ASD-related syndromes

GSK3 and Fragile X Syndrome

Fragile X Syndrome (FXS) is the most common inherited cause of intellectual disability and autism. It is caused by mutations in fragile X mental retardation 1 (*FMR1*) gene, leading to the loss of fragile X mental retardation protein (FMRP), an RNA-binding protein [139, 140]. FMRP is a translational repressor that acts as an inhibitor of mGluR-dependent long-term depression (LTD) and protein synthesis, and it is now known that the mGluR signaling is upregulated in FXS [141].

A growing body of evidence supports an increase in the activity of GSK3 in FXS due to the reduced levels of inhibitory serine phosphorylation [142–146], although total levels of both α and β isoforms are comparable between FXS and unaffected subjects [144]. One study used GSK3 knockin mice, in which inhibitory serines of both isoforms were mutated into alanines, rendering them constitutively active. These mice displayed decreased social preference to novel subjects, similar to what is observed in *Fmr1* KO mice, suggesting that decreased inhibition of GSK3 plays a role in social preference's impairment [147].

Moreover, multiple studies using *Fmr1* KO mice showed that lithium, 2-methyl-6-(phenylethynyl) pyridine (MPEP) (a mGluR inhibitor), and several GSK3 inhibitors increased

the serine phosphorylation of GSK3 in various brain regions [143, 144, 146, 147]. Furthermore, the co-administration of MPEP and GSK3 inhibitor did not produce an additive effect, which suggests that GSK3 acts as a downstream effector of mGluR [144]. The administration of lithium or various GSK3 inhibitors was shown to improve the aberrant phenotypes observed in FXS mice, including social anxiety, susceptibility to audiogenic seizures, astrogliosis, hyperactivity, and/or alterations in elevated plus-maze and passive avoidance [144, 146–148]. In a novel approach, Pardo et al. reported that the intranasal administration of siRNAs targeting GSK3β restored learning and memory impairments in FXS mice [149].

One study reported that FMRP loss caused an impairment in adult NPC proliferation and fate specification in the hippocampus of FXS mice, which is partly mediated by Wnt signaling downregulation due to GSK3β upregulation [150]. Consistently, the GSK3 inhibitor SB216763 rescued hippocampus-dependent learning and ameliorated neurogenesis deficits, dendritic morphogenesis, and integration of newly formed neurons into the hippocampus of FXS mice [143]. Moreover, FMRP deletion impaired late-phase LTP in the anterior cingulate cortex, which was rescued by the inhibition of mGluR5 or GSK3 [151]. Also, GSK3 inhibition rescued the cognitive deficits and LTP impairments observed at the medial perforant path-dentate granule cell synapses in the hippocampi of FX mice, and also improved several cognitive tasks in FX mouse model, such as object detection, spatial learning task and visual object task [142]. The nicotine metabolite cotinine emerged as a potential regulator of GSK3. Cotinine mediates its effects through binding to nicotinic acetylcholine receptors (nAChRs) [152], and its administration to *Fmr1* KO mice increased serine phosphorylation of GSK3β through activation of Akt in the mouse hippocampus, resulting in improvements in learning and memory of these animals [145].

However, the translation of preclinical findings into clinical trials has been challenging, due to the side effects mostly arising from GSK3β inhibition, and the subsequent β-catenin accumulation inducing increased gene expression. In an attempt to overcome this problem, McCamphill et al. developed paralog-selective GSK3 inhibitors, and found that, in addition to improving learning and memory, the inhibition of GSK3α, but not GSK3β, reversed the pathologically elevated protein synthesis, reduced susceptibility to audiogenic seizures, corrected cortical hyperexcitability, and impaired mGluR5-dependent and protein synthesis-dependent LTD in *FMR1*$^{-/y}$ mouse model of FXS [153].

GSK3 and Rett syndrome

Rett syndrome (RTT) is a neurodevelopmental disorder caused by loss-of-function mutations in the X-linked *MECP2* gene that encodes methyl-CpG binding protein 2 (MeCP2) [154]. It almost exclusively affects female offspring in a prevalence of 1 in 15,000 female birth, though mutations in *MECP2* gene were also reported in male patients [155]. Patients show normal mental and neurological development during their first year of life, which is gradually followed by developmental stagnation and regression of acquired motor and verbal skills, ataxia and seizure [156, 157].

As *MECP2* mutation is the main genetic factor underlying RTT [158], *MeCP2* mutant animal models have been used to provide more information about RTT and its treatment options. MeCP2 deficiency in *MeCP2* knockout mice was rescued upon using a specific GSK3β inhibitor [159]. Inhibition of GSK3β in these mice has prolonged the life span, alleviated motor deficits, decreased neuroinflammation, and rescued dendritic network and spine density [159]. Tang et al. reported that the injection of GSK3β inhibitors improved the disease-related behavioral deficits in *MeCP2* mutant mice [160]. Another study reported a low level of Ser9 GSK3β phosphorylation but high β-catenin phosphorylation level in MeCP2 T158A mice model of RTT, which were restored back to normal levels by lenti-Wnt6 transduction in addition to reduced locomotor impairment and social behavior deficits [161]. These data suggest that targeting GSK3β could be a promising strategy for RTT treatment.

Pharmacological regulation of GSK3

GSK3 was first identified as a key enzyme in glycogen synthesis in the late 1970s [38, 55]. However, this enzyme has gained a renewed interest due to the promising potential of its inhibitors for the treatment of a wide range of diseases including diabetes, inflammation, neurodegenerative diseases, psychiatric disorders and cancer [162]. There are several chemical categories of GSK3 inhibitors (Table 1) with varying selectivity and with noteworthy therapeutic effects for the treatment of diseases that have no current effective treatment [37].

Lithium, a cation GSK3 inhibitor, was medically prescribed for manic-depression before it was known to inhibit the activity of GSK3 [163, 164]. Lithium inhibits GSK3 directly by competition for magnesium (Mg^{2+}) [165], or indirectly through serine phosphorylation of both GSK3 isoforms [166]. Furthermore, lithium can inhibit GSK3 within protein complexes [167]. It has been in clinical use for a significant time despite the fact that it lacks target specificity and shows clinical side effects and a high toxicity level [162].

Another group of GSK3 inhibitors comprises ATP-competitive compounds that can establish hydrogen bonds with the backbone atoms of Asp133 and Val135 [168] and decrease the phosphorylation of Tyr279 and Tyr216 in GSK3α and GSK3β

Table 1 In vitro and in vivo studies on drugs targeting GSK3 in ASD, FXS, and RTT

Drug	Drug's category	Mode(s) of action	Disorder	In vitro studies: cell lines used	In vivo studies: Animal model used	Effect(s)	Reference
Lithium	Cation/small molecule inhibitor	Competes for Mg^{2+} Phosphorylates serine residue of GSK3 Forms protein complexes	ASD		Young adult rats with neonatal isolation	Rescued autistic-like behaviors induced by neonatal-isolation Ameliorated abnormal repetitive self-grooming behavior Reduced anxiety and depressive behaviors Restored hippocampal neurogenesis	[138]
					*Dixdc1*KO mice	Corrected forced swim test (FST) and social interactions in pairs test (SIP) behaviors Rescued dendritic spine density, spine morphology and glutamatergic synapse density in L5/6 pyramidal neurons within Dixdc1KO mice brains	[128]
			FXS		FX mice	Rescued the deficits in LTP at MPP-DGC synapses	[142]
			FXS		*Fmr1* knockout mice	Increased sociability Ameliorated several behavioral impairments Reduced anxiety-related behaviors	[147]
			FXS		*Fmr1* knockout mice	Restored elevated GSK3 activity to the normal Reduced the number and lethality of audiogenic seizures Shifted the behavior towards that of wild-type mice	[144]

Table 1 (continued)

Drug	Drug's category	Mode(s) of action	Disorder	In vitro studies: cell lines used	In vivo studies: Animal model used	Effect(s)	Reference
SB216763	ATP-competitive GSK3 inhibitor	Competes with ATP at the active center of GSK3	RTT	Whole-brain primary neuronal cultures from *Mecp2 knockout mice*	*Mecp2* knockout mice	Prolonged the survival of *Mecp2* knockout mice Improved RTT symptoms Decreased Nfkb1 signaling and neuroinflammation Rescued dendritic networks, spine density and synaptic activity in vitro	[159]
			FXS		*Fmr1* knockout mice	Reversed hippocampus-dependent learning deficits Rescued hippocampal neurogenesis Improved neuronal maturation of new neurons by enhancing dendritic complexity	[143]
			FXS	aNPC isolated from *Fmr1* knockout mice	*Fmr1* knockout mice	Enhanced Wnt signaling pathway Rescued the neuronal and astrocytes differentiation deficits	[150]
SB415286	ATP-competitive GSK3 inhibitor	Competes with ATP at the active center of GSK3	FXS		*Fmr1* knockout mice	Rescued L-LTP and network recruitment Improved trace fear memory	[151]
CT99021	ATP-competitive GSK3 inhibitor	Competes with ATP at the active center of GSK3	FXS		*Fmr1* knockout mice	Rescued L-LTP and network recruitment Improved trace fear memory	[151]

Table 1 (continued)

Drug	Drug's category	Mode(s) of action	Disorder	In vitro studies: cell lines used	In vivo studies: Animal model used	Effect(s)	Reference
KEECs (BIO: 6-bromoindirubin-3′-oxime)	ATP-competitive GSK3 inhibitor	Competes with ATP at the active center of GSK3	RTT	Human RTT syndrome neurons	*Mecp2* mutant mice	Enhanced KCC2 expression in human RTT neurons Rescued functional and morphological deficits in RTT neurons (GABA functional switch and excitatory glutamatergic synapses) Improved disease-associated respiratory and locomotion phenotypes in vivo	[160]
BRD0705 (Selective inhibitor of GSK3α)	ATP-competitive GSK3 inhibitor	Competes with ATP at the active center of GSK3 Impairs GSK3α Tyr279 phosphorylation	FXS		$Fmr1^{-/y}$ mice	Reduced susceptibility to audiogenic seizure Reduced FMRP synthesis Corrected cortical hyperexcitability Rescued deficits in learning and memory	[153]
Cotinine	ATP-competitive GSK3 inhibitor	Increases phosphorylation of GSK3 and AKT	FXS		$Fmr1^{-/-}$ knockout mice	Improved cognitive functions	[145]
Thiadiazolinones (TDZD)	ATP-noncompetitive GSK3 inhibitor	Phosphorylates Ser9 of GSK3β Interacts with Cys199	FXS		FX mice	Reversed learning deficits Ameliorated cognitive impairments	[142]

aNPCs: Adult neural progenitor/stem cells; *FMR1*: Fragile X mental retardation 1 gene; FMRP: Fragile X mental retardation protein; FXS: Fragile X syndrome; KCC2: K^+/Cl^- cotransporter 2; KEEC: KCC2 expression-enhancing compounds; LTP: Long term potentiation; L-LTP: Late phase-long term potentiation; Mecp2: Methyl CpG binding protein 2; MPP-DGC: Medial perforant path synapses onto dentate granule cells; RTT: Rett syndrome

respectively [169]. Such types of inhibitors target ATP binding site and compete with ATP at the active center of GSK3 [169, 170]. However, such inhibitors have adverse effects when used in chronic treatment and exhibit minimal activity toward other protein kinases such as protein kinase B (PKB) and 3-phosphoinositide-dependent protein kinase 1 (PDK1) [171]. On the other hand, ATP-noncompetitive GSK3 inhibitors show numerous advantages over the ATP-competitive inhibitors, mainly in selectivity since they bind to specific regions within the kinase providing more modulation of kinase activity than simply blocking ATP pocket [162]. This issue is of critical importance for GSK3-activity modulation since only the aberrant GSK3 activity should be targeted. Thiadiazolinones (TDZD) family were reported to inhibit GSK3 selectively in an ATP-noncompetitive manner and showed no interference with other kinases including PKA, PKC, casein kinase II (CK-2) and cyclin-dependent kinase1 (CDK1) [37]. Is has been postulated that the mechanism of TDZD action is mediated by the phosphorylation at ser9 and hence inhibition of GSK3 activity [172], or by covalent interaction with cys199 residue, a key amino acid in the active site of GSK3 [173]. Because ATP-noncompetitive compounds display a high selective mode of action, they may be a favorable choice for clinical use.

Challenges

Cumulative data now suggest a promising therapeutic potential of GSK3 inhibitors. However, many concerns have been raised regarding the safety profile and toxicity of these chemicals such as tumorigeneses and neuronal deregulation [174]. Moreover, our knowledge regarding the side effects of GSK3 inhibitors in humans is rather scarce because a very limited number of these compounds have reached the clinical phase. Nonetheless, GSK3 activity is dysregulated in many pathological conditions, so precise modulation to restore the activity into the normal physiological one would be enough to produce a potential therapeutic effect and avoid adverse effects [175]. Another challenge to overcome for GSK3 inhibitors in neurological disorders is the blood–brain barrier permeability [176]. In addition, the long-term effects of GSK3 inhibitors must be studied in the case of chronic treatment. Therefore, using selective GSK3 inhibitors with convenient pharmacokinetic/dynamic properties and excellent blood brain barrier permeability may hold a high therapeutic potential for the treatment of neurological disorders including ASD and its related disorders.

Conclusion and Future Perspectives

A number of key signaling molecules and hence signaling pathways are differentially perturbed in ASD. Several lines of evidence have identified the dysregulation of GSK3 as highly associated with the development of ASD. Accordingly, targeting GSK3 activity has been deemed an important approach for ASD treatment. However, the clinical translation of GSK3 therapy could be highly challenging since GSK3 is implicated in numerous signaling pathways and developmental processes. Despite the big gap in our understanding of ASD development, studies are still mandatory for a better characterization of GSK3 regulation in ASD in order to develop treatments taking into consideration that GSK3 targeting drugs should be highly selective, safe, and blood brain barrier penetrable. Nevertheless, further investigation is needed to elucidate the specific roles of the different GSK3 isoforms in ASD pathophysiology, leading to the development of isoform-specific interventions that avoid the toxicity caused by manipulating the activity of both isoforms.

Authors' contributions HFB and SN conceived the concept and idea of the present review. HFB and SN worked on the study design strategy and selected the topics to be discussed. MR, ZS, HFB and SN did literature searches and screened titles and abstracts for relevance. MR and ZS abstracted the data from the eligible full text articles, analyzed and interpreted the data, and drafted the manuscript. HH and YF revised the final draft of the manuscript. HFB and SN critically revised the manuscript with input from the entire team. All authors have read and approved the final draft.

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