

Function of GSK-3 signaling in spinal cord injury (Review)

XIONG DONG^{*}, HONGXIANG HONG^{*} and ZHIMING CUI

Department of Spinal Surgery, The Second Affiliated Hospital of Nantong University, Nantong, Jiangsu 226001, P.R. China

Received January 6, 2023; Accepted August 10, 2023

DOI: 10.3892/etm.2023.12240

Abstract. Spinal cord injury (SCI) is a major social problem with a heavy burden on patient physiology and psychology. Glial scar formation and irreversible neuron loss are the two key points during SCI progression. During the acute phase of spinal cord injury, glial scars form, limiting the progression of inflammation. However, in the subacute or chronic phase, glial scarring inhibits axon regeneration. Following spinal cord injury, irreversible loss of neurons leads to further aggravation of spinal cord injury. Several therapies have been developed to improve either glial scar or neuron loss; however, few therapies reach the stage of clinical trials and there are no mainstream therapies for SCI. Exploring the key mechanism of SCI is crucial for finding further treatments. Glycogen synthase kinase-3 (GSK-3) is a widely expressed kinase with important physiological and pathophysiological functions *in vivo*. Dysfunction of the GSK-3 signaling pathway during SCI has been widely discussed for controlling neurite growth *in vitro* and *in vivo*, improving the proliferation and neuronal differentiation of endogenous neural stem cells and functional recovery from spinal cord injury. SCI can decrease the phosphorylated (p)/total (t)-GSK-3 β ratio, which leads to an increase in apoptosis, whereas treatment with GSK-3 inhibitors can promote neurogenesis. In addition, several therapies for the treatment of SCI involve signaling pathways associated with GSK-3. Furthermore, signaling pathways associated with GSK-3 also participate in the pathological process of neuropathic pain that remains following SCI. The present review summarized the roles of GSK-3 signaling in SCI to aid in the understanding of GSK-3 signaling during the pathological processes of SCI and to provide evidence for the development of comprehensive treatments.

Contents

1. Introduction
2. Mechanisms involved in SCI
3. GSK-3
4. Function and role of GSK-3 in SCI
5. Conclusion

1. Introduction

The CNS contains the brain and spinal cord from which the peripheral nerves branch and is safeguarded by the spinal cord, which encompasses the meninges, cerebrospinal fluid and spine. The spinal cord exerts important functions, including the regulation of motor and sensory functions (1,2). Spinal cord injury (SCI) is the most common disabling spinal injury; For the last 30 years, its global prevalence has increased from 236 to 1,298 cases per million populations. The estimated global rate of SCI falls between 250,000 and 500,000 individuals every year.

It can damage the normal anatomy of the spinal cord, leading to axonal rupture, neuronal degeneration and necrosis, inflammatory response and demyelination, ultimately leading to severe neurological dysfunction (3,4). SCI frequently results in sensorimotor disorders, autonomic changes and intractable pain; Spinal cord injury can also affect respiratory, urinary, and gastrointestinal functions and is one of the factors leading to the development of infection. After spinal cord injury, a large number of inflammatory substances are released into the blood and cause inflammation throughout the body. Thus seriously affecting the quality of life of patients (5). SCI is categorized into two types: Traumatic and non-traumatic. The former is more common and mainly caused by external physical impacts, such as vehicle accidents, violence and falls (1,6), whereas the latter is usually caused by compression of a tumor; the enlargement of some tumors can compress the spinal cord tissue, resulting in the destruction of the spinal cord tissue, resulting in clinical symptoms, vascular ischemia or congenital disease such as Spinal Bifida (7). The current review mainly focused on traumatic SCI. Following spinal cord injury, axons of the CNS fail to regenerate. By contrast, peripheral nervous system axons regenerate after injury and show restored function. The lack of CNS regeneration after injury may be associated with abnormal expression of specific molecules in myelin and glial scars in the CNS, including Nogo, oligodendroglia-myelin glycoproteins and myelin-associated

Correspondence to: Professor Zhiming Cui, Department of Spinal Surgery, The Second Affiliated Hospital of Nantong University, 6 North Hai-er-xiang Road, Nantong, Jiangsu 226001, P.R. China
E-mail: czmntyy@163.com

^{*}Contributed equally

Key words: spinal cord injury, glycogen synthase kinase-3, neurogenesis, glial scar

glycoproteins (8). A previous study reported that these molecules induce the activity of the Rho-Rho-associated protein containing kinase 2 (ROCKII) and glycogen synthase kinase-3 β (GSK-3 β) signaling pathways, leading to inhibition of axonal regeneration in the CNS (9). Thus, the Rho-ROCKII and/or GSK-3 β signaling pathways may be targets for restoring axon regeneration.

2. Mechanisms involved in SCI

The CNS is composed of neurons and glial cells; glial cells include astrocytes, microglia, oligodendrocytes and Schwann cells, and are crucial for proper CNS development and function (10). The interaction between neurons and glial cells plays an important role in the physiological processes of the central nervous system. The dysfunction of neurons and glial cells is one of the pathogenesis of neuro developmental disorders (11). Glial cells, mainly astrocytes, collaborate with neurons and vasculature to harvest nutrients from the bloodstream, thus providing metabolic sustenance to neurons (12). The myelinating glia of the CNS and the peripheral nervous system, oligodendrocytes and Schwann cells, respectively, contribute to the electrical insulation of axons, thus enabling swift signal transmission (13). Microglial cells are innate immune cells that reside in the CNS; they dynamically monitor the microenvironment of the CNS and contribute to the CNS homeostasis in physiological conditions, and are closely associated with neuroinflammation in pathological conditions (14).

The pathophysiological process of SCI is quite complex, involving the dysfunction of neurons and glial cells, which includes vascular responses, abnormal neuroinflammation, neuronal loss and demyelination (15,16). In addition, traumatic SCI can be divided into two phases: i) Irreversible primary injury, which happens at the moment of injury, and ii) secondary injury, which occurs within minutes following the primary injury (17). Spinal cord compression is the most common pathogenesis of spinal cord injury and persists after injury (18). Bleeding can occur in the early stages of an SCI, followed by disruption of the blood supply. The most common clinical manifestations immediately following injury are disruption of the spinal vascular supply and hypotension/hypoperfusion, resulting in hypovolemia, neurogenic shock and bradycardia due to spinal cord ischemia (19). Disturbance of blood flow following SCI leads to hypoxia and ischemic infarction. Specifically, these two conditions damage the metabolically higher gray matter; white matter and gray matter metabolism show different basic properties, but the responses to neuronal activity are qualitatively similar. The neurons in the damaged area are physically broken and the thickness of the myelin sheath is reduced (20). In addition, edema and macrophage accumulation in the damaged tissue exacerbate the deterioration of neuronal transmission. Secondary injuries can be caused by primary injuries and several pathophysiological mechanisms can come into play hours or days after an SCI occurs (21,22). Energy deficiency caused by ischemia and impaired perfusion at the cellular level is the most influential factor (23). Key changes have been identified, such as bleeding, demyelination, edema, cavity formation with axon and neuron necrosis, and a series of pathological changes such as neuron death and axon breaking in nerve tissue following SCI can

further increase infarction (24). Following secondary injury, increased free radical damage and lipid peroxidation in the cell membrane, as well as secondary injury signal cascade in the damaged tissue area, can eventually lead to the death of neurons (25). In addition, during the second injury, released toxic compounds stimulate the differentiation of neural stem/progenitor cells into astrocytes, leading to reactive astrogliosis and the transition from the inflammation phase to glial scar formation (Fig. 1) (26).

The poor prognosis of SCI may be, in large part, due to two critical factors, including glial scar formation and irreversible neuron loss, which work together to interrupt the neural pathway and lead to the damage of axon regeneration (27). In patients with spinal cord injury and in rodent models, obstruction of axon regeneration and its functional recovery has been shown to permanently inhibit regeneration of the spinal cord (28). The central idea of alleviating SCI is preventing, attenuating and reversing secondary injury and improving spinal cord neurological functions (1). Common treatments used in clinical practice include traditional drug therapy (1), surgery (29,30), cell transplantation (31-34), tissue engineering (35), cell therapy and nanomedicine (36). However, these treatments rarely recover SCIs completely and can only improve symptoms and reduce complications.

Glial scar formation. Glial cells of the CNS (mainly astrocytes) are abundant and their roles in sustaining the dynamic balance of the neuronal microenvironment and controlling blood flow are fundamental. Preservation of the blood-brain barrier and the malleability and purpose of the synapses must be regulated (37). Following SCI, the trauma activates resident astrocytes and pericytes, and recruits infiltrating fibroblasts and Schwann cells from the peripheral nervous system, leading to the formation of glial scars in the injured spinal cord (38,39). Fibroblasts and Schwann cells migrate into the epicenter of the lesion and contribute to the production of extracellular matrix (ECM) proteins, such as nestin, glial fibrillary acidic protein and proteins transported by the veins (40,41). The deposition of ECM components and the accumulation and activation of glial cells contribute to the formation of a glial scar around the periphery of the lesion. Other cells such as activated microglia and NG2 glia form a dense boundary that isolates the damaged area (42). The lesion core includes a mixture of mononuclear macrophages, activated fibroblasts and ECM proteins (43,44).

For decades, glial scars have been considered the main factor against spinal cord regeneration (45). The primary inhibitory ECM molecules that are produced by reactive astrocytes during glial scar formation include the chondroitinase enzyme, which acts on chondroitin. In animal models, treatment with chondroitinase following SCI exhibited axonal regeneration and functional recovery (46). In non-mammalian vertebrates such as zebrafish, a restricted amount of glial scarring demonstrated the regeneration of the spinal cord, accompanied by a considerable restoration of motor function (28,47). However, glial scar formation is also an essential event during SCI recovery. In the acute phase of SCI, the formation of a glial scar serves an important role in restricting the size of the primary injury. The glial scar limits excessive inflammation from the lesion to normal tissue, clears debris and repairs the blood-spinal cord barrier, which prevents the spread of toxic

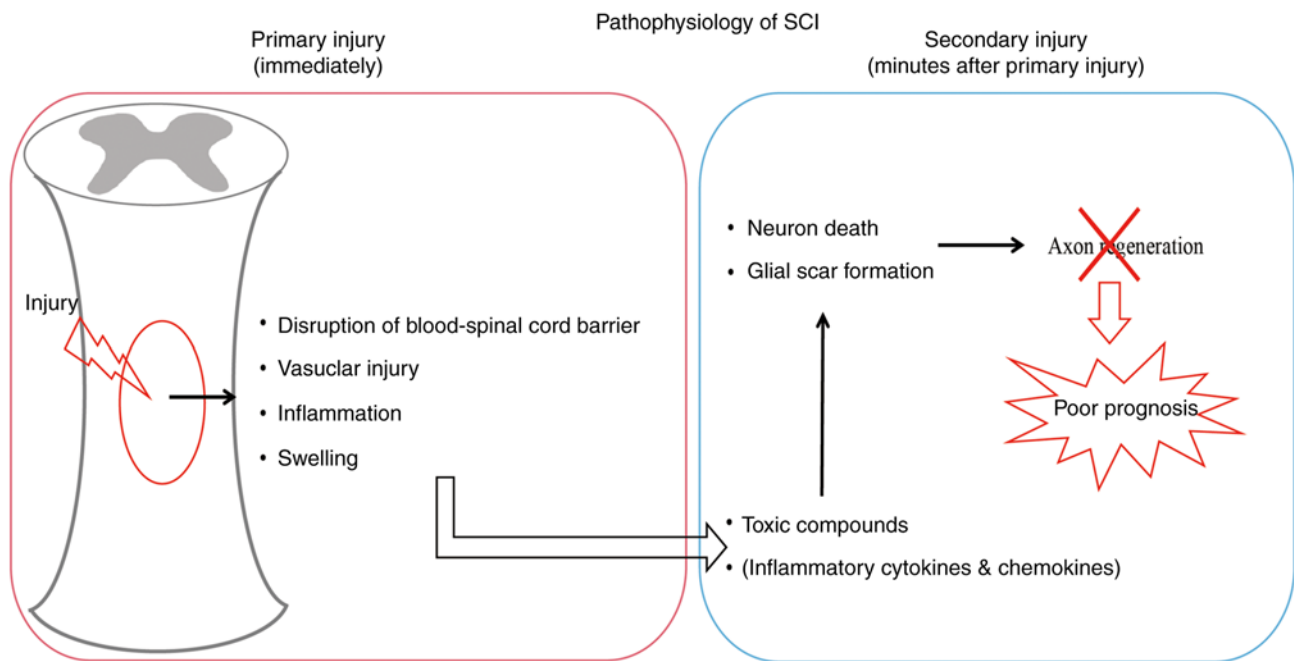


Figure 1. Mechanisms involved in SCI. Traumatic SCI can be divided into two phases, irreversible primary injury that happens immediately at the moment of injury, and secondary injury that occurs within minutes following the primary injury. Irreversible primary injury induced by mechanical injury will lead to the disruption of blood-spinal cord barrier, vascular injury, swelling and inflammation. Subsequently, the damaged neurons and glial cells will release toxic compounds, such as pro-inflammatory cytokines and chemokines, which in turn leads to the second injury with the death of most of cells. In addition, during the second injury, the released toxic compounds will stimulate the differentiation of neural stem/progenitor cells into astrocyte, leading to reactive astrogliosis and the transition from the inflammation phase to glial scar formation, resulting in a poor prognosis. SCI, spinal cord injury.

compounds to the surrounding tissue and the production of neurotrophins (48-50). In the sub-acute or chronic phase, the glial scar inhibits axonal regeneration, which has been shown to be harmful to the regeneration of the spinal cord (45). The dual role of the glial scar (both harmful and protective) during SCI makes it difficult to target the glial scar for therapeutic purposes (Fig. 2) (51).

Neuron loss. Irreversible neuron loss is another crucial part of SCI recovery. A combination of multiple causes, such as direct injury, inflammation, ischemia/reperfusion injury and neurotoxic cells, can lead to neuron loss (52,53). The primary sites of active neurogenesis in the adult brain are the subventricular zone of lateral ventricles and the subgranular zone of the dentate gyrus, which possess the capacity to generate all major neuronal phenotypes (54,55). However, neurons in the spinal cord have low regeneration and proliferation potential, and the vast majority of the adult spinal cord is composed of nerve cells, which mainly produce astrocytes and oligodendrocytes (56). Microglia are resident macrophages of the CNS and are essential in the control of damage repair, brain development and the upkeep of neuronal networks (57). Microglia activation is strongly associated with delayed neuronal loss in the peri-infarct area (58,59). Microglia are found only in the brain, retina and spinal cord (60). They are cells specialized in the phagocytosis and digestion of extracellular matter, including other cells. In normal tissues, microglia are highly differentiated, with elongated processes capable of engulfing smaller objects, such as synapses and fragments, but not larger objects such as neurons (61). However, when microglia are activated by inflammatory stimuli, they increase the expression of

opsonins, lysosomes and phagocytic receptors; in addition, the microglia process is retracted, thus producing a large moving cell body capable of phagocytosing neurons (62).

Insufficient neurogenesis in the adult spinal cord is a key challenge in reconstructing original neuronal networks; as such, neural repair and neuroregeneration after nerve repair is a key step in tissue repair following SCI. Various types of stem/progenitor cell therapy have been shown to have great development potential (63,64). Transplantation of cells is considered to be one of the most promising therapies for neuronal regeneration following SCI; this process includes direct injection/transplantation of olfactory ensheathing cells (65), intramedullary Schwann cell (66), embryonic (67) and mesenchymal stem cells (64,68). Although these therapies have demonstrated good therapeutic effects in several preclinical studies, some adverse reactions were found during clinical application. For instance, direct injection of olfactory ensheathing cells had serious side effects, such as syrinx formation, myelomalacia and perioperative morbidity, which limited its clinical application (69); in addition, intramedullary transplantation of Schwann cells can induce unsatisfactory motor and functional improvement (66), and the transplantation of embryonic stem cells also had severe risks such as the formation of teratomas (67), whereas mesenchymal stem cell transplantation could induce tumor formation (70,71). Neuronal reprogramming is a novel technology that can regenerate functional neurons from glial cells by overexpressing neurogenic transcription factors (such as NeuroD1) in several neurodegenerative disorders, including Huntington's and Alzheimer's diseases (72-75). Here, an adeno-associated virus is used to overexpress NeuroD1 to the convert reactive

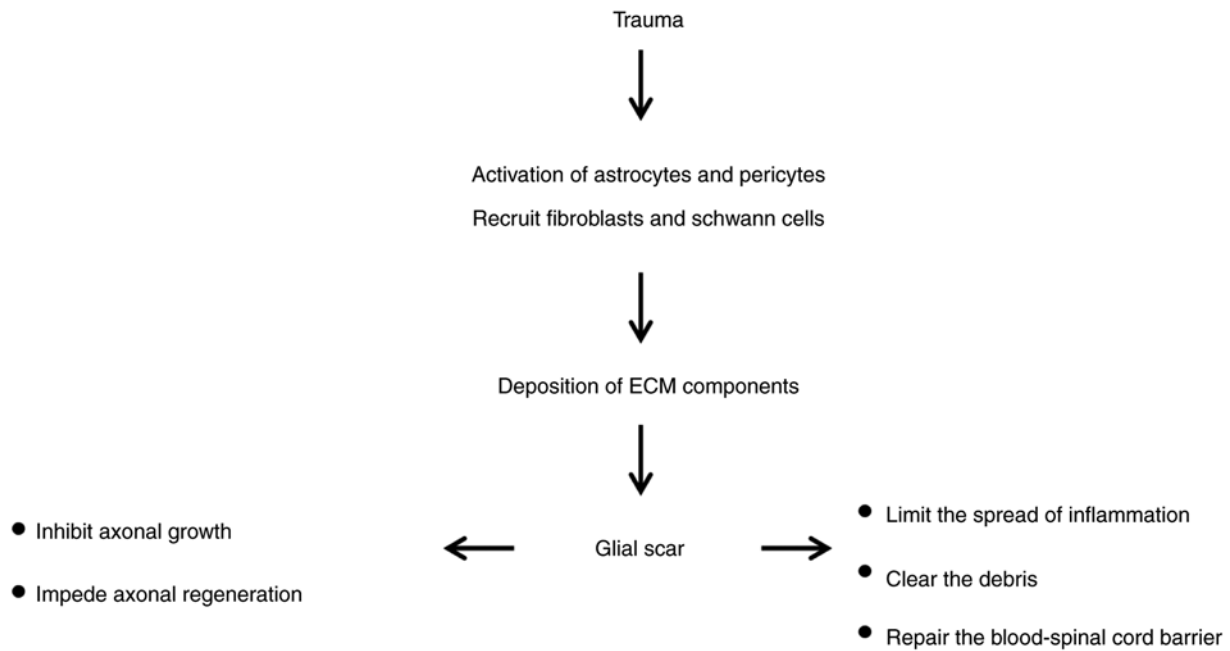


Figure 2. A schematic representation of glial scar formation and its double-sided effects. Following SCI, trauma activates resident astrocytes and pericytes, and recruits infiltrating fibroblasts and Schwann cells from periphery. Fibroblasts and Schwann cells migrate into the lesion epicenter and contribute to the deposition of ECM proteins, such as GFAP, nestin and vimentin. The deposition of ECM components and the accumulation and activation of glial cells work together in the formation of a glial scar around the periphery of the lesion. The formation of glial scars can limit the spread of inflammation, remove debris and repair the blood-spinal barrier, but can also inhibit axon growth and hinder axon regeneration. ECM, extracellular matrix; GFAP, glial fibrillary acidic protein; SCI, spinal cord injury.

astrocytes into neurons in the dorsal horn of the injured spinal cord, thus providing a novel possibility for the treatment of SCI.

3. GSK-3

GSK-1, GSK-2 and GSK-3 are highly conserved serine/threonine kinases in the GSK protein family; they were initially identified as negative regulators of glycogen metabolism (76). Among them, GSK-3 is the most studied as it has pivotal roles in numerous cellular functions, including regulating gene expression, cell survival and neuronal polarity (77). GSK-3 has two isoforms, GSK-3 α and GSK-3 β , and one splice variant (GSK-3 β 2), which is expressed specifically in the nervous system (78). These two isoforms share ~95% amino acid identity, thus, GSK-3 α and GSK-3 β have unique and overlapping functions (79). GSK-3 has a large number of interacting substrates, including CREB (80), the Nfat family of proteins (81), neurogenin 2 (82), SMAD1 (83) and β -catenin (84), all of which are part of the cyclic AMP response element-binding protein family. Among the two isoforms, GSK-3 β may have more predicted substrates than GSK-3 α , so GSK-3 β has traditionally received more attention (85).

GSK-3 is mainly localized in the cytoplasm where it regulates transcription factors by regulating their protein concentrations, DNA attachment capabilities and/or nuclear positioning (86). Most kinases are inactive in resting cells and become active after phosphorylation. In contrast with other kinases, GSK-3 is highly active in unstimulated cells and it is rendered inactive after phosphorylation following stimulation from various sources, including growth factors (87). Growth factor-mediated phosphorylation of GSK-3 inhibits

its activation and leads to the activation of its downstream substrates.

GSK-3 is ubiquitously expressed in the human body, and its dysfunction has been confirmed in several disorders such as cancer, cardiovascular diseases, diabetes and inflammatory conditions. GSK-3 is also expressed in the CNS and participates in several physiological and pathological functions (88). There is evidence of a close association between the disruption of GSK-3 signaling and the emergence of neuroinflammation, neurodegenerative illnesses and psychiatric disorders. For example, GSK-3 is a key role in the pathogenesis of Alzheimer's disease, as it participates in the abnormal phosphorylation of τ protein and the production of amyloid- β (89-92). Dysfunction of the GSK-3 β signaling pathway has also been demonstrated in neuropsychiatric disorders, such as schizophrenia (93). In postmortem tissues of patients with schizophrenia, GSK-3 β mRNA expression was reduced in the active frontal cortex and dorsolateral prefrontal cortex, although there was no difference in occipital cortical protein expression (93,94). GSK-3 also regulates rhythms in hippocampal clock gene expression and synaptic plasticity (95). During brain development, GSK-3 and its upstream and downstream regulators serve key roles in the fundamental processes of neurodevelopment, and the disruption of GSK-3 signaling is associated with several neurodevelopmental disorders such as delayed development and intellectual disability (78).

Along with its role in neurodegenerative and neurodevelopmental diseases, GSK-3 also serves an important role in neurogenesis. Behavioral deficits and neuroprogenitor cell proliferation in schizophrenia are regulated by the GSK-3/ β -catenin signaling pathway (96). The hippocampal neurons of adults display heightened neurogenesis, as well

as migration, differentiation, proliferation and neurophenotypic formation, which are linked to the inhibition of GSK-3 in rats (97). A correlation between GSK-3 inhibition and an increase in neurogenesis was established *in vitro* and *in vivo* in adult mouse neural progenitors (97-99). Neurogenesis in the dentate gyrus of the hippocampus of adult rats can be induced by the small molecule NP03112 or lithium-induced inhibition of GSK-3 (97,100). Conditional deletion of GSK-3 in mouse neural progenitors increases proliferation (101). Considering the close relationship between GSK-3 and neurogenesis, the role of GSK-3 signaling pathway in SCI is further discussed below.

4. Function and role of GSK-3 in SCI

SCI decreases the ratio of p-GSK-3 β /t-GSK-3 β and increases the number of apoptotic cells in the spinal dorsal horn. Increasing this ratio may be a useful strategy for reducing apoptosis and subsequent neuropathic pain associated with SCI (102). PI3K-mediated activation of GSK-3 β can reduce dorsal root ganglia neurite outgrowth associated with excitotoxic spinal cord injury dysesthesias (103). The development of GSK-3 signaling pathway in spinal cord injury is shown in Fig. 3.

Role of GSK-3 inhibitors in SCI. The aforementioned hypothesis, that GSK-3 regulates SCI, can first be demonstrated using GSK-3 inhibitors. The function of several GSK-3 inhibitors in spinal cord injury has been extensively studied. For example, GSK-3 inhibitor Ro3303544 was demonstrated to stimulate neurogenesis in cultured multipotent stem cells and in SCI rat model (104), as also demonstrated using 4-benzyl-2-methyl-1,2,4-thiadiazolidine-3 (TDZD-8). GSK-3 is most effectively and precisely inhibited by a 5-dione non-ATP inhibitor. Treatment with TDZD-8, one of these inhibitors, following SCI could significantly inhibit neuronal apoptosis and increases the density of cortical spinal tract fibers around the injured area (105). Combination therapy with TDZD-8 and Y27632 (a Rho-associated coiled-coil kinase 2 inhibitor) could improve the protective effect on axonal regeneration in a rat SCI model (106). Lithium, a traditional inhibitor of GSK-3 β , has been extensively utilized in the treatment of mood disorders, particularly manic depression (107). Neurotrophic factors, such as nerve growth factor, neurotrophic factor-3, brain-derived neurotrophic factor (BDNF) and receptors in the brain are all involved in the increase in the concentration and amount of lithium in animals (108). Lithium also stimulates stem cells proliferation, including neural stem cells in the subventricular area, striatum, spinal cord and forebrain (103). Animal models of stroke and brain injury, as well as Huntington's, Alzheimer's, Parkinson's and amyotrophic lateral sclerosis diseases, show that lithium (107) increases the incidence of these diseases. Li *et al* (109) showed that in spinal cord neurons, lithium inhibits GSK-3 activity through two different signaling pathways; lithium activates phosphorylation of AKT in the acute phase and upregulates the expression of Na⁺/K⁺-ATPase α 1 in the chronic phase (109). A hypoxic environment is often generated around the SCI tissue, so that single therapy with gene or stem cells becomes inefficient. Combination treatment with the GSK-3 inhibitor, CHIR99021, and a histone

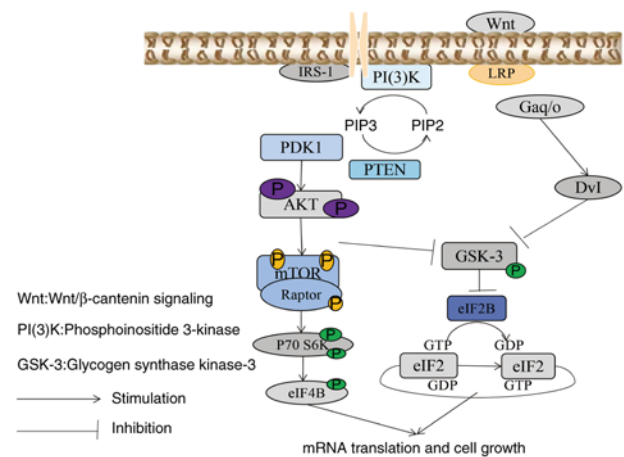


Figure 3. The main lipid substrate of PTEN is PIP3, and PTEN is a negative regulator of PI3K/AKT signaling. In the upstream signaling network, the activation of mTOR by AKT leads to phospholipid activation of GSK-3, which transduces signals from various growth factors and cytokines into intracellular information. PTEN, phosphatase and tensin homolog; PIP3, phosphatidylinositol-3,4,5-trisphosphate; GSK-3, glycogen synthase kinase-3.

deacetylase inhibitor, such as valproic acid, can significantly boost gene expression through hypoxia/neuron-inducible gene expression system and human-induced neural therapy such as additive stimulus induction. SCI tends to damage nerve tissue and create a hypoxic environment (110). A previous study (56) confirmed that gene or stem cell therapy alone is inefficient, but studies of combination stem cell and gene therapy to treat tissue damage have begun to overcome associated limitations, including inefficient gene delivery and poor treatment effectiveness. Therefore, the combination of stem cells, gene therapy and hypoxia-specific systems may contribute to the reconstruction of SCI (104). Endoplasmic reticulum (ER) stress-induced apoptosis serves an important role in SCI. The AKT/GSK-3 β signaling pathway was demonstrated to be able to reduce ER stress-induced apoptosis in SH-SY5Y cells when valproate, a well-known medication for treating epilepsy and mania in clinics, is administered (111). Table I outlines the dosage and effects of GSK-3 inhibitors.

Treatments through GSK-3 in SCI. Alongside the inhibitors, the therapeutic effects of several other treatments in SCI that also target GSK-3 signaling pathways have been investigated. Basic fibroblast growth factor (bFGF) is a potential neuroprotective factor that can promote regeneration and repair of SCI, especially in the early stage of the injury (112-114). Adrenomedullin (AM) is highly expressed in the spinal cord; it can increase p-AKT, p-GSK-3 β , p-CREB and BDNF expression levels and promote cAMP accumulation in dorsal root ganglion, which indicates the possible beneficial role of AM in the protection, survival and regeneration of sensory neurons during SCI (115). The potential neuroprotective effects of astaxanthin, a powerful antioxidant and anti-inflammatory agent, on spinal cord ischemia-reperfusion injury may be due to activation of the PI3K/Akt/GSK-3 β pathway (116), although the mechanism remains to be elucidated. Loureirin B is a constituent of Traditional Chinese Medicine that is extracted from Dragon's blood tree and has been shown to

Table I. A summary of doses and effects of GSK-3 inhibitors.

First author/s, year	GSK-3 inhibitor	Dose	Effects	(Refs.)
Rodriguez-Jimenez <i>et al</i> , 2021	Ro3303544 ^a	1 μ M, 24 h	Promotes ependymal stem/progenitor cells and human embryonic stemcell-derived neural progenitor differentiation to mature neurons; enhances neurogenesis in ependymal stem/progenitor cells.	(104)
Lei <i>et al</i> , 2019	TDZD-8 ^b	1 mg/kg/d, 3 weeks	Promotes neuronal cell regeneration and functional recovery in SCI model rats	(105)
Zhang <i>et al</i> , 2016	Y27632 + TDZD-8 ^c	Y27632 1.6 mg/kg, 2 weeks; TDZD-8 1 mg/kg, 3 weeks	Protects against secondary SCI by inhibiting apoptosis in SCI rats	(106)
Burgess <i>et al</i> , 2001	Lithium ^d	1 mM, 1-48 h, <i>in vitro</i> ; 20 mg/kg/d, 3 days, <i>in vivo</i>	Activates phosphorylation of AKT in the acute phase, and upregulates the expression of Na ⁺ /K ⁺ -ATPase α 1 in the chronic phase in primarily cell cultured spinal cord neurons	(107)

^aRo3303544 can promote the neurogenesis in both cultured multipotent stem cells and in SCI model. ^bTDZD-8 is the most effective and specific non-ATP-competitive inhibitor of GSK-3; treatment with TDZD-8 following SCI can significantly inhibited neuronal apoptosis and increased density of cortical spinal tract fibers around areas of injury. ^cY27632 + TDZD-8; a combination therapy of TDZD-8 and Y27632 (a ROCKII inhibitor) can improve the protective effect on axonal regeneration in rats SCI models. ^dLithium is a traditional inhibitor of GSK-3 β , which has been widely used to treat mood disorders, especially manic depression; in animals, lithium upregulates neurotrophins, including brain-derived neurotrophic factor and nerve growth factor, neurotrophin-3, as well as receptors to these growth factors in brain. Lithium also stimulates proliferation of stem cells, including bone marrow and neural stem cells in the subventricular zone, striatum and forebrain. GSK-3, glycogen synthase kinase-3; SCI, spinal cord injury; TDZD-8, 4-benzyl-2-methyl-1,2,4-thiadiazolidine-3,5-dione.

affect insulin secretion stimulation, blood glucose reduction and immune suppression (117,118). In addition to these functions, Loureirin B also promotes neuron polarization and axon regeneration by regulating the Akt/GSK-3 β pathway following SCI (119). Analysis of gene expression profiles can reveal several essential pathways and genes linked to neuropathic pain in those suffering from spinal cord injury. Among them, GSK-3 β is identified in human umbilical cord-derived mesenchymal stem cell (HUCMSC) transplantation has been confirmed to be an effective therapy to alleviate the symptoms of neuropathic pain and to improve motor recovery following SCI (120). Stable bFGF-overexpressing HUCMSC transplantation exhibited improved therapeutic outcomes, such as reduction of glial scar formation, improvement of nerve regeneration and proliferation of endogenous neural stem cells and increased locomotion functional recovery of posterior limbs in a mouse SCI model (121). In addition, the promotion of the proliferation and neuronal differentiation of neural stem cells was demonstrated to operate through the PI3K-Akt-GSK-3 β pathway (116). Neuropathic pain is a common complication following SCI experienced by 75-80% of patients with SCI (121,122). GSK-3 β protein is in the protein-protein interaction network (123). Furthermore, the signaling pathways of GSK-3 β have been reported to closely participate in nerve

injuries, such as neurodegenerative diseases, inflammation and neuropathic pain (102). Therefore, GSK-3 signaling pathways may also participate in the pathological process of neuropathic pain following SCI.

Relationship between neuropathic pain and GSK-3 in SCI. Intrathecal injection of ghrelin can significantly suppress the activation of GSK-3 β in the spinal dorsal horn and alleviate neuropathic pain (124). Activation of the GSK-3 signaling pathway significantly enhances motor function, as well as reducing SCI-induced allodynia and hyperalgesia when laser treatment and human adipose-derived stem cell transplantation are combined (125). Intrathecal injection of SB216763, a selective GSK-3 β inhibitor, has been shown to increase the level of p-GSK-3 β in the dorsal lumbar sections of the spinal cord and to completely inhibit the tolerance to morphine analgesia in rats (126).

Neuroinflammation has been identified to be crucial in the development of neuropathic pain (127). Chemokine CXCL5, which participates in the inflammatory process of CNS, regulates neuropathic pain after injury by modulating GSK-3 β phosphorylation and activity in rats (128). Valproate can inhibit pAKT/pGSK-3 β -mediated neuronal death induced by neuropathic pain (129). Spinal nerve ligation could induce

mechanical allodynia and thermal hyperalgesia (130). The administration of GSK-3 β selective inhibitor AR-014418 decreased mechanical allodynia by increasing the p-/t-GSK-3 β ratio and decreasing apoptosis in spinal nerve ligation model rats; however, it did not affect thermal hyperalgesia (101). However, there are also reports (98) showing that GSK-3 β activity was enhanced in the hippocampus but reduced in the spinal dorsal horn following spared nerve injury. Induced neuropathic pain can cause short-term memory deficits and treatment with selective GSK-3 β inhibitors, such as SB216763 and AR-A014418, can prevent short-term memory deficits but does not affect neuropathic pain (131). These discrepancies may be due to the use of different animal models, although they all lead to neuropathic pain.

5. Conclusion

The pathophysiological process of SCI is quite complex; nonetheless, the poor prognosis of patients with SCI may mainly be due to glial scar formation and irreversible neuron loss. Glial scar formation and concomitant inflammatory responses, on the one hand, inhibit the spread of lesions; on the other hand, they limit the injury repair. The dual role of glial scars makes it difficult to be used as a therapeutic target (46). In addition, irreversible neuron loss is another critical part of SCI recovery. The importance of neuron loss has led researchers to develop corresponding treatments; therefore, several stem/progenitor cells therapies have been developed (57-59). Unfortunately, only a few therapies reach the clinical trial stage, and their therapeutic effects are debatable. Exploring the key mechanism of SCI is crucial for finding improved treatments.

The dysfunction of GSK-3 signaling pathway during SCI has been widely investigated. SCI decreases the ratio of p-/t-GSK-3 β . Treatment with GSK-3 inhibitors can promote neurogenesis; in addition, several therapies for the treatment of SCI also act through GSK-3 signaling pathways. In addition, GSK-3 signaling pathways also participate in the pathological process of neuropathic pain, which is one of the common complications of SCI. Based on the current body of evidence, GSK-3 signaling can be considered a potential therapeutic target for SCI. However, the data of GSK-3 inhibitors promoting neurogenesis in SCI are mainly generated from *in vitro* experiments. The development of therapies based on GSK-3 still needs further study. Nonetheless, the present review summarized the participation of GSK-3 signaling in SCI and may help understand the role GSK-3 signaling during the pathological processes of SCI.

Acknowledgements

Not applicable.

Funding

The present study was supported by The Project of Nantong Municipal Health Commission, Project of Nantong First People's Hospital (grant nos. MS2022016 and YPYJJZD008), Postgraduate Research & Practice Innovation Program of Jiangsu Province (grant no. KYCX21_3107) and Project of

Jiangsu Administration of Traditional Chinese Medicine (grant no. MS2022090).

Availability of data and materials

Not applicable.

Authors' contributions

XD and HH were responsible for the literature search and discussion. ZC made substantial contributions to conception and design, conducted a thorough review of the manuscript for its significant intellectual content and gave his approval to the final version. Data authentication is not applicable. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

- Ahuja CS, Wilson JR, Nori S, Kotter MRN, Druschel C, Curt A and Fehlings MG: Traumatic spinal cord injury. *Nat Rev Dis Primers* 3: 17018, 2017.
- Silva NA, Sousa N, Reis RL and Salgado AJ: From basics to clinical: A comprehensive review on spinal cord injury. *Prog Neurobiol* 114: 25-57, 2014.
- Vismara I, Papa S, Veneruso V, Mauri E, Mariani A, De Paola M, Affatato R, Rossetti A, Sponchioni M, Moscatelli D, *et al*: Selective modulation of A1 astrocytes by drug-loaded nano-structured gel in spinal cord injury. *ACS Nano* 14: 360-371, 2020.
- Huang X, Gu YK, Cheng XY and Su ZD: Astrocytes as therapeutic targets after spinal cord injury. *Sheng Li Xue Bao* 69: 794-804, 2017 (In Chinese).
- Erlach S, Alexandrovich A, Shohami E and Pinkas-Kramarski R: Rapamycin is a neuroprotective treatment for traumatic brain injury. *Neurobiol Dis* 26: 86-93, 2007.
- Wu Q, Li YL, Ning GZ, Feng SQ, Chu TC, Li Y, Hao Y and Wu QL: Epidemiology of traumatic cervical spinal cord injury in Tianjin, China. *Spinal Cord* 50: 740-744, 2012.
- McKinley WO, Seel RT and Hardman JT: Nontraumatic spinal cord injury: Incidence, epidemiology, and functional outcome. *Arch Phys Med Rehabil* 80: 619-623, 1999.
- Lu P, Wang Y, Graham L, McHale K, Gao M, Wu D, Brock J, Blesch A, Rosenzweig ES, Havton LA, *et al*: Long-distance growth and connectivity of neural stem cells after severe spinal cord injury. *Cell* 150: 1264-1273, 2012.
- Mar FM, Simões AR, Rodrigo IS and Sousa MM: Inhibitory injury signaling represses axon regeneration after dorsal root injury. *Mol Neurobiol* 53: 4596-4605, 2016.
- Prinz M and Priller J: The role of peripheral immune cells in the CNS in steady state and disease. *Nat Neurosci* 20: 136-144, 2017.
- Kim YS, Choi J and Yoon BE: Neuron-glia interactions in neurodevelopmental disorders. *Cells* 9: 2176, 2020.
- Pellerin L, Bouzier-Sore AK, Aubert A, Serres S, Merle M, Costalat R and Magistretti PJ: Activity-dependent regulation of energy metabolism by astrocytes: An update. *Glia* 55: 1251-1262, 2007.
- Taveggia C: Schwann cells-axon interaction in myelination. *Curr Opin Neurobiol* 39: 24-29, 2016.

14. Casano AM and Peri F: Microglia: Multitasking specialists of the brain. *Dev Cell* 32: 469-477, 2015.
15. Norenberg MD, Smith J and Marcillo A: The pathology of human spinal cord injury: Defining the problems. *J Neurotrauma* 21: 429-440, 2004.
16. Sofroniew MV: Molecular dissection of reactive astrogliosis and glial scar formation. *Trends Neurosci* 32: 638-647, 2009.
17. Alizadeh A, Dyck SM and Karimi-Abdolrezaee S: Traumatic spinal cord injury: An overview of pathophysiology, models and acute injury mechanisms. *Front Neurol* 10: 282, 2019.
18. Wasner G, Naleschinski D and Baron R: A role for peripheral afferents in the pathophysiology and treatment of at-level neuropathic pain in spinal cord injury? A case report. *Pain* 131: 219-225, 2007.
19. Anjum A, Yazid MD, Fauzi Daud M, Idris J, Ng AMH, Selvi Naicker A, Ismail OHR, Athi Kumar RK and Lokanathan Y: Spinal cord injury: Pathophysiology, multimolecular interactions, and underlying recovery mechanisms. *Int J Mol Sci* 21: 7533, 2020.
20. Nickel M and Gu C: Regulation of central nervous system myelination in higher brain functions. *Neural Plast* 2018: 6436453, 2018.
21. Schwartz G and Fehlings MG: Secondary injury mechanisms of spinal cord trauma: A novel therapeutic approach for the management of secondary pathophysiology with the sodium channel blocker riluzole. *Prog Brain Res* 137: 177-190, 2002.
22. Zhang Y, Al Mamun A, Yuan Y, Lu Q, Xiong J, Yang S, Wu C, Wu Y and Wang J: Acute spinal cord injury: Pathophysiology and pharmacological intervention (Review). *Mol Med Rep* 23: 417, 2021.
23. Sharma HS, Patnaik R, Sharma A, Sjöquist PO and Lafuente JV: Silicon dioxide nanoparticles (SiO₂, 40-50 nm) exacerbate pathophysiology of traumatic spinal cord injury and deteriorate functional outcome in the rat. An experimental study using pharmacological and morphological approaches. *J Nanosci Nanotechnol* 9: 4970-4980, 2009.
24. Dimitrijevic MR, Danner SM and Mayr W: Neurocontrol of movement in humans with spinal cord injury. *Artif Organs* 39: 823-833, 2015.
25. Zhang JX, Wang R, Xi J, Shen L, Zhu AY, Qi Q, Wang QY, Zhang LJ, Wang FC, Lü HZ and Hu JG: Morroniside protects SK-N-SH human neuroblastoma cells against H₂O₂-induced damage. *Int J Mol Med* 39: 603-612, 2017.
26. Leal-Filho MB: Spinal cord injury: From inflammation to glial scar. *Surg Neurol Int* 2: 112, 2011.
27. Yang T, Xing L, Yu W, Cai Y, Cui S and Chen G: Astrocytic reprogramming combined with rehabilitation strategy improves recovery from spinal cord injury. *FASEB J* 34: 15504-15515, 2020.
28. Lee-Liu D, Edwards-Faret G, Tapia VS and Larraín J: Spinal cord regeneration: Lessons for mammals from non-mammalian vertebrates. *Genesis* 51: 529-544, 2013.
29. Yilmaz T and Kaptanoğlu E: Current and future medical therapeutic strategies for the functional repair of spinal cord injury. *World J Orthop* 6: 42-55, 2015.
30. Ahuja CS, Nori S, Tetreault L, Wilson J, Kwon B, Harrop J, Choi D and Fehlings MG: Traumatic spinal cord injury-repair and regeneration. *Neurosurgery* 80 (3S): S9-S22, 2017.
31. Sabapathy V, Tharion G and Kumar S: Cell therapy augments functional recovery subsequent to spinal cord injury under experimental conditions. *Stem Cells Int* 2015: 132172, 2015.
32. Yousefifard M, Rahimi-Movaghar V, Nasirinezhad F, Baikpour M, Safari S, Saadat S, Moghadas Jafari A, Asady H, Razavi Tousi SM and Hosseini M: Neural stem/progenitor cell transplantation for spinal cord injury treatment: A systematic review and meta-analysis. *Neuroscience* 322: 377-397, 2016.
33. Ide C and Kanekiyo K: Points regarding cell transplantation for the treatment of spinal cord injury. *Neural Regen Res* 11: 1046-1049, 2016.
34. Lin XY, Lai BQ, Zeng X, Che MT, Ling EA, Wu W and Zeng YS: Cell transplantation and neuroengineering approach for spinal cord injury treatment: A summary of current laboratory findings and review of literature. *Cell Transplant* 25: 1425-1438, 2016.
35. Dumont CM, Margul DJ and Shea LD: Tissue engineering approaches to modulate the inflammatory milieu following spinal cord injury. *Cells Tissues Organs* 202: 52-66, 2016.
36. Raspa A, Pugliese R, Maleki M and Gelain F: Recent therapeutic approaches for spinal cord injury. *Biotechnol Bioeng* 113: 253-259, 2016.
37. Gradišnik L, Bošnjak R, Maver T and Velnar T: Advanced bio-based polymers for astrocyte cell models. *Materials (Basel)* 14: 3664, 2021.
38. Orr MB and Gensel JC: Spinal cord injury scarring and inflammation: Therapies targeting glial and inflammatory responses. *Neurotherapeutics* 15: 541-553, 2018.
39. Beck KD, Nguyen HX, Galvan MD, Salazar DL, Woodruff TM and Anderson AJ: Quantitative analysis of cellular inflammation after traumatic spinal cord injury: Evidence for a multiphasic inflammatory response in the acute to chronic environment. *Brain* 133: 433-447, 2010.
40. Buss A, Pech K, Kakulas BA, Martin D, Schoenen J, Noth J and Brook GA: Growth-modulating molecules are associated with invading Schwann cells and not astrocytes in human traumatic spinal cord injury. *Brain* 130: 940-953, 2007.
41. Zhang SX, Huang F, Gates M and Holmberg EG: Role of endogenous Schwann cells in tissue repair after spinal cord injury. *Neural Regen Res* 8: 177-185, 2013.
42. Tran AP, Warren PM and Silver J: The biology of regeneration failure and success after spinal cord injury. *Physiol Rev* 98: 881-917, 2018.
43. Adams KL and Gallo V: The diversity and disparity of the glial scar. *Nat Neurosci* 21: 9-15, 2018.
44. Pang QM, Chen SY, Xu QJ, Fu SP, Yang YC, Zou WH, Zhang M, Liu J, Wan WH, Peng JC and Zhang T: Neuroinflammation and scarring after spinal cord injury: Therapeutic roles of MSCs on inflammation and glial scar. *Front Immunol* 12: 751021, 2021.
45. Silver J and Miller JH: Regeneration beyond the glial scar. *Nat Rev Neurosci* 5: 146-156, 2004.
46. Bradbury EJ, Moon LD, Popat RJ, King VR, Bennett GS, Patel PN, Fawcett JW and McMahon SB: Chondroitinase ABC promotes functional recovery after spinal cord injury. *Nature* 416: 636-640, 2002.
47. Diaz Quiroz JF and Echeverri K: Spinal cord regeneration: Where fish, frogs and salamanders lead the way, can we follow? *Biochem J* 451: 353-364, 2013.
48. Okada S, Nakamura M, Katoh H, Miyao T, Shimazaki T, Ishii K, Yamane J, Yoshimura A, Iwamoto Y, Toyama Y and Okano H: Conditional ablation of Stat3 or Socs3 discloses a dual role for reactive astrocytes after spinal cord injury. *Nat Med* 12: 829-834, 2006.
49. Herrmann JE, Imura T, Song B, Qi J, Ao Y, Nguyen TK, Korsak RA, Takeda K, Akira S and Sofroniew MV: STAT3 is a critical regulator of astrogliosis and scar formation after spinal cord injury. *J Neurosci* 28: 7231-7243, 2008.
50. Sofroniew MV: Astrocyte barriers to neurotoxic inflammation. *Nat Rev Neurosci* 16: 249-263, 2015.
51. Yang T, Dai Y, Chen G and Cui S: Dissecting the dual role of the glial scar and scar-forming astrocytes in spinal cord injury. *Front Cell Neurosci* 14: 78, 2020.
52. Sofroniew MV: Dissecting spinal cord regeneration. *Nature* 557: 343-350, 2018.
53. Wang L, Pei S, Han L, Guo B, Li Y, Duan R, Yao Y, Xue B, Chen X and Jia Y: Mesenchymal stem cell-derived exosomes reduce A1 astrocytes via downregulation of phosphorylated NFκB p65 subunit in spinal cord injury. *Cell Physiol Biochem* 50: 1535-1559, 2018.
54. Alvarez-Buylla A and Lim DA: For the long run: Maintaining germinal niches in the adult brain. *Neuron* 41: 683-686, 2004.
55. Ming GL and Song H: Adult neurogenesis in the mammalian brain: Significant answers and significant questions. *Neuron* 70: 687-702, 2011.
56. Horner PJ, Power AE, Kempermann G, Kuhn HG, Palmer TD, Winkler J, Thal LJ and Gage FH: Proliferation and differentiation of progenitor cells throughout the intact adult rat spinal cord. *J Neurosci* 20: 2218-2228, 2000.
57. Borst K, Dumas AA and Prinz M: Microglia: Immune and non-immune functions. *Immunity* 54: 2194-2208, 2021.
58. Park JH, Cho JH, Ahn JH, Choi SY, Lee TK, Lee JC, Shin BN, Hong S, Jeon YH, Kim YM, *et al*: Neuronal loss and gliosis in the rat striatum subjected to 15 and 30 min of middle cerebral artery occlusion. *Metab Brain Dis* 33: 775-784, 2018.
59. Lee Y, Lee SR, Choi SS, Yeo HG, Chang KT and Lee HJ: Therapeutically targeting neuroinflammation and microglia after acute ischemic stroke. *Biomed Res Int* 2014: 297241, 2014.
60. Wolf SA, Boddeke HWGM and Kettenmann H: Microglia in physiology and disease. *Annu Rev Physiol* 79: 619-643, 2017.
61. Savage JC, Carrier M and Tremblay ME: Morphology of microglia across contexts of health and disease. *Methods Mol Biol* 2034: 13-26, 2019.

62. Brown GC: Neuronal loss after stroke due to microglial phagocytosis of stressed neurons. *Int J Mol Sci* 22: 13442, 2021.
63. Mothe AJ and Tator CH: Proliferation, migration, and differentiation of endogenous ependymal region stem/progenitor cells following minimal spinal cord injury in the adult rat. *Neuroscience* 131: 177-187, 2005.
64. Park JH, Kim DY, Sung IY, Choi GH, Jeon MH, Kim KK and Jeon SR: Long-term results of spinal cord injury therapy using mesenchymal stem cells derived from bone marrow in humans. *Neurosurgery* 70: 1238-1247, 2012.
65. Lima C, Escada P, Pratas-Vital J, Branco C, Arcangeli CA, Lazzeri G, Maia CA, Capucho C, Hasse-Ferreira A and Peduzzi JD: Olfactory mucosal autografts and rehabilitation for chronic traumatic spinal cord injury. *Neurorehabil Neural Repair* 24: 10-22, 2010.
66. Saberi H, Firouzi M, Habibi Z, Moshayedi P, Aghayan HR, Arjmand B, Hosseini K, Razavi HE and Yekaninejad MS: Safety of intramedullary Schwann cell transplantation for postrehabilitation spinal cord injuries: 2-Year follow-up of 33 cases. *J Neurosurg Spine* 15: 515-525, 2011.
67. Ronaghi M, Erceg S, Moreno-Manzano V and Stojkovic M: Challenges of stem cell therapy for spinal cord injury: Human embryonic stem cells, endogenous neural stem cells, or induced pluripotent stem cells? *Stem Cells* 28: 93-99, 2010.
68. Osaka M, Honmou O, Murakami T, Nonaka T, Houkin K, Hamada H and Kocsis JD: Intravenous administration of mesenchymal stem cells derived from bone marrow after contusive spinal cord injury improves functional outcome. *Brain Res* 1343: 226-235, 2010.
69. Chhabra HS, Lima C, Sachdeva S, Mittal A, Nigam V, Chaturvedi D, Arora M, Aggarwal A, Kapur R and Khan TAH: Autologous olfactory [corrected] mucosal transplant in chronic spinal cord injury: An Indian pilot study. *Spinal Cord* 47: 887-895, 2009.
70. Liu C, Chen Z, Chen Z, Zhang T and Lu Y: Multiple tumor types may originate from bone marrow-derived cells. *Neoplasia* 8: 716-724, 2006.
71. Tolar J, Nauta AJ, Osborn MJ, Panoskaltsis Mortari A, McElmurry RT, Bell S, Xia L, Zhou N, Riddle M, Schroeder TM, *et al*: Sarcoma derived from cultured mesenchymal stem cells. *Stem Cells* 25: 371-379, 2007.
72. Wu Z, Parry M, Hou XY, Liu MH, Wang H, Cain R, Pei ZF, Chen YC, Guo ZY, Abhijeet S and Chen G: Gene therapy conversion of striatal astrocytes into GABAergic neurons in mouse models of Huntington's disease. *Nat Commun* 11: 1105, 2020.
73. Chen YC, Ma NX, Pei ZF, Wu Z, Do-Monte FH, Keefe S, Yellin E, Chen MS, Yin JC, Lee G, *et al*: A NeuroDI AAV-based gene therapy for functional brain repair after ischemic injury through in vivo astrocyte-to-neuron conversion. *Mol Ther* 28: 217-234, 2020.
74. Li H and Chen G: In vivo reprogramming for CNS repair: Regenerating neurons from endogenous glial cells. *Neuron* 91: 728-738, 2016.
75. Guo Z, Zhang L, Wu Z, Chen Y, Wang F and Chen G: In vivo direct reprogramming of reactive glial cells into functional neurons after brain injury and in an Alzheimer's disease model. *Cell Stem Cell* 14: 188-202, 2014.
76. Hemmings BA, Yellowlees D, Kernohan JC and Cohen P: Purification of glycogen synthase kinase 3 from rabbit skeletal muscle. Copurification with the activating factor (FA) of the (Mg-ATP) dependent protein phosphatase. *Eur J Biochem* 119: 443-451, 1981.
77. Jope RS: Lithium and GSK-3: One inhibitor, two inhibitory actions, multiple outcomes. *Trends Pharmacol Sci* 24: 441-443, 2003.
78. Hur EM and Zhou FQ: GSK3 signalling in neural development. *Nat Rev Neurosci* 11: 539-551, 2010.
79. Force T and Woodgett JR: Unique and overlapping functions of GSK-3 isoforms in cell differentiation and proliferation and cardiovascular development. *J Biol Chem* 284: 9643-9647, 2009.
80. Grimes CA and Jope RS: CREB DNA binding activity is inhibited by glycogen synthase kinase-3 beta and facilitated by lithium. *J Neurochem* 78: 1219-1232, 2001.
81. Neal JW and Clipstone NA: Glycogen synthase kinase-3 inhibits the DNA binding activity of NFATc. *J Biol Chem* 276: 3666-3673, 2001.
82. Ma YC, Song MR, Park JP, Henry Ho HY, Hu L, Kurtev MV, Zieg J, Ma Q, Pfaff SL and Greenberg ME: Regulation of motor neuron specification by phosphorylation of neurogenin 2. *Neuron* 58: 65-77, 2008.
83. Fuentealba LC, Eivers E, Ikeda A, Hurtado C, Kuroda H, Pera EM and De Robertis EM: Integrating patterning signals: Wnt/GSK3 regulates the duration of the BMP/Smad1 signal. *Cell* 131: 980-993, 2007.
84. Kazi A, Xiang S, Yang H, Delitto D, Trevino J, Jiang RHY, Ayaz M, Lawrence HR, Kennedy P and Sebt SM: GSK3 suppression upregulates β -catenin and c-Myc to abrogate KRas-dependent tumors. *Nat Commun* 9: 5154, 2018.
85. Lindner R, Jensen LJ, Ostheimer GJ, van Vugt MA, Jørgensen C, Miron IM, Diella F, Colwill K, Taylor L, Elder K, *et al*: Systematic discovery of in vivo phosphorylation networks. *Cell* 129: 1415-1426, 2007.
86. Beurel E, Grieco SF and Jope RS: Glycogen synthase kinase-3 (GSK3): Regulation, actions, and diseases. *Pharmacol Ther* 148: 114-131, 2015.
87. Mancinelli R, Carpino G, Petrungaro S, Mammola CL, Tomaipitina L, Filippini A, Facchiano A, Ziparo E and Giampietri C: Multifaceted roles of GSK-3 in cancer and autophagy-related diseases. *Oxid Med Cell Longev* 2017: 4629495, 2017.
88. Jaworski T, Banach-Kasper E and Gralec K: GSK-3 β at the intersection of neuronal plasticity and neurodegeneration. *Neural Plast* 2019: 4209475, 2019.
89. Hernandez F, Lucas JJ and Avila J: GSK3 and tau: Two convergence points in Alzheimer's disease. *J Alzheimers Dis* 33 (Suppl 1): S141-S144, 2013.
90. Albeely AM, Ryan SD and Perreault ML: Pathogenic feed-forward mechanisms in Alzheimer's and Parkinson's disease converge on GSK-3. *Brain Plast* 4: 151-167, 2018.
91. Manduca JD, Thériault RK and Perreault ML: Glycogen synthase kinase-3: The missing link to aberrant circuit function in disorders of cognitive dysfunction? *Pharmacol Res* 157: 104819, 2020.
92. Wu YY, Wang X, Tan L, Liu D, Liu XH, Wang Q, Wang JZ and Zhu LQ: Lithium attenuates scopolamine-induced memory deficits with inhibition of GSK-3 β and preservation of postsynaptic components. *J Alzheimers Dis* 37: 515-527, 2013.
93. Kozlovsky N, Belmaker RH and Agam G: Low GSK-3 activity in frontal cortex of schizophrenic patients. *Schizophr Res* 52: 101-105, 2001.
94. Kozlovsky N, Shanon-Weickert C, Tomaskovic-Crook E, Kleinman JE, Belmaker RH and Agam G: Reduced GSK-3 β mRNA levels in postmortem dorsolateral prefrontal cortex of schizophrenic patients. *J Neural Transm (Vienna)* 111: 1583-1592, 2004.
95. Besing RC, Rogers CO, Paul JR, Hablitz LM, Johnson RL, McMahon LL and Gamble KL: GSK3 activity regulates rhythms in hippocampal clock gene expression and synaptic plasticity. *Hippocampus* 27: 890-898, 2017.
96. Mao Y, Ge X, Frank CL, Madison JM, Koehler AN, Doud MK, Tassa C, Berry EM, Soda T, Singh KK, *et al*: Disrupted in schizophrenia 1 regulates neuronal progenitor proliferation via modulation of GSK3 β /beta-catenin signaling. *Cell* 136: 1017-1031, 2009.
97. Morales-Garcia JA, Luna-Medina R, Alonso-Gil S, Sanz-Sancristobal M, Palomo V, Gil C, Santos A, Martinez A and Perez-Castillo A: Glycogen synthase kinase 3 inhibition promotes adult hippocampal neurogenesis in vitro and in vivo. *ACS Chem Neurosci* 3: 963-971, 2012.
98. Lange C, Mix E, Frahm J, Glass A, Müller J, Schmitt O, Schmölle AC, Klemm K, Ortinau S, Hübner R, *et al*: Small molecule GSK-3 inhibitors increase neurogenesis of human neural progenitor cells. *Neurosci Lett* 488: 36-40, 2011.
99. Lie DC, Colamarino SA, Song HJ, Désiré L, Mira H, Consiglio A, Lein ES, Jessberger S, Lansford H, Dearie AR and Gage FH: Wnt signalling regulates adult hippocampal neurogenesis. *Nature* 437: 1370-1375, 2005.
100. Wexler EM, Geschwind DH and Palmer TD: Lithium regulates adult hippocampal progenitor development through canonical Wnt pathway activation. *Mol Psychiatry* 13: 285-292, 2008.
101. Kim WY, Wang X, Wu Y, Doble BW, Patel S, Woodgett JR and Snider WD: GSK-3 is a master regulator of neural progenitor homeostasis. *Nat Neurosci* 12: 1390-1397, 2009.
102. Rashvand M, Danyali S and Manaeji H: The potential role of glycogen synthase kinase-3 β in neuropathy-induced apoptosis in spinal cord. *Basic Clin Neurosci* 11: 15-30, 2020.
103. Bareiss SK, Dugan E and Brewer KL: PI3K mediated activation of GSK-3 β reduces at-level primary afferent growth responses associated with excitotoxic spinal cord injury dysesthesias. *Mol Pain* 11: 35, 2015.

104. Rodriguez-Jimenez FJ, Vilches A, Perez-Arago MA, Clemente E, Roman R, Leal J, Castro AA, Fustero S, Moreno-Manzano V, Jendelova P, *et al*: Activation of neurogenesis in multipotent stem cells cultured in vitro and in the spinal cord tissue after severe injury by inhibition of glycogen synthase kinase-3. *Neurotherapeutics* 18: 515-533, 2021.
105. Lei F, He W, Tian X, Zhou Q, Zheng L, Kang J, Song Y and Feng D: GSK-3 inhibitor promotes neuronal cell regeneration and functional recovery in a rat model of spinal cord injury. *Biomed Res Int* 2019: 9628065, 2019.
106. Zhang G, Lei F, Zhou Q, Feng D and Bai Y: Combined application of Rho-ROCKII and GSK-3 β inhibitors exerts an improved protective effect on axonal regeneration in rats with spinal cord injury. *Mol Med Rep* 14: 5180-5188, 2016.
107. Burgess S, Geddes J, Hawton K, Townsend E, Jamison K and Goodwin G: Lithium for maintenance treatment of mood disorders. *Cochrane Database Syst Rev*: CD003013, 2001.
108. Young W: Review of lithium effects on brain and blood. *Cell Transplant* 18: 951-975, 2009.
109. Li B, Ren J, Yang L, Li X, Sun G and Xia M: Lithium inhibits GSK3 β activity via two different signaling pathways in neurons after spinal cord injury. *Neurochem Res* 43: 848-856, 2018.
110. Pan Z, Oh J, Huang L, Zeng Z, Duan P, Li Z, Yun Y, Kim J, Ha Y and Cao K: The combination of forskolin and VPA increases gene expression efficiency to the hypoxia/neuron-specific system. *Ann Transl Med* 8: 933, 2020.
111. Li Z, Wu F, Zhang X, Chai Y, Chen D, Yang Y, Xu K, Yin J, Li R, Shi H, *et al*: Valproate attenuates endoplasmic reticulum stress-induced apoptosis in SH-SY5Y cells via the AKT/GSK3 β signaling pathway. *Int J Mol Sci* 18: 315, 2017.
112. Zhou Y, Wang Z, Li J, Li X and Xiao J: Fibroblast growth factors in the management of spinal cord injury. *J Cell Mol Med* 22: 25-37, 2018.
113. Rabchevsky AG, Fugaccia I, Turner AF, Blades DA, Mattson MP and Scheff SW: Basic fibroblast growth factor (bFGF) enhances functional recovery following severe spinal cord injury to the rat. *Exp Neurol* 164: 280-291, 2000.
114. Rabchevsky AG, Fugaccia I, Fletcher-Turner A, Blades DA, Mattson MP and Scheff SW: Basic fibroblast growth factor (bFGF) enhances tissue sparing and functional recovery following moderate spinal cord injury. *J Neurotrauma* 16: 817-830, 1999.
115. Sisakht M, Khoshdel Z, Mahmoodazadeh A, Shafiee SM and Takhshid MA: Adrenomedullin increases cAMP accumulation and BDNF expression in rat DRG and spinal motor neurons. *Iran J Basic Med Sci* 24: 978-985, 2021.
116. Fu J, Sun H, Wei H, Dong M, Zhang Y, Xu W, Fang Y and Zhao J: Astaxanthin alleviates spinal cord ischemia-reperfusion injury via activation of PI3K/Akt/GSK-3 β pathway in rats. *J Orthop Surg Res* 15: 275, 2020.
117. Ding Y, Xia S, Fang H, Niu B and Chen Q: Loureirin B attenuates insulin resistance in HepG2 cells by regulating gluconeogenesis signaling pathway. *Eur J Pharmacol* 910: 174481, 2021.
118. Shi S, Zhao Q, Ke C, Long S, Zhang F, Zhang X, Li Y, Liu X, Hu H and Yin S: Loureirin B exerts its immunosuppressive effects by inhibiting STIM1/Orai1 and K_v1.3 channels. *Front Pharmacol* 12: 685092, 2021.
119. Wang Q, Cai H, Hu Z, Wu Y, Guo X, Li J, Wang H, Liu Y, Liu Y, Xie L, *et al*: Loureirin B promotes axon regeneration by inhibiting endoplasmic reticulum stress: Induced mitochondrial dysfunction and regulating the Akt/GSK-3 β pathway after spinal cord injury. *J Neurotrauma* 36: 1949-1964, 2019.
120. Yousefifard M, Nasirinezhad F, Shardi Manaheji H, Janzadeh A, Hosseini M and Keshavarz M: Human bone marrow-derived and umbilical cord-derived mesenchymal stem cells for alleviating neuropathic pain in a spinal cord injury model. *Stem Cell Res Ther* 7: 36, 2016.
121. Schieweck R, Schöneweiss EC, Harner M, Rieger D, Illig C, Saccà B, Popper B and Kiebler MA: Pumilio2 promotes growth of mature neurons. *Int J Mol Sci* 22: 8998, 2021.
122. Huang F, Gao T, Wang W, Wang L, Xie Y, Tai C, Liu S, Cui Y and Wang B: Engineered basic fibroblast growth factor-overexpressing human umbilical cord-derived mesenchymal stem cells improve the proliferation and neuronal differentiation of endogenous neural stem cells and functional recovery of spinal cord injury by activating the PI3K-Akt-GSK-3 β signaling pathway. *Stem Cell Res Ther* 12: 468, 2021.
123. New PW, Lim TC, Hill ST and Brown DJ: A survey of pain during rehabilitation after acute spinal cord injury. *Spinal Cord* 35: 658-663, 1997.
124. Störmer S, Gerner HJ, Grüniger W, Metzmacher K, Föllinger S, Wienke C, Aldinger W, Walker N, Zimmermann M and Paeslack V: Chronic pain/dysaesthesiae in spinal cord injury patients: Results of a multicentre study. *Spinal Cord* 35: 446-455, 1997.
125. Peng Z, Zha L, Yang M, Li Y, Guo X and Feng Z: Effects of ghrelin on pGSK-3 β and β -catenin expression when protects against neuropathic pain behavior in rats challenged with chronic constriction injury. *Sci Rep* 9: 14664, 2019.
126. Sarveazad A, Janzadeh A, Taheripak G, Dameni S, Yousefifard M and Nasirinezhad F: Co-administration of human adipose-derived stem cells and low-level laser to alleviate neuropathic pain after experimental spinal cord injury. *Stem Cell Res Ther* 10: 183, 2019.
127. Parkitna JR, Obara I, Wawrzczak-Bargiela A, Makuch W, Przewlocka B and Przewlocki R: Effects of glycogen synthase kinase 3 β and cyclin-dependent kinase 5 inhibitors on morphine-induced analgesia and tolerance in rats. *J Pharmacol Exp Ther* 319: 832-839, 2006.
128. Wang X, Lin C, Jin S, Wang Y, Peng Y and Wang X: Cannabidiol alleviates neuroinflammation and attenuates neuropathic pain via targeting FKBP5. *Brain Behav Immun* 111: 365-375, 2023.
129. Xu W, Zhu M, Yuan S and Yu W: Spinal CXCL5 contributes to nerve injury-induced neuropathic pain via modulating GSK-3 β phosphorylation and activity in rats. *Neurosci Lett* 634: 52-59, 2016.
130. Chen JY, Chu LW, Cheng KI, Hsieh SL, Juan YS and Wu BN: Valproate reduces neuroinflammation and neuronal death in a rat chronic constriction injury model. *Sci Rep* 8: 16457, 2018.
131. Cheng H, Zhang L, Xia F, Jin L, Liu S, Ren H, Zhu C, Ji Q and Tang J: Astrocytic NDRG2 is critical in the maintenance of neuropathic pain. *Brain Behav Immun* 89: 300-313, 2020.



Copyright © 2023 Dong et al. This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.