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Review

Therapeutic Strategies for Ischemic Stroke: Modulating the Adult Neural Stem Cell Niche through the Wnt/β-catenin Pathway

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Abstract

Stroke is a prominent contributor to mortality and impairment on a global scale. Ischemic stroke accounts for approximately 80% of stroke cases and is caused by occlusion of cerebral blood vessels. Enhancing neurogenesis through the modulation of the neural stem cell niche in the adult brain is a promising therapeutic strategy for individuals afflicted with ischemic stroke. Neurogenesis results in the generation of newborn neurons that serve as replacements for deceased neural cells within the ischemic core, thereby playing a significant role in the process of neural restoration subsequent to cerebral ischemia. Research has shown that activation of the Wnt/β-catenin pathway can augment neurogenesis following cerebral ischemia, suggesting that this pathway is a potentially beneficial therapeutic target for managing ischemic stroke. This review provides an extensive analysis of the current knowledge regarding the involvement of the Wnt/β-catenin pathway in promoting neurogenesis, thereby offering a promising avenue for therapeutic intervention in the context of ischemic stroke or other neurological impairments.

Keywords: Wnt/β-catenin signaling pathway; neurogenesis; neural stem cells; ischemic stroke; lineage tracing

1. Introduction

Stroke is a prevalent cerebrovascular disorder characterized by significant rates of disability and mortality. The two primary classifications of stroke are hemorrhagic stroke and ischemic stroke. Ischemic strokes account for approximately 80% of all stroke cases, with middle cerebral artery thromboembolism as the principal etiology [1]. Patients who experience ischemic stroke frequently exhibit significant neurological impairments. At present, the sole interventions that have demonstrated efficacy during the acute phase of ischemic stroke are the administration of tissue plasminogen activator (tPA) and mechanical thrombectomy [2]. Unfortunately, the therapeutic window is narrow, and only a small proportion of patients are eligible [3–5]. Therefore, potential drugs for ischemic stroke treatment are urgently needed. The pathophysiology of ischemic stroke involves complicated molecular mechanisms, such as oxidative stress, inflammation and apoptosis. Available pharmacological therapies targeting crucial stages in the pathophysiology of ischemic stroke aim to promote optimal post-stroke neurological recovery by reducing neuronal apoptosis, inhibiting inflammation, promoting angiogenesis, and removing free radicals [6,7]. However, the performance of these therapies is not clinically satisfactory. In recent years, promoting neurogenesis from endogenous neural stem cells (NSCs) has emerged as a promising therapeutic approach for treating ischemic stroke [8].

Josef Altman was the first to present evidence of neurogenesis in the adult brain when he identified newly formed neurons and glial cells through the use of tritiated thymidine to label proliferating cells [9]. Since then, accumulating evidence has revealed that neurogenesis persists in the adult mammalian brain. Although research on adult neurogenesis in humans is controversial, the occurrence of neurogenesis in the adult human brain is widely acknowledged [10,11]. Presently, two extensively documented neurogenic niches housing adult NSCs exist within the adult brain: the subventricular zone (SVZ) located in the lateral ventricle (LV) and the subgranular zone (SGZ) situated in the dentate gyrus (DG) of the hippocampus. Under normal physiological conditions, SVZ NSCs are responsible for the production of transit-amplifying cells (TACs), subsequently leading to the emergence of neuroblasts. These neuroblasts undergo migration along the rostral migratory stream (RMS) to reach the olfactory bulb (OB), where they differentiate into interneurons that play a crucial role in the sense of smell. NSCs located in the SGZ are responsible for generating excitatory glutamatergic neurons, which subsequently integrate into the granule cell layer of the DG. These neurons play a crucial role in spatial and temporal memory processing. During ischemic stroke, SGZ NSCs proliferate and migrate toward the granule cell layer within the DG, although their migration remains confined within the boundaries of the hippocampus [12]. Simultaneously, ischemic injury drastically increases neurogenesis in both rodent and human SVZs, and neuroblasts deviate from the conventional pathway toward the adjacent parenchyma and striatal ischemic penumbra. Within the ischemic penumbra, a limited population of neuroblasts produces neurons, potentially facilitating tissue restoration and

the recovery of locomotor function. However, a significant proportion of NSCs undergo differentiation into astrocytes, which subsequently transform into reactive astrocytes that play a crucial role in the process of glial scar formation. This impedes the effective repair of nerve injuries [8,13,14]. A prior investigation employed a genetic approach utilizing the herpes simplex virus thymidine kinase/ganciclovir (HSV-TK/GCV) suicide system to eliminate doublecortin $(DCX)^+$ neuroblasts, which led to increased infarct size and exacerbated neurological impairments in mice that underwent middle cerebral artery occlusion (MCAO) [15]. These findings indicate that neurogenesis induced by ischemia appears to play a role in alleviating histological and neurobehavioral impairments.

Taken together, these findings indicate that enhancing neurogenesis and augmenting neuronal regeneration within the ischemic penumbra subsequent to cerebral ischemia can potentially enhance the restoration of neurological function. Multiple pathways are believed to be implicated in adult neurogenesis, including the Notch signaling pathway [16], Sonic Hedgehog signaling (SHH) pathway [17,18], and bone morphogenetic protein (BMP) signaling pathway [19,20]. This review primarily concentrates on the role of the Wnt/β-catenin pathway in adult neurogenesis and its potential therapeutic implications for the management of cerebral ischemia (Fig. 1).

2. The Wnt Signaling Pathway

The Wnt signaling pathway primarily consists of Wnt ligands, which are secreted glycoproteins, and cell-surface receptors called Frizzled. The mammalian genome harbors a total of 19 *Wnt* genes that encode Wnt ligands. Wnt signaling pathways can be categorized into three different types: the Wnt/β-catenin pathway (also referred to as the canonical Wnt signaling pathway), the planar cell polarity pathway (Wnt/PCP), and the Wnt/Ca^{2+} signaling pathway.

In the canonical Wnt signaling pathway, Wnt ligands bind to the Frizzled receptor (Fzd) and low-density lipoprotein receptor-related protein 5/6 (LRP5/6). Then, the cytoplasmic protein Dishevelled (Dvl) is activated. Activation of Dvl leads to the degradation of a complex containing glycogen synthase kinase-3β (GSK-3β), adenomatous polyposis coli (APC), the protein kinase casein kinase 1α (CK1α), and Axin. In the absence of Wnt pathway activation, the GSK-3β/APC/CK1α/Axin complex phosphorylates cytosolic β-catenin, leading to its ubiquitination and subsequent degradation by the proteasome. Conversely, upon activation of the Wnt pathway, the degradation machinery of the GSK-3β/APC/CK1α/Axin complex is hindered, resulting in the cytosolic accumulation of β-catenin and its subsequent transportation into the nucleus. Intranuclear β-catenin participates in interactions with T-cell factor/lymphoid enhancer-binding factor (TCF/LEF) proteins, thereby promoting the transcription of downstream genes (Fig. 2) [21–23]. In addition to the canonical pathway, Wnt ligands can initiate various signaling cascades that do not rely on β-catenin, namely, the Wnt/PCP pathway and the Wnt/Ca^{2+} signaling pathway. The Wnt/PCP pathway begins with the interaction between a Wnt ligand and Fzd, subsequently leading to the activation of Dvl. The activation of Dvl, in turn, initiates the activation of small G proteins, including Rac and Rho, which subsequently activate Rho-associated kinase (ROCK) and c-Jun N-terminal kinase (JNK). This intricate series of events ultimately contributes to the establishment of cellular polarity and the facilitation of cell migration [24,25]. The initiation of the Wnt/Ca^{2+} pathway occurs when a Wnt ligand interacts with Fzd, leading to the subsequent activation of phospholipase C (PLC). This activation subsequently modulates the release of calcium from the endoplasmic reticulum, thereby governing the regulation of intracellular calcium concentrations [26].

Research has demonstrated that the Wnt signaling pathway, particularly the Wnt/β-catenin signaling pathway, is important for neurogenesis. This review aimed to consolidate existing studies pertaining to the role of the Wnt/β-catenin signaling pathway in neurogenesis throughout developmental stages and in the adult brain. Additionally, we explored the potential therapeutic implications of the Wnt/β-catenin pathway in the treatment of cerebral ischemia.

3. The Role of the Wnt/β-catenin Pathway in Neurogenesis during Brain Development

The Wnt/β-catenin signaling pathway has diverse functions throughout various stages of neural development. Overall, stimulation of the Wnt/β-catenin signaling pathway promotes neurogenesis and suppresses gliogenesis [27,28].

Chenn *et al*. [29] employed transgenic mice to induce excessive activation of β-catenin in neural precursors, resulting in the identification of enlarged brains characterized by augmented cerebral cortical surface area and folds, in addition to enlarged lateral ventricles lined with neuroepithelial precursor cells. This outcome can be attributed to an increased population of proliferative precursor cells [29]. Machon *et al*. [30] employed a *D6-Cre* mouse strain to conditionally inactivate *β-catenin* (*Ctnnb1*) within the murine cerebral cortex and hippocampus after embryonic day (E) 10.5. The results showed that β-catenin is needed for hippocampal progenitor proliferation, cortical neuronal migration, late-embryonic cortical proliferation, and cortical radial glial cell maintenance [30]. Additionally, several other studies have demonstrated the indispensability of the Wnt pathway in the development of the hippocampus [31,32]. Furthermore, the Wnt/β-catenin pathway plays a crucial role in the early stages of cerebellar development and assumes a pivotal function in governing differentiation within the cerebellar ventricular zone in the subsequent stages of embryonic development [33].

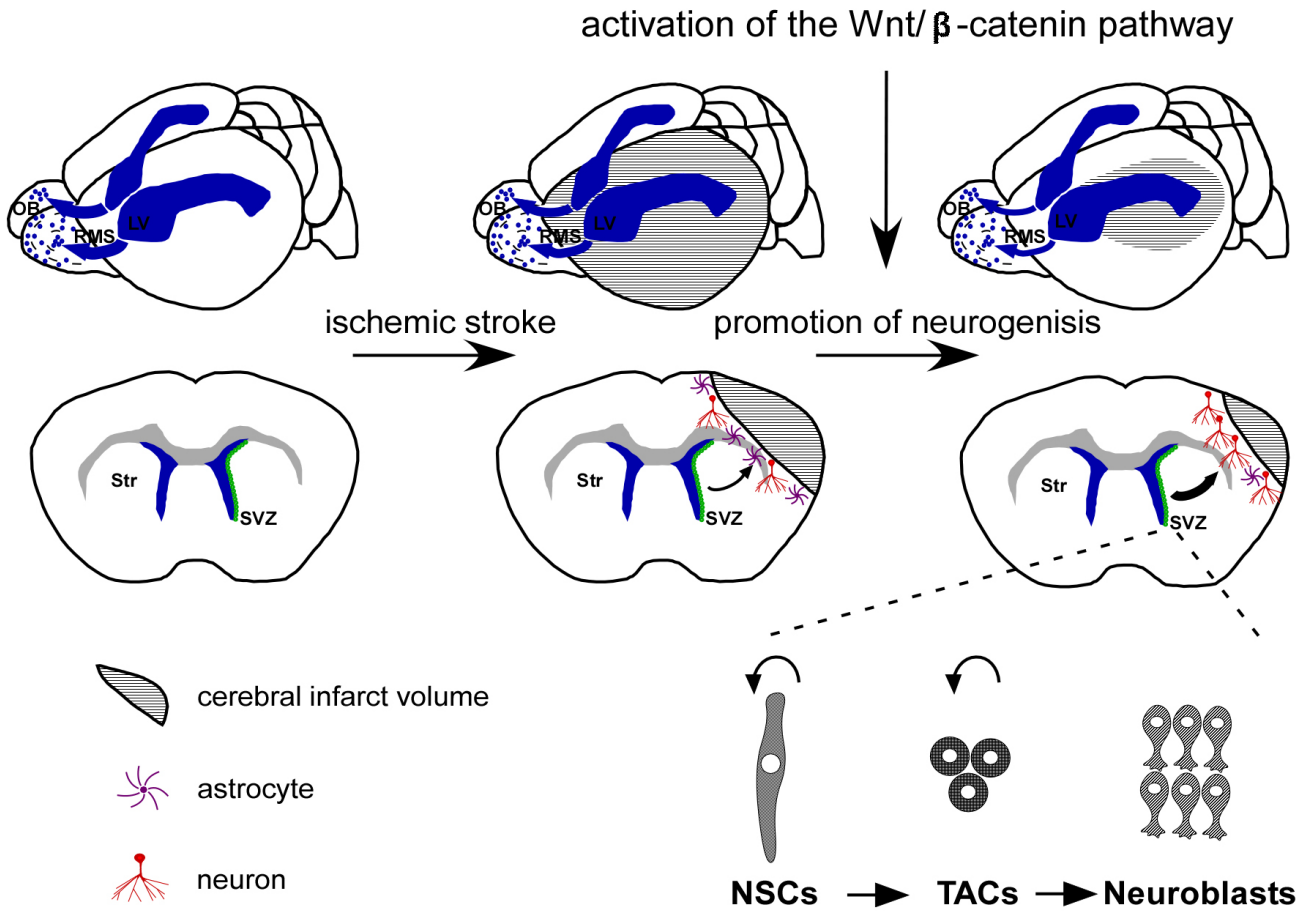

Fig. 1. Activation of the Wnt/β-catenin pathway offers a promising therapeutic strategy for treating ischemic stroke by promoting neurogenesis. Under typical physiological circumstances, SVZ NSCs are responsible for producing TACs, which possess the capacity to generate neuroblasts that subsequently migrate through the RMS toward the OB, where they undergo differentiation into interneurons. However, in the event of ischemic stroke, neuroblasts deviate from their conventional pathway toward the ischemic penumbra. Within the ischemic penumbra, a limited population of neuroblasts produces neurons. Activation of the Wnt/β-catenin pathway has been shown to facilitate neurogenesis and augment neuronal regeneration within the ischemic penumbra following cerebral ischemia, leading to an improvement in nerve function impairment. SVZ, subventricular zone; NSCs, neural stem cells; TACs, transit-amplifying cells; RMS, rostral migratory stream; OB, olfactory bulb; LV, lateral ventricle; Str, Striatum.

In vitro, the introduction of exogenous Wnt3a protein enhanced the proliferation and differentiation of NSCs isolated from the forebrain of E14.5 mice [34]. The inhibition of GSK-3β or the overexpression of β-catenin in ventral midbrain (VM) precursors leads to an increase in neuronal differentiation and the quantity of dopaminergic (DA) neurons [35]. Furthermore, Wnt1 and Wnt3a promoted the proliferation of VM precursors obtained from E14.5 rats, and Wnt5a induced the differentiation of DA precursors (from either the cortex or VM) into DA neurons [36,37]. These findings indicate that Wnts serve as pivotal regulators of neurogenesis in the VM.

As described above, the Wnt/β-catenin signaling pathway is known to play a crucial role in brain development, particularly in neurogenesis. However, the precise mechanism and downstream target genes involved in the promotion of neurogenesis by Wnt/β-catenin remain uncertain. Furthermore, the complexity of the mechanism is further compounded by considerations of spatial heterogeneity. A study revealed that β-catenin plays a crucial role in the proliferation and neuronal differentiation of neural progenitor cells. Chromatin immunoprecipitation (ChIP) and luciferase reporter assays revealed that β-catenin binds directly to the promoter or proximal enhancer regions of proneural genes, including *neurogenin 1*, *neurogenin 2*, *mash1*, and *myoD*, resulting in their activation [38]. A separate ChIP investigation revealed that the β-catenin/T-cell factor 1 (TCF1) complex plays a direct role in modulating the expression of Sox1, a marker of neural precursor cells, in the context of neuronal differentiation in embryonic stem cells [39]. In addition, some articles suggest that the Wnt/β-catenin signaling pathway facilitates the proliferation of NSCs while inhibiting their differentiation, thereby playing a role in the preservation of stem cell characteris-

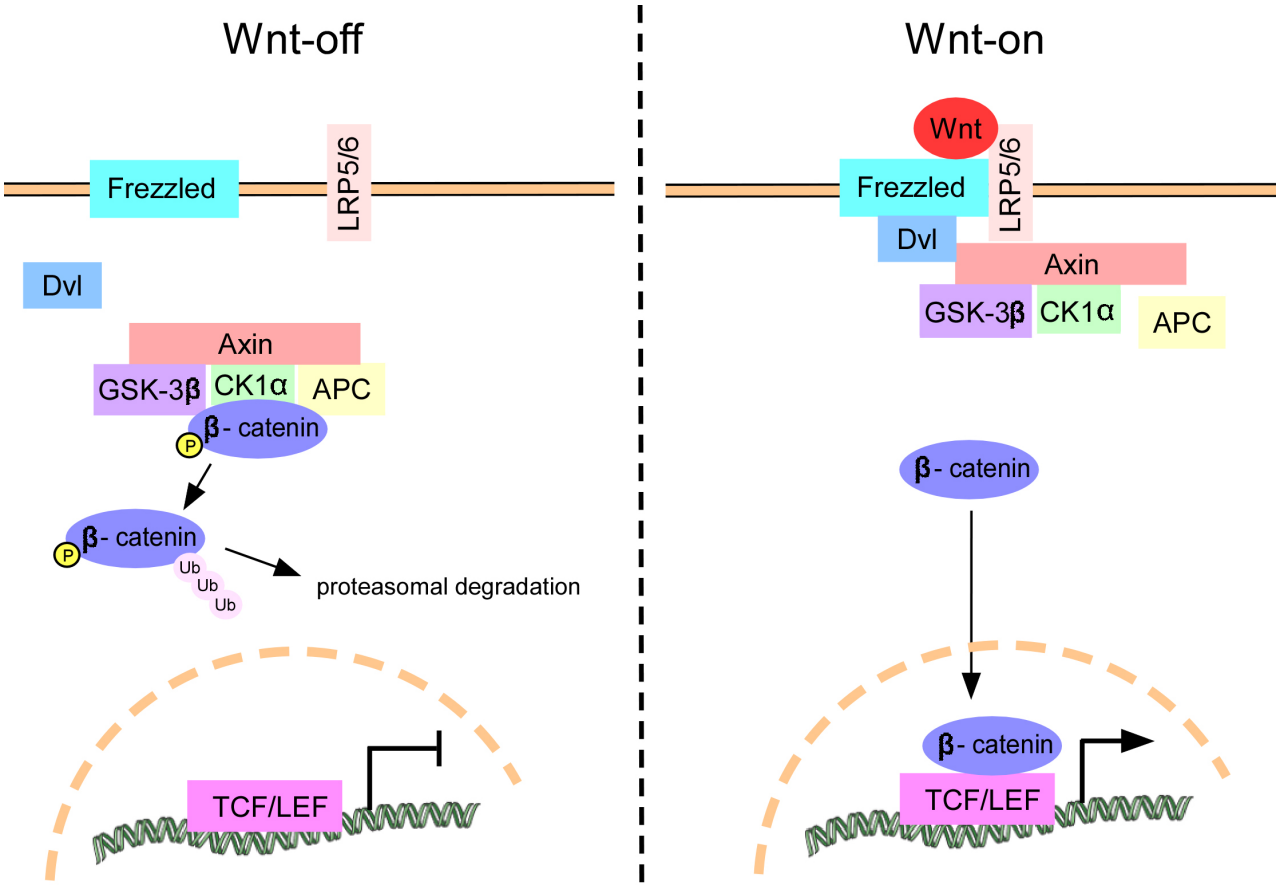

Fig. 2. Schematic diagram of the Wnt/β-catenin pathway. In the absence of Wnt ligand stimulation, β-catenin undergoes phosphorylation by the GSK-3β/APC/CK1α/Axin complex, leading to its ubiquitination and subsequent removal via the proteasome (Wnt-off). Upon activation of the Wnt/β-catenin pathway, the Wnt ligand binds to the Frizzled receptor and LRP5/6, resulting in activation of the cytoplasmic protein Dvl. This activation of Dvl triggers the degradation of the GSK-3β/APC/CK1α/Axin complex, leading to the accumulation of β-catenin in the cytosol and its subsequent translocation into the nucleus. In the nuclear compartment, β-catenin engages in interactions with TCF/LEF proteins, thereby facilitating the transcription of genes located downstream (Wnt-on). Dvl, Dishevelled; GSK-3β, glycogen synthase kinase-3β; APC, adenomatous polyposis coli; CK1α, casein kinase 1α; LRP5/6, low-density lipoprotein receptor-related protein 5/6; TCF/LEF, T-cell factor/lymphoid enhancer-binding factor.

tics [40,41]. This disparity could be attributed to variations in the culture conditions of neural progenitor cells, specifically the presence or absence of Fibroblast growth factor 2 (FGF2) [38].

Taken together, these findings highlight the involvement of Wnt/β-catenin signaling in NSCs during development and imply that this pathway may also facilitate adult neurogenesis and contribute to nerve regeneration following cerebral ischemia.

4. The Role of the Wnt/β-catenin Pathway in Adult Brain Neurogenesis

4.1 Inhibition of the Wnt/β-catenin Pathway Blocks Adult Brain Neurogenesis

Wnt/β-catenin signaling is activated in adult NSCs [42,43]. Numerous published studies have shown that the ablation of Wnt/β-catenin signaling impedes the proliferation of adult NSCs and their subsequent differentiation into neurons. The overexpression of mutant Wnt1 protein in the DG led to reduced proliferation of adult hippocampal NSCs and impaired generation of new neurons by suppressing Wnt signaling, consequently impacting spatial memory and object recognition memory [44,45]. Knockout of *Wnt7a* led to a substantial decrease in the abundance of NSCs and an increase in the rate of cell cycle termination in neural progenitors located in the DG and SVZ of adult mice. Furthermore, Wnt7a plays a pivotal role in the process of neuronal differentiation and maturation. The absence of Wnt7a expression led to a noteworthy decrease in the number of newly formed neurons in the DG region of the hippocampus [46,47]. Another study showed that the suppression of Wnt signaling through the knockdown of the Frizzled-1 receptor in the hippocampus resulted in reduced neuronal differentiation of NSCs and altered migration patterns of newly generated neurons [48].

Table 1. Evidence of the role of the Wnt/β-catenin pathway in adult brain neurogenesis.

Signaling molecule	Manipulation	Effect on Wnt/β-catenin pathway	*In vivo*/*In vitro*	Phenotype	Reference
Wnt1	overexpression of mutant Wnt1	inhibits	*in vivo*	inhibits hippocampal neurogenesis	[44,45]
Wnt7a	knockout	inhibits	*in vivo*	inhibits neurogenesis in DG and SVZ	[46,47]
Frizzled-1	knockdown	inhibits	*in vivo*	inhibits hippocampal neurogenesis	[48]
β-catenin	knockout	inhibits	*in vivo*	inhibits hippocampal neurogenesis	[49]
Dickkopf-1	overexpression	inhibits	*in vivo*	decreases proliferation of Mash1$^+$ progenitor cells within the SVZ	[50]
β-catenin	knockout	inhibits	*in vivo*	inhibits dendritic development	[55]
β-catenin	stabilization	activates	*in vivo*	promotes NSCs proliferation and their displacement from their correct SGZ location	[42]
GSK-3β	inhibition by CHIR99021	activates	*in vitro*	promotes neuronal differentiation of active NSCs and promotes the activation of quiescent NSCs	[42]
β-catenin GSK-3β	overexpression of stabilized β-catenin or inhibition of GSK-3β by Ro3303544	activates	*in vivo*	increases proliferation of Mash1$^+$ progenitor cells within the SVZ	[50]
Wnt7a β-catenin	overexpression of Wnt7a or stabilized β-catenin	activates	*in vitro*	promotes NSCs self-renewal	[46]
β-catenin	overexpression of stabilized β-catenin	activates	*in vivo*	increases numbers of type B NSCs in SVZ	[46]
Wnt3	overexpression	activates	*in vivo*/*in vitro*	promotes neuroblasts proliferation and neuronal differentiation	[44]
Wnt3a Wnt5a	overexpression	activates	*in vitro*	enhances the proliferation and neuronal differentiation of neural progenitor cells	[51]
Dickkopf-1	knockout	activates	*in vivo*	enhances hippocampal neurogenesis	[52]
secreted frizzled-related protein 3	knockout	activates	*in vivo*	promotes hippocampal neurogenesis, dendritic growth and spine formation	[53]
β-catenin	stabilization	activates	*in vivo*	accelerates dendritic growth, but eventually causes dendritic defects and excessive spine numbers	[54]

DG, dentate gyrus; SGZ, subgranular zone.

Table 2. Evidence of the role of the Wnt/β-catenin pathway in neurogenesis after cerebral ischemia.

Signaling molecule	Manipulation	Effect on Wnt/β-catenin pathway	*In vivo*/*In vitro*	Phenotype	Reference
β-catenin	knockdown	inhibits	*in vivo*	inhibits neurogenesis in SVZ and increases infarct volume	[56]
Dickkopf-1	overexpression	inhibits	*in vitro*	inhibits neuronal differentiation of NSCs derived from the SVZ of middle cerebral artery occlusion (MCAO) mice	[57]
Wnt3a	overexpression	activates	*in vivo*	increases neurogenesis and promotes neurological function recovery	[58]
Wnt3a	overexpression	activates	*in vivo*	promotes neurogenesis in SGZ and SVZ, decreases infarct volume, and enhances sensorimotor functions	[59]

However, Austin *et al*. [42] reported that conditional deletion of *β-catenin* in adult Glast-positive NSCs did not have any impact on their maintenance, activation, or differentiation. Interestingly, a separate investigation indicated that conditional knockout of *β-catenin* in Sox2-positive NSCs in the DG inhibited newborn neuron generation and the survival of neuronal progenitors [49].

4.2 Activation of the Wnt/β-catenin Pathway Promotes Adult Brain Neurogenesis

In contrast to knockout of *β-catenin*, conditional stabilization of β-catenin in mice resulted in the displacement of NSCs from the SGZ. Additionally, in an *in vitro* model of hippocampal NSCs, the activation of the Wnt/β-catenin signaling pathway facilitated the differentiation of active NSCs into neurons while also inducing the proliferation or differentiation of quiescent NSCs in a dose-dependent manner [42]. Another study indicated that the activation of the Wnt/β-catenin signaling pathway within the SVZ through the suppression of GSK-3β resulted in an elevated quantity of nascent neurons within the olfactory bulb. This phenomenon can be attributed to the increased proliferation of $Mash1^+$ progenitor cells within the SVZ [50].

The observed phenotypes resulting from the activation of Wnt/β-catenin signaling in adult NSCs are consistent with other reports in the literature. Activation of the Wnt/β-catenin signaling pathway leads to increased expression of NeuroD1 and long interspersed nuclear element 1 (LINE-1), both of which are pivotal in the process of neuronal differentiation [49]. Overexpression of *Wnt7a* and stabilized β-catenin promote NSC self-renewal *in vitro*. The introduction of *β-catenin* through lentiviral transduction resulted in an increase in the population of type B NSCs within the SVZ of adult brains [46]. The overexpression of *Wnt3* in NSCs *in vitro* or in the DG *in vivo* promoted neuroblast proliferation and neuronal differentiation [44]. In another study, the overexpression of *Wnt3a* or *Wnt5a* was found to enhance the proliferation and neuronal differentiation of neural progenitor cells derived from postnatal and adult mouse brains [51]. In addition, the augmentation of neurogenesis in the hippocampus was also observed upon the removal of Wnt inhibitors [52,53]. Moreover, the Wnt/β-catenin signaling pathway plays a significant role in the maturation of newborn neurons, as well as in the growth of dendrites and the formation of dendritic spines in adult hippocampal neurons [53–55].

In summary, the available evidence suggests that activation of the Wnt/β-catenin pathway in adult NSC niches promotes adult neurogenesis, whereas the inhibition of this pathway hinders adult neurogenesis (Table 1, Ref. [42,44–55]).

5. The Role of the Wnt/β-catenin Pathway in Neurogenesis after Cerebral Ischemia

The occurrence of cerebral ischemia leads to the stimulation of NSCs in the SVZ, resulting in their increased proliferation and subsequent asymmetric division into migratory neuroblasts. These neuroblasts subsequently migrate toward ischemic regions, where they undergo differentiation into neurons, potentially facilitating functional recovery. Additionally, NSCs in the SGZ can migrate to the granular cell layer and differentiate into new neurons, potentially reversing the learning and memory deficits caused by ischemia. However, it should be noted that this reparative mechanism is insufficient to fully compensate for the extensive damage caused by severe cerebral ischemia [8,13]. Studies have indicated that the Wnt/β-catenin pathway within adult NSC niches facilitates neurogenesis following cerebral ischemia and contributes to the restoration of neurological function (Table 2, Ref. [56–59]).

5.1 Inhibition of the Wnt/β-catenin Pathway Impedes Neurogenesis Subsequent to Cerebral Ischemia

Lei *et al*. [56] administered *β-catenin* Small interfering RNA (siRNA) intracerebroventricularly to mice subjected to transient middle cerebral artery occlusion (tMCAO) to deactivate β-catenin, leading to an increase in infarct volume and a decrease in neurogen-

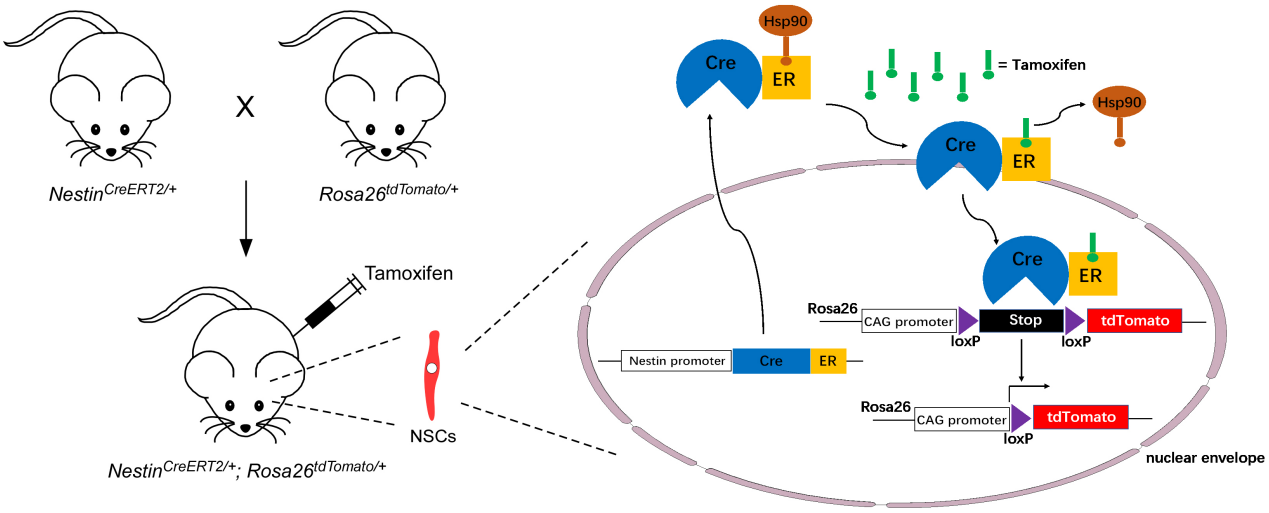

Fig. 3. Schematic protocol for lineage tracing of Nestin-positive adult NSCs. *Nestin*$^{CreERT2/+}$; *Rosa26*$^{tdTomato/+}$ mice are generated by crossing *Nestin-CreERT2* mice with *Rosa26-CAG-tdTomato* mice. Upon the administration of tamoxifen to *Nestin*$^{CreERT2/+}$; *Rosa26*$^{tdTomato/+}$ mice, Hsp90 is displaced from CreER, allowing Cre recombinase to enter the nucleus of Nestin-positive adult NSCs. Subsequently, the Cre recombinase removes the *loxP-Stop-loxP cassette*, resulting in permanent tdTomato expression in adult NSCs. ER, Estrogen receptor; CAG, cytomegalovirus enhancer plus chicken beta-actin promotor.

esis within the SVZ. The administration of *β-catenin* siRNA resulted in a significant reduction in the populations of 5-Bromo-2′-deoxyuridine (BrdU)$^+$/β3-tubulin (Tuj1)$^+$ cells, BrdU$^+$/Doublecortin (DCX)$^+$ cells, and BrdU$^+$/Microtubule-associated protein 2 (MAP2)$^+$ cells within the ischemic striatum, which serve as markers for newborn immature neurons, proliferating progenitors, and newborn mature neurons, respectively [56]. Furthermore, the use of a genetic approach to induce the overexpression of the Wnt inhibitor Dickkopf-1 (DKK1) in NSCs derived from the SVZs of mice subjected to MCAO suppressed neuronal differentiation [57].

5.2 Activation of the Wnt/β-catenin Pathway Promotes Neurogenesis after Cerebral Ischemia and is Beneficial for Ameliorating Nerve Function Injury following Cerebral Ischemia

The increase in Wnt3a levels within the SVZ or striatum of mice afflicted with focal ischemic injury was found to play a significant role in facilitating functional recovery subsequent to the ischemic event. This was achieved through the promotion of neurogenesis or the enhancement of neuronal viability [58]. A separate study demonstrated that the intranasal administration of Wnt3a subsequent to focal ischemic stroke in mice reduced infarct volume, augmented sensorimotor functions, stimulated neurogenesis in the SVZ and SGZ, increased the number of DCX$^+$/BrdU$^+$ colocalized cells migrating from the SVZ toward the peri-infarct area, and increased the quantity of newly formed neurons (BrdU$^+$/NeuN$^+$ cells) in the peri-infarct zone. Conversely, intranasal administration of a Wnt inhibitor hindered neurogenesis and decreased the quantity of newly generated neurons in the peri-infarct area [59].

In addition, studies have revealed that some treatments and mechanisms can further upregulate the Wnt/β-catenin pathway after cerebral ischemia, thereby promoting neurogenesis and contributing to the reinstatement of neurological function [60–64]. Taken together, these findings indicate that the activation of the Wnt/β-catenin pathway subsequent to cerebral ischemia has the potential to stimulate neurogenesis and ameliorate nerve function impairment, making this pathway a viable therapeutic target for the management of cerebral ischemia.

6. Limitations in the Available Studies on the Role of the Wnt/β-catenin Pathway in Postischemic Neurogenesis

As stated above, considerable advancements have been made in the field of research pertaining to genetic or pharmacological interventions in the Wnt/β-catenin pathway aimed at increasing neurogenesis following cerebral ischemia. However, most studies lack a quality study design. Many published studies utilized immunofluorescence colocalization of BrdU with neuronal markers or neuroblastic markers to evaluate neurogenesis and the *de novo* generation of neurons. However, it is unclear whether these proliferative cells were derived from NSCs. Previous studies have reported that astrocytes can transdifferentiate into morphologically mature and functional neurons after cerebral ischemia [65]. Therefore, it is recommended that researchers employ lineage tracing techniques in future studies to track the fate of NSCs to elucidate their potential contributions to brain neurogenesis and postischemic stroke recovery. Lineage tracing serves as a valuable tool for investigating the origin of new neurons in is-

chemic regions, specifically whether they originate from NSCs within the adult NSC niche. Additionally, it enables the examination of the impact of the Wnt/β-catenin signaling pathway on the cellular fate of different NSC populations. Currently, numerous techniques are available for lineage tracing (for review, see [66]), with one of the most prevalent approaches involving the integration of the *Cre* mouse line in combination with the *Rosa26-CAG-reporter* mouse line. For adult NSC lineage tracing, researchers can cross inducible NSC-specific Cyclization recombination enzyme (Cre) lines, such as *Nestin-CreERT2* mice [67] and *Ascll-CreERT2* mice [68], with *Rosa26-CAG-reporter* lines, such as *Rosa26-CAG-tdTomato* mice [69], to generate $Nestin^{CreERT2/+}$; $Rosa26^{tdTomato/+}$ mice or $Ascll^{CreERT2/+}$; $Rosa26^{tdTomato/+}$ mice. With tamoxifen administration, the NSCs in lineage-traced mice exhibit stable expression of tdTomato, enabling the tracking of the fate of cells originating from the NSC niche (Fig. 3).

There are additional limitations in studies on the role of the Wnt/β-catenin pathway in postischemic neurogenesis. All current studies on the Wnt/β-catenin pathway in postischemic neurogenesis have been conducted using animal models. Given the ongoing debate surrounding human adult neurogenesis, the transition from preclinical research to clinical trials for the treatment of ischemic stroke through the activation of the Wnt/β-catenin pathway presents significant obstacles. In addition, the potential of agonists of Wnt/β-catenin signaling to enhance neurogenesis following cerebral ischemia remains uncertain. The preclinical exploration of Wnt/β-catenin signaling agonists holds significance for their eventual clinical utility.

7. Conclusion

Ischemic stroke is characterized by increased morbidity, disability, recurrence, and mortality. Intravenous thrombolysis and thrombectomy are approved medical treatments for acute stroke. However, it is crucial to acknowledge the limited timeframe within which these therapies can be administered, as well as the potential for intracerebral hemorrhage and other bleeding complications that may arise from the utilization of intravenous thrombolysis and mechanical thrombectomy. Thus, there is a need for the development of new treatments. Researchers have focused on endogenous NSC-induced neurogenesis following ischemic stroke due to the demonstrated positive impact of NSCs on neural repair. According to existing studies, the modulation of numerous signaling pathways has been found to facilitate NSC proliferation and differentiation while also augmenting angiogenesis and synaptic plasticity. These mechanisms collectively contribute to the process of neural repair subsequent to ischemic brain injury. Based on the existing evidence, the Wnt/β-catenin pathway is a promising therapeutic target for alleviating the consequences of ischemic stroke. The activation of this pathway facilitates neurogenesis, leading to the generation of newly formed neurons that can replace deceased neural cells within the ischemic core. Future studies should further investigate whether the newly generated neurons are functional *in vivo* and whether they can integrate with existing neural circuits. Furthermore, additional in-depth lineage tracing experiments should be conducted.

Author Contributions

JDX and LSC designed the study. JDX wrote the manuscript and draw Figures and Tables; LSC revised the manuscript; SYL, LXX, YNZ, and WRJ collected and sorted references, as well as provided comments to improve the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

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Conflict of Interest

The authors declare no conflict of interest.

References

[1] Virani SS, Alonso A, Aparicio HJ, Benjamin EJ, Bittencourt MS, Callaway CW, *et al*. Heart Disease and Stroke Statistics-2021 Update: A Report from the American Heart Association. Circulation. 2021; 143: e254–e743.

[2] Powers WJ. Acute Ischemic Stroke. The New England Journal of Medicine. 2020; 383: 252–260.

[3] Aroor SR, Asif KS, Potter-Vig J, Sharma A, Menon BK, Inoa V, *et al*. Mechanical Thrombectomy Access for All? Challenges in Increasing Endovascular Treatment for Acute Ischemic Stroke in the United States. Journal of Stroke. 2022; 24: 41–48.

[4] Zerna C, Thomalla G, Campbell BCV, Rha JH, Hill MD. Current practice and future directions in the diagnosis and acute treatment of ischaemic stroke. Lancet (London, England). 2018; 392: 1247–1256.

[5] Nogueira RG, Jadhav AP, Haussen DC, Bonafe A, Budzik RF, Bhuva P, *et al*. Thrombectomy 6 to 24 Hours after Stroke with a Mismatch between Deficit and Infarct. The New England Journal of Medicine. 2018; 378: 11–21.

[6] Qin C, Yang S, Chu YH, Zhang H, Pang XW, Chen L, *et al*. Signaling pathways involved in ischemic stroke: molecular mechanisms and therapeutic interventions. Signal Transduction and Targeted Therapy. 2022; 7: 215.

[7] Mao R, Zong N, Hu Y, Chen Y, Xu Y. Neuronal Death Mechanisms and Therapeutic Strategy in Ischemic Stroke. Neuroscience Bulletin. 2022; 38: 1229–1247.
[8] Tang H, Li Y, Tang W, Zhu J, Parker GC, Zhang JH. Endogenous Neural Stem Cell-induced Neurogenesis after Ischemic Stroke: Processes for Brain Repair and Perspectives. Translational Stroke Research. 2023; 14: 297–303.
[9] ALTMAN J. Are new neurons formed in the brains of adult mammals? Science (New York, N.Y.). 1962; 135: 1127–1128.
[10] Moreno-Jiménez EP, Terreros-Roncal J, Flor-García M, Rábano A, Llorens-Martín M. Evidences for Adult Hippocampal Neurogenesis in Humans. The Journal of Neuroscience: the Official Journal of the Society for Neuroscience. 2021; 41: 2541–2553.
[11] Ernst A, Alkass K, Bernard S, Salehpour M, Perl S, Tisdale J, *et al*. Neurogenesis in the striatum of the adult human brain. Cell. 2014; 156: 1072–1083.
[12] Jin K, Sun Y, Xie L, Peel A, Mao XO, Batteur S, *et al*. Directed migration of neuronal precursors into the ischemic cerebral cortex and striatum. Molecular and Cellular Neurosciences. 2003; 24: 171–189.
[13] Passarelli JP, Nimjee SM, Townsend KL. Stroke and Neurogenesis: Bridging Clinical Observations to New Mechanistic Insights from Animal Models. Translational Stroke Research. 2024; 15: 53–68.
[14] Faiz M, Sachewsky N, Gascón S, Bang KWA, Morshead CM, Nagy A. Adult Neural Stem Cells from the Subventricular Zone Give Rise to Reactive Astrocytes in the Cortex after Stroke. Cell Stem Cell. 2015; 17: 624–634.
[15] Jin K, Wang X, Xie L, Mao XO, Greenberg DA. Transgenic ablation of doublecortin-expressing cells suppresses adult neurogenesis and worsens stroke outcome in mice. Proceedings of the National Academy of Sciences of the United States of America. 2010; 107: 7993–7998.
[16] Lampada A, Taylor V. Notch signaling as a master regulator of adult neurogenesis. Frontiers in Neuroscience. 2023; 17: 1179011.
[17] Yao PJ, Petralia RS, Mattson MP. Sonic Hedgehog Signaling and Hippocampal Neuroplasticity. Trends in Neurosciences. 2016; 39: 840–850.
[18] Álvarez-Buylla A, Ihrie RA. Sonic hedgehog signaling in the postnatal brain. Seminars in Cell & Developmental Biology. 2014; 33: 105–111.
[19] Choe Y, Pleasure SJ, Mira H. Control of Adult Neurogenesis by Short-Range Morphogenic-Signaling Molecules. Cold Spring Harbor Perspectives in Biology. 2015; 8: a018887.
[20] Bond AM, Bhalala OG, Kessler JA. The dynamic role of bone morphogenetic proteins in neural stem cell fate and maturation. Developmental Neurobiology. 2012; 72: 1068–1084.
[21] Rim EY, Clevers H, Nusse R. The Wnt Pathway: From Signaling Mechanisms to Synthetic Modulators. Annual Review of Biochemistry. 2022; 91: 571–598.
[22] Clevers H, Nusse R. Wnt/β-catenin signaling and disease. Cell. 2012; 149: 1192–1205.
[23] Nusse R, Clevers H. Wnt/β-Catenin Signaling, Disease, and Emerging Therapeutic Modalities. Cell. 2017; 169: 985–999.
[24] Koca Y, Collu GM, Mlodzik M. Wnt-frizzled planar cell polarity signaling in the regulation of cell motility. Current Topics in Developmental Biology. 2022; 150: 255–297.
[25] Shi DL. Wnt/planar cell polarity signaling controls morphogenetic movements of gastrulation and neural tube closure. Cellular and Molecular Life Sciences: CMLS. 2022; 79: 586.
[26] Kohn AD, Moon RT. Wnt and calcium signaling: beta-catenin-independent pathways. Cell Calcium. 2005; 38: 439–446.
[27] Kriska J, Honsa P, Dzamba D, Butenko O, Kolenicova D, Janeckova L, *et al*. Manipulating Wnt signaling at different subcellular levels affects the fate of neonatal neural stem/progenitor cells. Brain Research. 2016; 1651: 73–87.
[28] Kunke D, Bryja V, Mygland L, Arenas E, Krauss S. Inhibition of canonical Wnt signaling promotes gliogenesis in P0-NSCs. Biochemical and Biophysical Research Communications. 2009; 386: 628–633.
[29] Chenn A, Walsh CA. Regulation of cerebral cortical size by control of cell cycle exit in neural precursors. Science (New York, N.Y.). 2002; 297: 365–369.
[30] Machon O, van den Bout CJ, Backman M, Kemler R, Krauss S. Role of beta-catenin in the developing cortical and hippocampal neuroepithelium. Neuroscience. 2003; 122: 129–143.
[31] Galceran J, Miyashita-Lin EM, Devaney E, Rubenstein JL, Grosschedl R. Hippocampus development and generation of dentate gyrus granule cells is regulated by LEF1. Development (Cambridge, England). 2000; 127: 469–482.
[32] Lee SM, Tole S, Grove E, McMahon AP. A local Wnt-3a signal is required for development of the mammalian hippocampus. Development (Cambridge, England). 2000; 127: 457–467.
[33] Selvadurai HJ, Mason JO. Activation of Wnt/β-catenin signalling affects differentiation of cells arising from the cerebellar ventricular zone. PloS One. 2012; 7: e42572.
[34] Kalani MYS, Cheshier SH, Cord BJ, Bababeygy SR, Vogel H, Weissman IL, *et al*. Wnt-mediated self-renewal of neural stem/progenitor cells. Proceedings of the National Academy of Sciences of the United States of America. 2008; 105: 16970–16975.
[35] Castelo-Branco G, Rawal N, Arenas E. GSK-3beta inhibition/beta-catenin stabilization in ventral midbrain precursors increases differentiation into dopamine neurons. Journal of Cell Science. 2004; 117: 5731–5737.
[36] Castelo-Branco G, Wagner J, Rodriguez FJ, Kele J, Sousa K, Rawal N, *et al*. Differential regulation of midbrain dopaminergic neuron development by Wnt-1, Wnt-3a, and Wnt-5a. Proceedings of the National Academy of Sciences of the United States of America. 2003; 100: 12747–12752.
[37] Castelo-Branco G, Sousa KM, Bryja V, Pinto L, Wagner J, Arenas E. Ventral midbrain glia express region-specific transcription factors and regulate dopaminergic neurogenesis through Wnt-5a secretion. Molecular and Cellular Neurosciences. 2006; 31: 251–262.
[38] Israsena N, Hu M, Fu W, Kan L, Kessler JA. The presence of FGF2 signaling determines whether beta-catenin exerts effects on proliferation or neuronal differentiation of neural stem cells. Developmental Biology. 2004; 268: 220–231.
[39] Kim H, Kim S, Song Y, Kim W, Ying QL, Jho EH. Dual Function of Wnt Signaling during Neuronal Differentiation of Mouse Embryonic Stem Cells. Stem Cells International. 2015; 2015: 459301.
[40] Shimizu T, Kagawa T, Inoue T, Nonaka A, Takada S, Aburatani H, *et al*. Stabilized beta-catenin functions through TCF/LEF proteins and the Notch/RBP-Jkappa complex to promote proliferation and suppress differentiation of neural precursor cells. Molecular and Cellular Biology. 2008; 28: 7427–7441.
[41] Wexler EM, Paucer A, Kornblum HI, Palmer TD, Geschwind DH. Endogenous Wnt signaling maintains neural progenitor cell potency. Stem Cells (Dayton, Ohio). 2009; 27: 1130–1141.
[42] Austin SHL, Gabarró-Solanas R, Rigo P, Paun O, Harris L, Guillemot F, *et al*. Wnt/β-catenin signalling is dispensable for adult neural stem cell homeostasis and activation. Development (Cambridge, England). 2021; 148: dev199629.
[43] Bowman AN, van Amerongen R, Palmer TD, Nusse R. Lineage tracing with Axin2 reveals distinct developmental and adult populations of Wnt/β-catenin-responsive neural stem cells. Proceedings of the National Academy of Sciences of the United States of America. 2013; 110: 7324–7329.
[44] Lie DC, Colamarino SA, Song HJ, Désiré L, Mira H, Consiglio

A, *et al*. Wnt signalling regulates adult hippocampal neurogenesis. Nature. 2005; 437: 1370–1375.

[45] Jessberger S, Clark RE, Broadbent NJ, Clemenson GD, Jr, Consiglio A, Lie DC, *et al*. Dentate gyrus-specific knockdown of adult neurogenesis impairs spatial and object recognition memory in adult rats. Learning & Memory (Cold Spring Harbor, N.Y.). 2009; 16: 147–154.

[46] Qu Q, Sun G, Li W, Yang S, Ye P, Zhao C, *et al*. Orphan nuclear receptor TLX activates Wnt/beta-catenin signalling to stimulate neural stem cell proliferation and self-renewal. Nature Cell Biology. 2010; 12: 31–39.

[47] Qu Q, Sun G, Murai K, Ye P, Li W, Asuelime G, *et al*. Wnt7a regulates multiple steps of neurogenesis. Molecular and Cellular Biology. 2013; 33: 2551–2559.

[48] Mardones MD, Andaur GA, Varas-Godoy M, Henriquez JF, Salech F, Behrens MI, *et al*. Frizzled-1 receptor regulates adult hippocampal neurogenesis. Molecular Brain. 2016; 9: 29.

[49] Kuwabara T, Hsieh J, Muotri A, Yeo G, Warashina M, Lie DC, *et al*. Wnt-mediated activation of NeuroD1 and retro-elements during adult neurogenesis. Nature Neuroscience. 2009; 12: 1097–1105.

[50] Adachi K, Mirzadeh Z, Sakaguchi M, Yamashita T, Nikolcheva T, Gotoh Y, *et al*. Beta-catenin signaling promotes proliferation of progenitor cells in the adult mouse subventricular zone. Stem Cells (Dayton, Ohio). 2007; 25: 2827–2836.

[51] Yu JM, Kim JH, Song GS, Jung JS. Increase in proliferation and differentiation of neural progenitor cells isolated from postnatal and adult mice brain by Wnt-3a and Wnt-5a. Molecular and Cellular Biochemistry. 2006; 288: 17–28.

[52] Seib DRM, Corsini NS, Ellwanger K, Plaas C, Mateos A, Pitzer C, *et al*. Loss of Dickkopf-1 restores neurogenesis in old age and counteracts cognitive decline. Cell Stem Cell. 2013; 12: 204–214.

[53] Jang MH, Bonaguidi MA, Kitabatake Y, Sun J, Song J, Kang E, *et al*. Secreted frizzled-related protein 3 regulates activity-dependent adult hippocampal neurogenesis. Cell Stem Cell. 2013; 12: 215–223.

[54] Heppt J, Wittmann MT, Schäffner I, Billmann C, Zhang J, Vogt-Weisenhorn D, *et al*. β-catenin signaling modulates the tempo of dendritic growth of adult-born hippocampal neurons. The EMBO Journal. 2020; 39: e104472.

[55] Gao X, Arlotta P, Macklis JD, Chen J. Conditional knock-out of beta-catenin in postnatal-born dentate gyrus granule neurons results in dendritic malformation. The Journal of Neuroscience: the Official Journal of the Society for Neuroscience. 2007; 27: 14317–14325.

[56] Lei ZN, Zhang LM, Sun FY. Beta-catenin siRNA inhibits ischemia-induced striatal neurogenesis in adult rat brain following a transient middle cerebral artery occlusion. Neuroscience Letters. 2008; 435: 108–112.

[57] Kriska J, Janeckova L, Kirdajova D, Honsa P, Knotek T, Dzamba D, *et al*. Wnt/β-Catenin Signaling Promotes Differentiation of Ischemia-Activated Adult Neural Stem/Progenitor Cells to Neuronal Precursors. Frontiers in Neuroscience. 2021; 15: 628983.

[58] Shruster A, Ben-Zur T, Melamed E, Offen D. Wnt signaling enhances neurogenesis and improves neurological function after focal ischemic injury. PloS One. 2012; 7: e40843.

[59] Wei ZZ, Zhang JY, Taylor TM, Gu X, Zhao Y, Wei L. Neuroprotective and regenerative roles of intranasal Wnt-3a administration after focal ischemic stroke in mice. Journal of Cerebral Blood Flow and Metabolism: Official Journal of the International Society of Cerebral Blood Flow and Metabolism. 2018; 38: 404–421.

[60] Yang X, Song D, Chen L, Xiao H, Ma X, Jiang Q, *et al*. Curcumin promotes neurogenesis of hippocampal dentate gyrus via Wnt/β-catenin signal pathway following cerebral ischemia in mice. Brain Research. 2021; 1751: 147197.

[61] Cheng J, Shen W, Jin L, Pan J, Zhou Y, Pan G, *et al*. Treadmill exercise promotes neurogenesis and myelin repair via upregulating Wnt/β catenin signaling pathways in the juvenile brain following focal cerebral ischemia/reperfusion. International Journal of Molecular Medicine. 2020; 45: 1447–1463.

[62] Xu D, Hou K, Li F, Chen S, Fang W, Li Y. XQ-1H alleviates cerebral ischemia in mice through inhibition of apoptosis and promotion of neurogenesis in a Wnt/β-catenin signaling dependent way. Life Sciences. 2019; 235: 116844.

[63] Qiu CW, Liu ZY, Zhang FL, Zhang L, Li F, Liu SY, *et al*. Post-stroke gastrodin treatment ameliorates ischemic injury and increases neurogenesis and restores the Wnt/β-Catenin signaling in focal cerebral ischemia in mice. Brain Research. 2019; 1712: 7–15.

[64] Yu Z, Cheng C, Liu Y, Liu N, Lo EH, Wang X. Neuroglobin promotes neurogenesis through Wnt signaling pathway. Cell Death & Disease. 2018; 9: 945.

[65] Yang JL, Fan H, Fu FF, Guo BL, Huang Y, Sun L, *et al*. Transient neurogenesis in ischemic cortex from Sox2$^+$ astrocytes. Neural Regeneration Research. 2023; 18: 1521–1526.

[66] Baron CS, van Oudenaarden A. Unravelling cellular relationships during development and regeneration using genetic lineage tracing. Nature Reviews. Molecular Cell Biology. 2019; 20: 753–765.

[67] Lagace DC, Whitman MC, Noonan MA, Ables JL, DeCarolis NA, Arguello AA, *et al*. Dynamic contribution of nestin-expressing stem cells to adult neurogenesis. The Journal of Neuroscience: the Official Journal of the Society for Neuroscience. 2007; 27: 12623–12629.

[68] Kim EJ, Leung CT, Reed RR, Johnson JE. In vivo analysis of Ascl1 defined progenitors reveals distinct developmental dynamics during adult neurogenesis and gliogenesis. The Journal of Neuroscience: the Official Journal of the Society for Neuroscience. 2007; 27: 12764–12774.

[69] Madisen L, Zwingman TA, Sunkin SM, Oh SW, Zariwala HA, Gu H, *et al*. A robust and high-throughput Cre reporting and characterization system for the whole mouse brain. Nature Neuroscience. 2010; 13: 133–140.