HIGHLIGHTS

Regenerative potential of targeting glycogen synthase kinase-3 signaling in neural tissues

Multiple roles of glycogen synthase kinase-3 (GSK-3) in neural tissues: GSK-3 is a serine/threonine kinase that has two isoforms encoded by two different genes, GSK-3α and GSK-3β, in mammals. GSK-3 has several sites of serine and tyrosine phosphorylation. Its activity is negatively regulated by phosphorylation of serine 21 for GSK-3α and serine 9 for GSK-3β, while it is positively regulated by phosphorylation of tyrosine 279 for GSK-3α and tyrosine 216 for GSK-3β. GSK-3 was initially found to be an important component of glycogen metabolism. However, recent studies have revealed that GSK-3 is a multifunctional kinase in various cell types, including neural cells. GSK-3α and GSK-3β are highly expressed in neural tissues such as the cerebral cortex, the hippocampus, the cerebellum, and the spinal cord. In particular, GSK-3β is elevated in the aged hippocampus, and more abundant than GSK-3α in rodents (Salcedo-Tello et al., 2011). Also, GSK-3β is highly expressed in neurons and astrocytes in the developing brain and spinal cord. In neurons, GSK-3 directly leads to the phosphorylation of several neuronal microtubule-associated proteins (MAPs), especially microtubule plus-end tracking proteins (+TIP), including collapsin response mediator protein-2 (CRMP-2), adenomatous polyposis coli (APC), cytoplasmic linker associated protein (CLASP), MAP1B, MAP2, microtubule actin cross-linking factor 1 (MACF1), and Tau (Kim and Snider, 2011). GSK-3 phosphorylation of primed-MAPs generally decreases their activity and thus leads to a decrease in microtubule stability in neurons. Localized inhibition of GSK-3 activity at the axon terminal is required for axon growth during development and regeneration after injury (Alabed et al., 2010). Meanwhile, phosphorylation by GSK-3 activates some unprimed-substrates such as MAP1B, which stabilizes microtubules for axon extension. This is why global inhibition of GSK-3 at a high degree using pharmacological inhibitors or genetic elimination of both isoforms suppresses axon growth (Kim et al., 2006). GSK-3 is also a master regulator of neural stem cell proliferation and differentiation. Loss of both GSK-3 alleles leads to an increase in neural stem cell and progenitor proliferation (Kim et al., 2009). Similarly, pharmacological inhibition of GSK-3 by SB-216763 maintains pluripotency in neural stem cells. Additionally, GSK-3 plays an important role in astrocyte and oligodendrocyte development. The rate of astrocyte apoptosis is increased by overexpression of a constitutively-active GSK-3β mutant in primary cortical astrocytes. Both the number and size of astrocytes are significantly increased in GSK-3 mutant mice when both GSK-3 isoforms are genetically eliminated in astrocyte progenitors and mature astrocytes using a GFAP-cre driver (Jung et al., 2015). Also, pharmacological inhibition of GSK-3 with lithium and indirubin results in increased numbers of oligodendrocyte progenitors and mature oligodendrocytes. Finally, elevated GSK-3 activity is correlated with neuronal death. For example, overexpression of GSK-3β significantly increases neuronal cell death, and pharmacological inhibition of GSK-3 promotes the survival of several types of neural cells. Therefore, GSK-3 is a major factor in many facets of neural cell regulation, such as neurogenesis, neural stem cell proliferation, neural cell death, neuronal differentiation, and gliogenesis (Kim and Snider, 2011).

GSK-3 signaling in neural cells: GSK-3 is a key molecule in multiple signaling pathways including WNT/β-catenin, growth factor, hedgehog, phosphoinositide 3-kinase (PI3K)/AKT, and mammalian target of rapamycin (mTOR) signaling pathways in the nervous system (**Figure 1**) (Kim et al., 2009; Kim and Snider, 2011; Ka et al., 2014). The WNT/β-catenin pathway has been established as a traditional player in GSK-3 signaling. Recently, however, the AKT/mTOR pathway has emerged as another crucial mediator of GSK-3 signaling in neural cell survival, proliferation, and differentiation. In GSK-3 null and conditional knockout brains, phosphorylation levels of mTOR downstream targets are significantly increased (Ka et al., 2014). Treatment of wild type mice with GSK-3 inhibitors likewise leads to increases in mTOR-target phosphorylation. Furthermore, overexpression of GSK-3 decreases mTOR activity. In addition, inhibiting mTOR with rapamycin prevents hyperproliferation of GSK-3 mutant neural progenitors (Ka et al., 2014). Although GSK-3 and mTOR show no direct interaction, GSK-3 regulates mTOR *via* activation of the mTOR upstream negative regulator tuberous sclerosis complex 2 (TSC2) in neural progenitors (Ka et al., 2014). The mTOR pathway participates in the synthesis of several proteins through its phosphorylation and activation of ribosomal S6 kinase. GSK-3 participation in mTOR signaling is negatively regulated by PI3K-mediated activation of AKT. The AKT pathway induces phosphorylation of GSK-3α at serine 21 and of GSK-3β at serine 9, resulting in the inhibition of GSK-3 activity. Elimination of both GSK-3 isoforms in neural progenitors results in larger brains in mice (Kim et al., 2009), while overexpression of GSK-3β decreases brain size in transgenic mice (Spittaels et al., 2002). Similarly, GSK-3 deletion in astrocyte progenitors increases brain size and induces activation of the AKT/mTOR pathway and signal transducer and activator of transcription 3 (STAT3) (Jung et al., 2015). Loss of other AKT/mTOR components, such as phosphatase and tensin homolog (PTEN) and tuberous sclerosis-1 (TSC1), which are positively linked to GSK-3 signaling, also increase brain size in mice. Thus, in addition to the WNT pathway, the AKT/mTOR pathway is an integral part of GSK-3 signaling in neural cells and renders a potential target of manipulating GSK-3 signaling. Pharmacological targeting of other GSK-3-associated pathways such as the WNT, hedgehog and notch pathways has been unsuccessful. However, pharmacological drugs for the AKT/mTOR pathway are currently in clinical uses for multiple diseases. Disrupted in schizophrenia 1 (DISC1) is an important protein in the development of postmitotic neurons and is also associated with GSK-3 signaling. DISC1 regulates the proliferation and differentiation of embryonic and adult neural progenitors through the GSK-3/β-catenin pathway (Ming and Song, 2009). Interestingly, inactivation of GSK-3 by the selective GSK-3 inhibitor TDZD-8 reverses prepulse inhibition and rescues the hyperactivity of DISC1 mutants.

GSK-3 effects on behavior: GSK-3 plays an important role in the behavioral mechanisms underlying several psychiatric disorders. Specifically, the AKT/mTOR/GSK-3 signaling pathway is involved in the progression of depression, trauma, and bipolar disorder. Patients with bipolar disorder show higher GSK-3 levels than healthy people. Interestingly, circadian rhythm is implicated in the generation of bipolar disorder, and inhibition of GSK-3 activity by lithium is thought to alleviate bipolar symptoms by stabilizing circadian rhythms (Klemfuss, 1992). For example, lithium increases the period of their sleep/wake cycle (Hickie et al., 2013). Furthermore, lithium-treated cells show rapid proteasomal degradation of Rev-erbα, a negative component of the circadian clock, and activation of clock gene Bmal1 (Yin et al., 2006). Thus, GSK-3 inhibition may be an important tool for the treatment of abnormal circadian rhythms and bipolar disorder. Mice lacking GSK-3α show decreased social motivation and novelty, reduced aggression, and decreased locomotion (Kaidanovich-Beilin et al., 2009). They exhibit weaker interactions with unfamiliar mice in both sociability and social novelty tests, compared with wild-type mice. Heterozygote GSK-3β mice display increased anxiety and reduced exploration (Bersudsky et al., 2008). However, deletion of GSK-3β in forebrain pyramidal neurons with a CamKII-Cre driver induces anxiolytic and pro-social behavior (Latapy et al., 2012). It is unclear why global and regional-specific deletions of GSK-3β lead to seemingly opposite behavioral outcomes. Ventricular forebrain or midbrain areas may contribute to GSK-3-assoicated behavior. Inhibition of both GSK-3 isoforms using GSK-3 knock-in mice, in which the inhibitory serines of GSK-3α and GSK-3β are mutated to alanines, show decreased social novelty (Mines et al., 2010). Consistently, a recent study has shown that deletion of both GSK-3 isoforms in astrocytes using a GFAP-Cre driver results in a decrease in social interaction and social novelty in mice (Jung et al., 2015). It is interesting that these mice display reduced anxiety in elevated plus maze

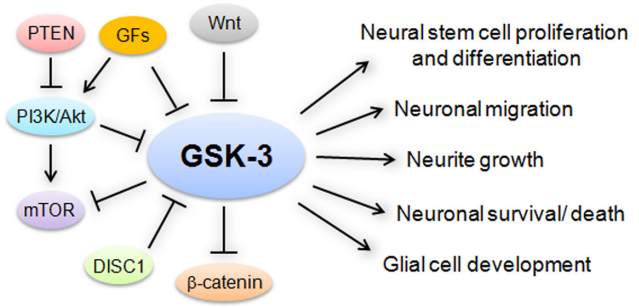

Figure 1 Glycogen synthase kinase-3 (GSK-3) and associated molecules in neural development.
GSK-3 is a key regulator in multiple aspects of neural development and mediates important cellular signaling including PI3K/AKT and WNT pathways. DISC1: Disrupted in schizophrenia 1; GFs: growth factors; mTOR: mammalian target of rapamycin; PI3K/Akt: phosphoinositide 3-kinase/Akt; PTEN: phosphatase and tensin homolog.

tests, which phenocopies the anxiety behavior of GSK-3β/CamKII-Cre mice but differs from the elevated anxiety behavior detected in heterozygote GSK-3β mice. Memory consolidation is reduced in heterozygote GSK-3β mice. GSK-3α null mice also have impaired associative memory, as seen in Pavlovian fear conditioning. GSK-3β overexpression in transgenic lines impairs spatial learning and decreases the acquisition of reference memory in a novel object recognition task. Thus, therapies modulating GSK-3 activities may be explored in nervous system disorders in the future.

Mechanisms of pharmacological GSK-3 inhibitors: Because of the role of GSK-3 in multiple facets of neural development, manipulation of GSK-3 activity may be an important therapeutic tool for neurodevelopmental and neurodegenerative diseases. The pharmaceutical industry has been developing pharmacological GSK-3 drugs. GSK-3 inhibitors can be classified into three basic types: ATP-competitive, non-ATP-competitive, and cationic inhibitors. Numerous inhibitors of each type have been developed and tested, but we will only discuss one or two representative examples from each category in this review, which demonstrate the known mechanisms for GSK-3 inhibition. ATP-competitive inhibitors of GSK-3, as their name suggests, act by blocking the ATP-binding site on GSK-3. One important group of ATP-competitive GSK-3 inhibitors is indirubin and its derivatives. Indirubins have been shown to inhibit neurite outgrowth and reduce tau phosphorylation in neurons. Indirubins also suppress the activity of cyclin-dependent kinases along a similar mechanism, though synthetic indirubin derivatives, such as 6-bromoindirubin-3′-oxime (6BIO) are substantially more selective for GSK-3 than for cyclin-dependent kinases (CDKs). Another relevant ATP-competitive inhibitor of GSK-3 is arylindole-maleimide SB-216763. This small molecule exhibits extensive neuroprotective properties and inhibits axon outgrowth in postnatal and embryonic dorsal root ganglion neurons. However, some studies have reported contradictory results that treatment with SB-216763 promotes axon outgrowth and axon regeneration following neuronal lesion. This discrepancy is probably dependent on the concentration of the inhibitor used. In addition to the multiple ATP-competitive GSK-3 inhibitors, a large assortment of non-ATP-competitive GSK-3 inhibitors has been discovered. One example from this group is TDZD-8, a thiadiazolidinone. TDZD-8 has proven to almost exclusively inhibit GSK-3 and has been shown to decrease tau phosphorylation, as well as to provide neuroprotective properties (Eldar-Finkelman and Martinez, 2011). A recent study has also demonstrated that TDZD-8 treatment improves the performance of mice with elevated levels of GSK-3 in behavioral tasks, such as novel object recognition and temporal ordering. Another widely-used non-ATP-competitive GSK-3 inhibitor is the peptide L803-mts, which binds to GSK-3 near its phosphate-binding pocket and blocks substrate-kinase interactions. Accordingly, L803-mts is very selective for GSK-3 and delivers both neuroprotective and antidepressive effects in mice. Among all inhibitors of GSK-3, a cation inhibitor lithium is most widely used. Lithium inhibits GSK-3 directly *via* competition with magnesium ions, thus inhibiting GSK-3-induced phosphorylation. Lithium was shown to modify axon length, increase growth cone area, and increase synapse formation in mouse brains. Moreover, lithium reduces the severity and frequency of mania in human bipolar disorder. Secretion of amyloid precursor protein and amyloid beta peptide are negatively regulated by lithiumin animal models. In patients with Alzheimer's disease, the treatment with microdose lithium slightly improves performance in the mini-mental state examination test (Pei et al., 1997). Some side effects have unfortunately been reported in patients treated with lithium, including ataxia, dysarthria, EEG changes, coma, sleep disturbances, and seizures. Mechanistic insights of GSK-3 modulation remain further elucidated with regard to the development of effective therapeutic tools for GSK-3-associated neurological diseases.

Eui-Man Jung, Jeffrey J. Moffat, Woo-Yang Kim*

Department of Developmental Neuroscience, Munroe-Meyer Institute, University of Nebraska Medical Center, Omaha, NE, USA

***Correspondence to:** *Woo-Yang Kim, Ph.D., wooyang.kim@unmc.edu.*
***Accepted:** *2015-09-18*
***orcid:** *0000-0003-3938-6062 (Woo-Yang Kim)*
***doi:** *10.4103/1673-5374.169627* **http**://www.nrronline.org/
Jung EM, Moffat JJ, Kim WY (2015) Regenerative potential of targeting glycogen synthase kinase-3 signaling in neural tissues. Neural Regen Res 10(12):1912-1913.

References

Alabed YZ, Pool M, Ong Tone S, Sutherland C, Fournier AE (2010) GSK3 beta regulates myelin-dependent axon outgrowth inhibition through CRMP4. J Neurosci 30:5635-5643.
Bersudsky Y, Shaldubina A, Kozlovsky N, Woodgett JR, Agam G, Belmaker RH (2008) Glycogen synthase kinase-3beta heterozygote knockout mice as a model of findings in postmortem schizophrenia brain or as a model of behaviors mimicking lithium action: negative results. Behav Pharmacol 19:217-224.
Eldar-Finkelman H, Martinez A (2011) GSK-3 inhibitors: preclinical and clinical focus on CNS. Front Mol Neurosci 4:32.
Hickie IB, Naismith SL, Robillard R, Scott EM, Hermens DF (2013) Manipulating the sleep-wake cycle and circadian rhythms to improve clinical management of major depression. BMC Med 11:79.
Jung EM, Ka M, Kim WY (2015) Loss of GSK-3 causes abnormal astrogenesis and behavior in mice. Mol Neurobiol DOI: 10.1007/s12035-015-9326-8.
Ka M, Condorelli G, Woodgett JR, Kim WY (2014) mTOR regulates brain morphogenesis by mediating GSK3 signaling. Development 141:4076-4086.
Kaidanovich-Beilin O, Lipina TV, Takao K, van Eede M, Hattori S, Laliberte C, Khan M, Okamoto K, Chambers JW, Fletcher PJ, MacAulay K, Doble BW, Henkelman M, Miyakawa T, Roder J, Woodgett JR (2009) Abnormalities in brain structure and behavior in GSK-3alpha mutant mice. Mol Brain 2:35.
Kim WY, Snider WD (2011) Functions of GSK-3 signaling in development of the nervous system. Front Mol Neurosci 4:44.
Kim WY, Wang X, Wu Y, Doble BW, Patel S, Woodgett JR, Snider WD (2009) GSK-3 is a master regulator of neural progenitor homeostasis. Nat Neurosci 12:1390-1397.
Kim WY, Zhou FQ, Zhou J, Yokota Y, Wang YM, Yoshimura T, Kaibuchi K, Woodgett JR, Anton ES, Snider WD (2006) Essential roles for GSK-3s and GSK-3-primed substrates in neurotrophin-induced and hippocampal axon growth. Neuron 52:981-996.
Klemfuss H (1992) Rhythms and the pharmacology of lithium. Pharmacol Ther 56:53-78.
Latapy C, Rioux V, Guitton MJ, Beaulieu JM (2012) Selective deletion of forebrain glycogen synthase kinase 3beta reveals a central role in serotonin-sensitive anxiety and social behaviour. Philos Trans R Soc Lond B Biol Sci 367:2460-2474.
Mines MA, Yuskaitis CJ, King MK, Beurel E, Jope RS (2010) GSK3 influences social preference and anxiety-related behaviors during social interaction in a mouse model of fragile X syndrome and autism. PLoS One 5:e9706.
Ming GL, Song H (2009) DISC1 partners with GSK3beta in neurogenesis. Cell 136:990-992.
Pei JJ, Tanaka T, Tung YC, Braak E, Iqbal K, Grundke-Iqbal I (1997) Distribution, levels, and activity of glycogen synthase kinase-3 in the Alzheimer disease brain. J Neuropathol Exp Neurol 56:70-78.
Salcedo-Tello P, Ortiz-Matamoros A, Arias C (2011) GSK3 function in the brain during development, neuronal plasticity, and neurodegeneration. Int J Alzheimers Dis 2011:189728.
Spittaels K, Van den Haute C, Van Dorpe J, Terwel D, Vandezande K, Lasrado R, Bruynseels K, Irizarry M, Verhoye M, Van Lint J, Vandenheede JR, Ashton D, Mercken M, Loos R, Hyman B, Van der Linden A, Geerts H, Van Leuven F (2002) Neonatal neuronal overexpression of glycogen synthase kinase-3 beta reduces brain size in transgenic mice. Neuroscience 113:797-808.
Yin L, Wang J, Klein PS, Lazar MA (2006) Nuclear receptor Rev-erbalpha is a critical lithium-sensitive component of the circadian clock. Science 311:1002-1005.