Therapeutic Potential of Wnt-3a in Neurological Recovery after Spinal Cord Injury

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Keywords

Spinal cord injury · Wnt-3a · Wnt/ β -catenin signaling pathway · Neuroprotection · Axon regeneration · Autophagy · Apoptosis · Mammalian target of rapamycin signaling pathway

Abstract

Background: Spinal cord injury (SCI) is a constant challenge in medical research and a global therapeutic problem. Treatment of this condition remains difficult in clinical practice. Hence, prevention, treatment, and rehabilitation of SCI have become imminent tasks in the medical field. **Summary:** Recent evidence suggest the important role of Wnt/ β -catenin signaling pathway, a canonical Wnt signaling pathway, in neural development, axon guidance, neuropathic pain relief, and neuronal survival. Wnt-3a is regarded as an activator of the canonical Wnt signaling pathway. This activator is expressed in the dorsal midline region and is responsible for spinal cord development. In addition, Wnt-3a plays a regulatory role in autophagy, apoptosis, and regeneration of neurons; neurogenic inflammation; and axon regeneration. Herein, we demonstrated that neuronal autophagy was regulated by Wnt-3a via β -catenin and mammalian target of rapamycin signaling pathways after SCI. Our study also discovered that the Wnt-3a provided a favorable microenviron-

ment for the recovery of nerve function after SCI. **Key Messages:** This study systematically elaborates the neuroprotective effect of Wnt-3a and its neuroprotection molecular mechanism after SCI. This study provides a new molecular mechanism and research basis for clinical treatment of SCI.

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Introduction

Spinal cord injury (SCI) is a severe central nervous system (CNS) traumatic disease with extremely high morbidity [1]. The main clinical manifestations of SCI include somatic sensation, voluntary movement, sphincter systolic and diastolic functions, and urinary dysfunction in the SCI segment, resulting in paraplegia. This condition devastates a patient's mental state, family economic status, labor ability, and social behavior [2]. In general, SCI is classified into primary and secondary injuries [3]. A primary SCI mainly involves mechanical damage of the spinal cord caused by trauma, including damage or breakage of blood vessels and nerves, which are irreversible

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damages. The secondary SCI injury is caused by spinal cord hemorrhage, edema, immunodeficiency, inflammatory reaction, ischemia reperfusion, apoptosis, oxidative stress, and lipid peroxidation. Primary injury is irreversible, whereas secondary injury is a dynamically modulating process that affects clinical outcomes of patients [4–6]. Therefore, the latter type of injury plays an important part in the process of SCI and offers a potential clinical therapeutic target for SCI.

Recent evidence has shown that the Wnt/ β -catenin signaling pathway, a canonical Wnt signaling pathway, plays a significant role in neural development, axonal guidance, neuropathic pain remission, and neuronal survival [7, 8]. The Wnt family of secreted cell signaling proteins, such as Wnt1, Wnt2, Wnt3, and Wnt-3a, consisting of at least 19 members in mammals plays an active role in cell growth and multiple processes during development [9, 10].

Wnt-3a, one of the Wnt family members that has been regarded as an activator of the canonical Wnt signaling pathway, is expressed in the dorsal midline region responsible for developing spinal cord, forming a dorsal-to-ventral concentration gradient. During vertebrate embryogenesis, Wnt-3a participates in multiple cellular functions, including self-renewal, proliferation, differentiation, and motility [11–14]. However, the specific molecular mechanism of Wnt-3a in the recovery of spinal motor function has not been elucidated after SCI. Hence, searching for the molecular mechanism underlying the neuroprotective effect of Wnt-3a will promote its application and conversion of clinical outcomes after SCI.

This review outlines the role of Wnt-3a in the pathogenesis and treatment of SCI and summarizes the latest findings focusing on the multiple roles of Wnt-3a, especially in SCI.

Expression of Wnt-3a after SCI

Previous studies have shown that Wnt signaling pathway is closely related to the development of the nervous system [15, 16]. Several studies have reported that 19 factors of the Wnt gene family are rarely expressed in uninjured spinal cord [9, 17]. The Wnt family comprises secreted glycoproteins, including Wnt1, Wnt3, Wnt-3a, and Wnt5, which play key roles in embryonic development, tissue regeneration, bone growth, stem cell differentiation, neurological diseases, and human cancers [18, 19]. In CNS diseases, Wnt family mainly affects the proliferation, composition, and sur-

vival of nerve cells. Wnt signaling pathways are mainly divided into 3 types: classical (Wnt/ β -catenin), noncanonical (Wnt/JNK), and Wnt- Ca^{2+} signaling pathways [20].

Wnt-3a is an important Wnt gene expressed in the development of the CNS [11]. This gene is mainly expressed in the dorsal region of the spinal cord, and it regulates the expansion and differentiation of spinal cord neurons, promotes continuous differentiation of spinal cord precursor cells, and inhibits the expression of protein kinase inhibitors [12]. Wnt-3a has been reported to promote axon conduction, stimulate axonal regeneration, reduce neuronal death, and ultimately promote the recovery of neurological function after SCI [13, 21, 22]. Wei et al. [23] revealed that within 7 days after SCI, the expression of Wnt-3a in the cephalic and caudal spinal cord segments significantly increased, remained at a high level, and declined. Wnt-3a causes a mitogenic effect on the development of spinal cord neural progenitor cells and plays an important role in specialization of dorsal spinal neurons [24]. From these findings, we can determine that Wnt gene expression increases after SCI, and the expression of Wnt-3a is consistent with nerve regeneration after SCI. Therefore, Wnt-3a may be involved in the recruitment of endogenous neural stem cells after SCI. The molecular mechanism may be due to spinal cord damage, which results in a rapid increase in Wnt-3a expression in the surrounding tissues, thereby recruiting a large number of neural stem cells to accumulate during lesion and repair and compensating the damaged neurons.

Wnt-3a expression level probably increases after SCI and changes with time. However, the specific molecular mechanism of Wnt-3a in the recovery of spinal neuromotor function after SCI has not been described. Future studies should provide more information on Wnt-3a expression in the spinal cord, especially in humans, whereas more in-depth research should focus on understanding the downstream molecular regulatory mechanisms involved in the expression of Wnt signaling molecules in the injured spinal cord and their roles.

Wnt-3a Promotes the Proliferation and Differentiation of Neural Stem Cells

Neural stem cells can differentiate into nerve cells, astrocytes, and oligodendrocytes; they are self-renewing and sufficiently provide a large number of brain tissue cells [25]. Neuronal damage, necrosis, axonal rupture, de-

myelination, and oligodendrocyte destruction during SCI directly cause spinal cord conduction dysfunction; on the other hand, neurons lack regenerative capacity, and the spinal cord itself inhibits regeneration and repair; late glial scar formation hinders axonal regeneration, which explains why the spinal cord cannot repair itself during SCI [26–29]. In response to the above mechanisms, neural stem cells feature potential in repairing spinal cord functions owing to 2 aspects: (1) cell replacement provides new neural connections and remyelination and (2) gene therapy supports injured neurons to survive, fights against unfavorable suppression of the environment, and promotes stability functional axon regeneration and synapse reformation [30]. To date, numerous problems are encountered in the treatment of SCI by neural stem cell transplantation mainly because of limited seed cell sources. The use of embryonic stem cells is inevitably blamed by ethics [31], and given the long-term in vitro culture and amplification and various stimulating factors, the biosafety of neural stem cells must also be considered. Previous studies have shown that neural stem cells cultured in vitro exhibit genetic abnormalities, including altered cell proliferation and differentiation traits and tumorigenicity, casting doubt on their safety [32]. Therefore, induction of endogenous neural stem cells for the treatment of SCI has become an important topic in neurology.

The Wnt signaling pathway transmits growth stimulation signals, which play important roles in the development of a variety of biological embryonic axes, especially the development of the embryonic nervous system [33, 34]. Jiao et al. [35] discovered that mir-124 can promote the proliferation of neural stem cells and induce their differentiation into neurons by targeting *dact1* to activate the Wnt/ β -catenin pathway, providing a potential target and contributing to the development of neurological cell therapy. Wehner et al. [36] delineated and experimentally studied the spinal cord regeneration of zebrafish larvae and its dependence on Wnt/ β -catenin signaling. Drug interference with Wnt/ β -catenin signaling (*Iwr-1*) impairs the recovery of spinal cord continuity, suggesting that Wnt-3a promotes neuronal cell division throughout the spinal cord. Lie et al. [37] showed that adult hippocampal stem cells express Wnt receptors and signal components. In vitro and in vivo experiments confirmed that Wnt signaling is involved in neuronal decisions and neuronal precursor cell-directed proliferation regulation during adult hippocampal stem cell neurogenesis. In addition, Du et al. [38] also noted that Wnt-3a is critical for endothelial progenitor cell-

mediated neural stem cell proliferation and differentiation.

The Wnt signal pathway is closed in healthy adult mammals, and the Wnt gene is hardly expressed in vivo, while expression of the Wnt gene is significantly increased when the Wnt signal pathway is activated by certain stimuli, such as damage [39]. Previous studies have shown that activation of the canonical Wnt signaling pathway is associated with the proliferation of bone marrow stromal cells (BMSCs) [40]. De Boer et al. [41] added lithium chloride and L cells containing Wnt-3a plasmid to BMSC culture medium. At low amount of lithium chloride, BMSC proliferation was stimulated, but at high levels, cell proliferation was inhibited; the Wnt-3a group promoted cell proliferation. Shang et al. [42] observed that addition of Wnt-3a also promoted the proliferation of BMSCs; the increase in β -catenin in cells was detected by immunofluorescence staining and transferred from the vicinity of the cell membrane to the nucleus. Wnt-3a was demonstrated to promote cell proliferation through the classical Wnt signaling pathway.

Therefore, Wnt-3a protein is believed to participate in the proliferation of endogenous neural stem cells after SCI and in promoting proliferation. Hence, the study of the exact mechanism of action of Wnt-3a may provide potential clinical value for SCI treatment.

Role of Wnt-3a in Axon Regeneration

SCI in humans leads to permanent functional deficits due to insufficient regeneration of injured axons in the adult CNS [43]. Therefore, promoting axonal regeneration is an essential prerequisite for effective recovery from SCI [44, 45]. Wnt ligands inhibit glycogen synthase kinase 3 (GSK-3) activity through their canonical signaling transduction pathway and facilitate axonal regeneration similar to that of neurotrophins; Wnt-3a induces neurite outgrowth in dorsal root ganglia neurons via Frizzled and Ryk receptor complex [21, 46]. Suh et al. [22] sought to demonstrate the therapeutic effect of Wnt-3a in a rat SCI model and tested the transplantation of Wnt-3a-expressing fibroblasts against spinal cord contusion that was created using a New York University impactor. Garcia et al. [47] reported that Wnt-induced pathways responsible for regulating axonal growth during embryogenesis could be repurposed to promote axonal growth after injury. The results strongly suggest that transplanted Wnt-3a-secreting fibroblasts promote axonal regeneration and functional improvement after SCI.

In Patel et al. [48] study, the regenerative and pro-survival properties of Wnt-3a after optic nerve injury were tested. They demonstrated that delivery of Wnt-3a to the retina led to increased Wnt signaling in *retinal ganglion cells* (RGCs), elevated RGC survival, and induced significant axonal regrowth following optic nerve crush. In addition, they used conditional Stat3 knock-out mice to demonstrate its contribution to the effects of Wnt-3a on RGCs. These results identify Wnt-3a as a novel molecular pathway for RGC survival and axonal regrowth after optic nerve crush.

The above experimental studies have shown that Wnt signaling pathways, especially that of Wnt-3a, are indispensable for axonal regeneration of the CNS. However, several scholars are skeptical and put forward an opposite view. Li et al. [49] study demonstrated that axonal outgrowth was inhibited by Wnt-3a overexpression, which then influenced the commissural axon projection and formation of nerve fibers. In addition, Wnt-3a ectopic expression resulted in impairment of neuronal migration and motor neuron positioning, ultimately leading to abnormal spinal cord. Selvaraj et al. [50] also showed that *nerve growth factor*-induced neurite outgrowth was inhibited by Wnt-3a, and this inhibition was prevented by neurotrophic factor (NF)- α 1. In addition, they showed that in PC12 cells, NF- α 1 acts by upregulating adenomatous polyposis coli accumulation at the neurite tips, thereby providing positive and negative Wnt-3a-/Wnt-5a-mediated cues to modulate neurite outgrowth, which is an important process during neurodevelopment.

The mechanism of Wnt-3a in axon regeneration is currently unclear, and researchers express different opinions regarding the matter. Although further investigation will be necessary to clarify the intracellular mechanism by which Wnt signaling/Wnt-3a promotes axonal regeneration and functional improvement, this approach could be a highly promising therapeutic strategy for SCI.

Regulation of Wnt-3a on Neurogenic Inflammation

Neuroinflammatory response is an important pathological basis for a number of neurodegenerative diseases; it plays an important role in the neurodegeneration of traumatized nervous system [51–53]. As the main mediator of innate CNS immune response, microglia play important roles in neuroinflammation and secondary injury after neurological trauma [54]. In recent years, microglia have been recognized to present a dual role in

brain and spinal cord trauma, promoting tissue recovery on the one hand and leading to neurodegeneration on the other [55, 56]. Microglia clear cell debris by phagocytosis, release nerve growth factors and anti-inflammatory factors to reduce nerve damage, and promote tissue repair [57]. However, the highly activated state of microglia can release high levels of proinflammatory and cytotoxic substances, resulting in neuronal disability and cell death [58].

Studies have reported that activated microglia-regulated neuroinflammatory responses are associated with the Wnt signaling pathway [59–61]. They demonstrated disease-associated β -catenin signaling in microglia in vivo by showing age-dependent accumulation of β -catenin in Alzheimer's disease-like pathology mice (APdE9). In cultured mouse microglia expressing the Wnt receptors *Fzd* 4, 5, 7, and 8 and low-density lipoprotein receptor-associated protein 5/6, Wnt-3a was found to stabilize β -catenin and dose-dependently induce lipoprotein receptor-associated protein 6 phosphorylation, featuring disordered downstream activation, β -catenin stabilization, and nuclear import. Gene expression profiling revealed that Wnt-stimulated microglia to specifically increase the expression of proinflammatory immune response genes in microglia, exacerbating the *de novo* release of interleukin-6, interleukin-12, and tumor necrosis factor [62]. Overall, their data suggest that the Wnt family of lipoproteins can direct the transformation of proinflammatory microglia and emphasize the pathological significance of β -catenin signaling network. This study suggests an increase in β -catenin levels in microglia, raising an important question: is β -catenin signaling in microglia in glial cells beneficial or harmful to disease outcomes?

Meaningfully, activation of microglia, particularly in Alzheimer's disease, is associated with a positive effect based on reducing Ab load by phagocytosis [63]. If this function is significantly affected by GSK3 inhibitor treatment, a beneficial therapeutic effect may be produced by supporting proinflammatory microglia [64]. Therefore, Halleskog et al. [65] concluded that selective blockade of Wnt signaling in microglia may exhibit therapeutic implications. However, treatment with GSK-3 inhibitors will inevitably enhance microglial Wnt signaling, which may exacerbate proinflammatory responses and enhance neurotoxicity as a long-term side effect. Di Liddo et al. [66] investigated the role of the Wnt pathway in the inflammation response of the enteric nervous system; neurons and glial cells were treated with exogenous Wnt-3a and/or *lipopolysaccharide* and with or without support-

ing NFs, such as basic fibroblast growth, epithelial growth, and glial cell-derived NFs. The results indicated the presence of neuronal monitoring in the intestinal myenteric plexus by FZD9 and Wnt-3a. In addition, experimental evidence was provided to elucidate the correlation between soluble trophic factors, Wnt signaling, and enteric nervous system anti-inflammatory protection.

The above studies confirmed that the Wnt signaling pathway and Wnt-3a are involved in the regulation of neurogenic inflammatory responses, which are associated with microglial activation. Although the specific mechanism of action is unclear, this finding provides sufficient potential clinical treatment value and may become a research hotspot in the future.

Wnt/ β -Catenin Signaling Pathway and Autophagy, Apoptosis, and Neuroprotection

As mentioned earlier, Wnt signaling regulates several biological processes, including brain development and stem cell proliferation, and has been implicated in a whole spectrum of diseases from cancer to neurodegeneration; Wnt-3a plays an important role in these processes [39, 67, 68].

Previous reports have demonstrated that suppression of the Wnt/ β -catenin signaling pathway increases apoptotic activity in cells [69, 70]. The effect Wnt/ β -catenin signaling pathway was also observed in brain injury. Zhang et al. [71] demonstrated the efficacy of intranasal Wnt therapy for suppressing autophagic and apoptotic cell death and promoting neurovascular repair, which ultimately resulted in improved functional outcomes after traumatic brain injury insult. In addition, recent reports have claimed that activation of the Wnt/ β -catenin signaling pathway after SCI improved neuronal survival, axonal guidance, and neuropathic pain remission [7, 18].

Meaningfully, studies have also reported that atorvastatin prevents calcification of vascular smooth muscle cells by inducing autophagy activity through inhibition of β -catenin signaling pathway [72]. In a study of head and neck squamous cell carcinoma, inhibition of β -catenin signaling pathway can induce autophagy activity, which inhibits cell apoptosis [73]. β -Catenin is considered a negative regulator of autophagy [74]. However, a study on rat hepatic ellipsoidal cell has reported that activation of the β -catenin signaling pathway promotes autophagy and proliferation and adaptability of hepatic ellipsoid cells [75].

However, the correlation between Wnt/ β -catenin signaling pathway and autophagy activity in neurons after SCI has not been elucidated. Although the mechanism has not been fully elucidated, the current findings warrant further examination of the Wnt/ β -catenin pathway for prospective treatment, particularly in the field of autophagy regulation.

New Discovery and Research Innovation in Our Study

Our previous study showed that at different time points after SCI, the expression levels of Wnt/ β -catenin classical signaling pathway proteins (Wnt-3a and β -catenin) increased first and then decreased with time. After 6, 12, and 18 h, all the proteins showed high expression levels. These results showed that the Wnt/ β -catenin classical signaling pathway was first activated and then suppressed over time after SCI. In addition, we observed changes in autophagy and autophagy mammalian target of rapamycin (mTOR) signaling pathway after SCI. We noted that the expression level of autophagy-tagged protein (Beclin-1) first increased and then decreased with time. The trend was higher at 6, 12, and 18 h after SCI and then gradually decreased, whereas the phosphorylation level of autophagy-specific mTOR pathway marker protein p70S6K after SCI was opposite to that of Beclin-1 (low levels at 6, 12, and 18 h). These results showed that autophagy of neurons after SCI increased first and then decreased. The mTOR signaling pathway showed the opposite trend. The above results also imply that the Wnt-3a/ β -catenin signaling pathway in neuronal cells after SCI may be positively correlated with the autophagy activity of neurons and negatively related with the mTOR signaling pathway.

In further experiments, we observed changes in autophagy, apoptosis, axonal regeneration, spinal cord anterior motoneuron number, and repair of SCI after Wnt-3a treatment. Wnt-3a was found to significantly activate the autophagy activity of neurons, inhibit the apoptotic activity of nerve cells, promote regeneration of axons, reduce the loss of motor neurons in the anterior horn of the spinal cord, and promote the repair of injured spinal cord tissue after SCI, providing a good microenvironment for the recovery of nerve function.

In addition, our study observed that the Wnt/ β -catenin signaling pathway was significantly activated in rats treated with methylprednisolone, rapamycin, and simvastatin

Fig. 1. The effect and molecular mechanism of wnt-3a/ β -catenin/mTOR signal pathway on autophagy after SCI. Human cell. Green: promote; Red: inhibit; P: phosphorylation; White number: references; Cytomembrane: extracellular membrane structure; Cytoplasm: intracellular structure. LRP5/6, lipoprotein receptor-associated protein 5/6; mTOR, mammalian target of rapamycin.

[76–78]. We also discovered that simvastatin activates the autophagic activity of neurons after SCI [79]. Furthermore, our study preliminarily verified the regulation of neural apoptosis activity by Wnt/ β -catenin signaling pathway after SCI.

Based on the above research, we propose the following hypotheses. (1) After SCI, the novel Wnt-3a/ β -catenin/mTOR signaling pathway positively regulates the autophagy activity of neurons. (2) Neuronal autophagy regulated by Wnt-3a/ β -catenin/mTOR signaling pathway exerts a neuroprotective effect on neurological recovery after SCI (Fig. 1). Given these hypotheses, the study of the molecular mechanism of autophagy after SCI may be promoted further. This study provides a new molecular mechanism and research basis for the treatment of SCI and promotes the research and clinical treatment of SCI.

Conclusion

Based on recent publications, Wnt-3a is expressed in the spinal cord dorsal midline region, which is responsible for developing spinal cord and forming a dorsal-to-ventral concentration gradient. Although the biological function of endogenous Wnt-3a in the spinal cord remains to be elucidated, recent evidence suggests that Wnt-3a may considerably influence the neuronal recovery following SCI. As shown in Figure 1, the activation of Wnt-3a in the spinal cord is neuroprotective and is closely related to the activation of autophagy, growth of neuron, inhibition of neuronal apoptosis, and axonal regen-

eration. This finding provides new insights into the study of SCI through Wnt-3a. However, relatively few studies focused on Wnt-3a in the spinal cord, and its specific mechanism on SCI has not been fully elucidated. Subsequent studies will further assess the effect of wnt-3a on different neurons, such as motor neurons, sensory neurons, central neurons, peripheral neurons and so on, and further verify the neuroprotective effect and its in-depth molecular mechanism of wnt-3a in different neurons after SCI through both in vivo and in vitro experiments.

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Disclosure Statement

The authors have declared that they have no conflict of interests for this work and project.

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