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Review

Targeting Neurogenesis: A Promising Therapeutic Strategy for Post-Stroke Treatment with Chinese Herbal Medicine

Jiangang Shen[a, b] Xingmiao Chen[a, b] Xi Chen[a] Ruixia Deng[a, b]

[a]School of Chinese Medicine, LKS Faculty of Medicine, and [b]Research Centre of Heart, Brain, Hormone and Healthy Aging, The University of Hong Kong, Hong Kong, SAR, China

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Neural stem cells · Chinese herbal medicine · Neurogenesis · Stroke

Abstract
Recent progress suggests that neural stem/progenitor cells can potentially develop into new functional neurons in adult brain, offering hope for regeneration therapies for stroke treatment. Targeting adult neurogenesis becomes a novel and promising therapeutic strategy for brain repair and recovery of neurological functions. Traditional Chinese Medicine (TCM) has a long history with accumulated experiences and case reports using herbal formulas to treat stroke disability. The combination of Chinese herbal medicine and stem cell biology approaches provides great potential for post-stroke rehabilitations. In the last decade, large efforts have been made to investigate the molecular targets for the regulation of adult neurogenesis and to explore the active compounds and molecular targets of herbal medicine for regeneration therapy. Herein, we reviewed the current progress concerning the molecular targets and cellular signaling pathways involved in adult neurogenesis after cerebral ischemia. We then briefly introduced Chinese medical theory and herbs for stroke treatment in TCM. Finally, we reviewed the current knowledge about the effects of Chinese herbal formulas, active fractions and active compounds on promoting adult neurogenesis as well as their molecular targets. Although the precise mechanisms and molecular targets of herbal medicine for neurogenesis are still unclear, current progress at least provides a cue for exploring the therapeutic principles of Chinese herbal medicine and developing new drugs for brain repair after stroke.

Jiangang Shen, MD, PhD
School of Chinese Medicine
The University of Hong Kong
10 Sassoon Road, Hong Kong, SAR (China)
E-Mail shenjg@hkucc.hku.hk

Background

Stroke is the second most common cause of death and a leading cause of adult disability in human diseases worldwide [1, 2]. In China, the age-adjusted stroke prevalence ranges from 259.86 to 719 per 100,000 people per year [3]. With increasing life expectancy, it is anticipated that stroke will become a major burden in public health worldwide.

Stroke involves a heterogeneous group of processes. Ischemic stroke accounts for about 85% of all stroke cases, while hemorrhagic stroke accounts for the remaining 15% of all stroke cases [4]. Ischemia is defined as a reduction in blood flow sufficient to result in an almost immediate lack of oxygen and glucose in the brain tissue. The brain is exquisitely sensitive to ischemia, such that even brief ischemic periods to neurons can initiate a complex sequence of events that may ultimately result in cell death [5]. Following neuronal cell death due to cerebral ischemia, the rapid degeneration of brain structure could induce severe neurological dysfunctions. The clinical symptoms of ischemic stroke include paralysis or numbness of one side of the body, loss of speech and vision, and trouble with balance or coordination. Early restoration of blood flow is extremely important in order to limit brain injury after stroke.

In the last decades, tremendous efforts have been made to develop therapeutic approaches for stroke treatment. Nevertheless, the clinical outcome is still not satisfying. Recombinant tissue plasminogen activator is an FDA-approved drug, but it has a critical time window within 4.5 h with the potential risk of hemorrhagic transformation. Although thrombolytic therapy has decreased the morbidity and mortality of stroke [6], most stroke patients could not catch up on the golden therapeutic window beginning from their initial clinical symptoms in order to reach a definite diagnosis. While many neuroprotective drugs are effective in animal models, they failed to pass clinical trials [7]. Thus, the development of novel therapeutic strategies and new drugs is important for stroke treatment. Therefore, post-stroke rehabilitation becomes a major therapeutic focus for most post-stroke patients. Unfortunately, the currently available therapies are only rarely successful in improving recovery from neurological deficits.

The discovery of adult neurogenesis sheds light on the development of new therapeutic approaches for stroke. Neural stem/progenitor cells (NSCs) can potentially develop into new functional neurons in the adult central nervous system (CNS) [8, 9]. Adult neurogenesis mainly occurs in the subgranular zone of the dentate gyrus of the hippocampus and the subventricular zone (SVZ) adjacent to the lateral ventricle [10, 11]. Enhanced neurogenesis was found in hypoxic NSCs in vitro [12, 13] and in ischemic brains of neonatal mice [14], adult rats [15] and aged humans in vivo [16]. Enhanced neurogenesis either by stem cell transplantation or stimulation of endogenous neurogenesis could partly amend the damaged brain functions, raising hopes for brain repair treatment. Recent progress in stem cell therapy proposes the approach of transplantation of NSCs targeting brain repair [17, 18]. Experimentally, the transplantation of NSCs is a promising strategy for the replacement of dead or injured neurons. Nevertheless, many remaining problems, such as ethical controversial, standardization, viability, purity of cell materials and safety issues, greatly block the clinical application at least in the near future. Thus, most efforts focus on stimulating the formation of and preventing the death of neurons and glial cells in the CNS.

Current Molecular Targets for Adult Neurogenesis

NSCs have limited capacities for growth, differentiation and generation of new neurons in order to repair the damaged CNS in adults [8, 9]. At 1–2 weeks after transient global ischemia, newly formed cells migrate into the granule cells and promote functional recovery

[19]. However, this spontaneous brain repair seems insufficient to amend neurological deficits in most stroke cases. The major obstacles include: (a) most of the newly proliferated NSCs are unable to form new functional neurons and integrate into the neurological network and (b) there is poor survival of new neuroblasts after 4 weeks of stroke [19]. To overcome those problems, pharmacological manipulation targeting NSCs is proposed for stimulating neurogenesis and promoting the recovery of neurological deficits. For example, treatment with basic fibroblast growth factor (bFGF) and epidermal growth factor (EGF) can stimulate massive regeneration and trigger brain repair after stroke [20].

NSCs within the adult brain germinal centers reside in a specialized microenvironmental niche. They are in close proximity to blood vessels and are surrounded by glial cells to form a microenvironmental niche. The proliferation and differentiation of NSCs and neural growth depend on the microenvironmental niche signals, which include bFGF, EGF, vascular endothelial growth factor (VEGF) and nerve growth factor among others [21–24]. Among them, VEGF plays a critical role in neural regeneration. It exerts its action via phosphotyrosine kinase receptors VEGFR1 and fms-like tyrosine kinase. VEGF can stimulate the proliferation and differentiation of NSCs and neurogenesis in vitro and in vivo [22, 24]. It was reported that VEGF enhanced neurogenesis, neuromigration and angiogenesis, and improved neurological functions in the ischemic brain and spinal cord injury [24–27]. VEGF functions as a niche signal for the proliferation of NSCs in response to the bFGF signal and mediates cross-talk between NSCs and endothelial cells in the niche [28–30].

Notch signaling plays a critical role in the maintenance, proliferation and differentiation of NSCs in the microenvironmental niche [31]. Notch proteins mediate multiple cellular functions through cell-cell interactions. Upon ligand binding, Notch is cleaved and releases a notch intracellular domain (NICD). NICD represses or activates transcription factors. Notch signaling can promote NSC proliferation and modulate glial and neural fates in a stepwise manner, first by inhibiting neuronal fate and promoting glial fate and second by inducing astrocyte differentiation. The Notch-mediated inhibition of neuronal differentiation is achieved by preventing the neighboring cells from becoming the same cell type. Focal cerebral ischemia activates Notch1 signaling and promotes the proliferation of NSCs in the SVZ of adult brains [31, 32]. The upregulation of Notch1 signaling, including Notch1, NICD and hairy and enhancer of split (Hes)1, can promote the proliferation of the NSCs and inhibit neuronal differentiation in ischemic brains [33, 34]. The Notch1 signal mediates the crosstalk of NSCs and endothelial cells in the microenvironmental niche. Endothelial cells can release soluble factors to stimulate the self-renewal of NSCs and inhibit their differentiation via activating Notch and Hes1 [35, 36]. The cross-talking of VEGF and Notch signaling can promote the tissue regeneration [36].

The Wnt/β-catenin pathway is another important regulator for neurogenesis and oligodendrogenesis. Wnt proteins are involved in multiple processes during the CNS development, including cell proliferation, migration, specification and differentiation. β-Catenin is a key downstream effector of Wnt. Activation of β-catenin could promote the proliferation of the neural progenitor pool. The Wnt/β-catenin pathway regulates the differentiation of neural progenitor cells into neurons and delays oligodendrocyte development [37, 38]. Wnt signaling contributes to the functional recovery after ischemic injury by increasing neurogenesis or neuronal survival in the striatum after focal ischemic injury [39].

The sonic hedgehog (Shh) signaling pathway also regulates neurogenesis [40]. Shh acts through a receptor complex composed of PTCH and SMO for the activation of the target gene Gli [41]. Shh promotes hippocampal progenitor cell proliferation [42]. Disruption of Shh signaling by conditional knockout of its downstream mediator SMO resulted in the reduction of NSC proliferation [43]. Shh can also regulate migration. Inhibiting Shh in the adult SVZ prevented neuroblasts (type A cells) from migrating to the olfactory bulb [44].

Our recent works explored the roles of caveolins (Cav) in the regulation of neurogenesis. Caveolins are a group of 22-kDa structural proteins including Cav-1, Cav-2 and Cav-3. Cav-1 and Cav-2 are widely expressed in neuronal cell types and brain regions [45–47], whereas Cav-3 is muscle specific [48]. Caveolins interact with proteins via the caveolin scaffold domain [49]. They negatively regulate a variety of signal pathways, such as G proteins, nitric oxide synthases, Src tyrosine kinases, ras, estrogen receptors, protein kinase C, integrins, MAP kinase and EGF-R [50–52]. Cav-1 decreased neurite outgrowth and branching, and neurite density in injured differentiated PC12 cells [53] and blocked the formation of neurites and phosphorylation of extracellular signal-regulated kinase (ERK) upon bFGF treatment in N2a cells [54]. We found that Cav-1 knockout mouse brains displayed an increased proliferation and differentiation of adult NSCs in the SVZ area [55–57]. The major discoveries include: (1) Cav-1 promoted astroglial differentiation of NSCs through modulating Notch1/NICD and Hes1 expressions [55]; (2) Cav-1 inhibited oligodendroglial differentiation of NSCs through modulating β-catenin expression [56], and (3) Cav-1 inhibited neuronal differentiation via downregulations of VEGF, p44/42 MAPK, Akt and Stat3 signaling pathways. Downregulation of Cav-1 contributed to hypoxia-mediated neuronal differentiation in neural progenitor cells [57]. Therefore, Cav-1 plays a critical role in neural progenitor cell proliferation and cell fate decision in the post-stroke brain.

Phosphatidylinositol 3-kinase (PI3K)/Akt and ERK signaling pathways are also involved in the process of neurogenesis. They can regulate hypoxia-inducible factor (HIF)-1α, GSK-3β, and cAMP response element-binding protein (CREB). HIF-1α deletion was reported to impair hippocampal Wnt-dependent processes, including NSC proliferation, differentiation and neuronal maturation [58]. GSK-3β can function as signaling nodes to regulate and orchestrate the diverse cellular responses in neurogenesis via affecting HIF-1α, HIF-2α and β-catenin. CREB is required for EGF-induced cell proliferation and serum response element activation in NSCs of adult mouse brain [59, 60]. Vanadium, a stimulator of PI3K/Akt and ERK, increased NSC proliferation and promoted the migration of newborn neurons in the ischemic brain [61]. PI3K/Akt and ERK also regulate many mitogenes including bFGF, Shh, and insulin-like growth factor 1. Thus, PI3K/Akt and ERK signaling pathways are important targets for drug discovery for adult neurogenesis.

A recent review article summarized the major cellular signaling pathways in adult neurogenesis: Shh, miR-124, Sox2, Tlx and Wnt/β-catenin signaling pathways are regulators for cell proliferation; basic helix-loop-helix transcription factors such as Asc1, Neurog2 and Tbr2 and epigenetic factors like Gadd45b, MBD1, MeCP2 and Mll1 are necessary for neuronal differentiation and maturation; insulin-like growth factor 1 and Shh are essential for neuroblast migration; extrinsic factors including BDNF, FGF-2, GABA, glutamate and NT-3 play important roles in regulating neuronal survival and dendritic arborization, synaptic plasticity and synapse formation; intrinsic factors including DISC1, Klf-9, NeuroD1, Cdk and CREB participate in neuronal survival, dendritic arborization, synaptic integration and maturation [62].

In summary, adult neurogenesis is a multistep process that requires the proliferation of NSCs, the differentiation into the specific neuronal cell types, migration, and that the new cells differentiate, survive and integrate into existing neural networks. Signaling pathways involved in the regulations of adult neurogenesis are very complex. The signal molecules not only come from the NSCs themselves but also from the microenvironmental or neurogenic niche. Therefore, targeting critical cellular signaling pathways is an important therapeutic strategy for drug discovery for brain repair for post-stroke treatment.

Classic Concepts and Representative Therapeutics for Stroke in Traditional Chinese Medicine

With the progress of stem cell biology, we can expect that more and more cellular signaling pathways will be discovered, making the underlying mechanisms of brain repair complicated. The question arises as how to find a 'magic drug' which can regulate overall profiles of cellular signaling and genes for neurogenesis. Given that Chinese herbal medicines generally include multiple herbal items and therapeutically target multiple signaling pathways, Chinese herbal medicine therapy may provide a solution to regulate the complex network systems to promote brain repair. Since Chinese herbal medicine has been used for thousands of years and produced a huge amount of case reports, intensive investigations on the neurogenesis-promoting effects of the active compounds, individually and synergistically, and their related molecular targets would create new directions for drug discovery to promote neurogenesis in post-stroke studies.

In Traditional Chinese Medicine (TCM) theory, stroke is named as 'wind stroke' with the definition of a condition mainly characterized by sudden collapse and loss of consciousness, deviation of the tongue and mouth, hemiplegia, slurred speech, or only deviation of the tongue and mouth and hemiplegia without collapses. According to TCM concepts, 'wind stroke' is caused by a long-term exposure to multiple pathological conditions including abnormal diet and lifestyles, emotional stress, abnormal psychological stress, and constitution factors, resulting in the deficiency of *qi* and abnormal movement of *qi* and blood. The patients with a particular constitution named deficiency of *gan-ying* and *shen-ying* is susceptible to pathological factors and easy to be attacked by 'wind stroke'. The attack of 'wind stroke' could be attributed to the reversed flow of *qi* and blood, and subsequently produce wind, fire, phlegm and blood stasis, inducing the formation of cerebral thrombosis or cerebral hemorrhage. According to the patient's clinical conditions, 'wind stroke' can be divided into three subtypes: 'meridian stroke', '*zhang-fu* stroke' and sequela. 'Meridian stroke' is considered as the mild one, but '*zhang-fu* stroke' is the most severe clinical pattern, characteristically with obnubilation. Accordingly, different therapeutic approaches and formulas are specifically designed to treat different TCM clinical patterns based on TCM syndrome differentiation. Based on the histological descriptions and clinical observations, the clinical patterns of 'meridian stroke' in TCM are generally equivalent to the clinical characteristics of primary hypertension and transient ischemic attack. *Tianma gouteng* decoction and *zhengang xifen* decoction are representative formulas for 'meridian stroke' treatment. As for '*zhang-fu* stroke', its clinical pattern is similar to acute ischemic stroke or hemorrhagic stroke with serious brain damage showing clinical symptoms of obnubilation or coma. '*Zhang-fu* stroke' has two clinical subtypes including excessive syndrome and deficiency syndrome. *Angong niuhuang wan* and *lingjiao guoteng decoction* are two representative formulas for 'excessive syndrome with *gang-yang and gang* wind hyperactivity and upwards', whereas *shenfu* decoction is particularly designed for the '*zhang-fu* stroke' with the deficiency of *yang qi*. For sequela, *jieyu dan* is a representative formula for relieving the symptoms of deviation of the tongue and mouth, hemiplegia and slurred speech. This clinical pattern is generally considered as the syndrome of phlegm and blood stasis. Another representative formula is *buyang huanwu* decoction (BYHWD). This formula is specifically designed for neurological deficits and dysfunctions with the types of *qi* deficiency-induced blood stasis.

In recent years, large efforts have been made to understand the therapeutic principles, molecular targets and active compounds of the TCM herbal formulas. For example, by using experimental stroke animal models and conducting clinical trials, *tianma gouteng* decoction, a representative formula for the treatment of primary hypertension and transient ischemic attack, revealed to decrease blood pressure, ameliorate cognitive impairment and protect

neural cells in ischemic brains [63–65]. Due to the poor design of clinical trials, however, a recent systematic review challenged its clinical efficacy for the treatment of primary hypertension [66]. BYHWD is one of the classic formulas for post-stroke disability with intensive investigations. Several recent systematic reviews and meta-analysis studies conducted by us and others provide experimental and clinical evidence to support the application of BYHWD in the post-stroke treatments [67, 68]. Although the quality of clinical trials needs to be improved, the effects of this formula on improving neurological functions have been well accepted. The active constitutions and the metabolites of BYHWD have been reported as well [69, 70]. In the following sections, we will focus on the current progress in the studies of Chinese herbal medicine for promoting neurogenesis and improving neurological functions by using different in vivo and in vitro experimental systems, providing opportunities for post-stroke treatment.

Current Progress in Chinese Herbal Medicine for Promoting Neurogenesis and Their Related Molecular Targets

In the last decades, great efforts have been made to investigate the effects of Chinese herbal medicine on promoting neurogenesis and their related molecular targets. In TCM practice, therapeutic approaches are generally designed based on unique TCM theory and the prescriptions include multiple herbs; the items can be changed with the different clinical syndrome patterns during the different phases of a disease. From the angle of photochemistry, the TCM formulas generally consist of thousands of chemical ingredients which may target multiple cellular signaling pathways. With comprehensive and advanced analytic approaches, scientists are trying to explore the therapeutic principles of TCM formulas by identifying their active compounds and molecular targets involved in the regulation of neurogenesis. Herein, we summarize and review the current progress in those aspects.

Representative TCM Formulas and Their Molecular Targets for Neurogenesis

Buyang Huanwu Decoction

BYHWD has been a classic TCM formula for post-stroke disability treatment for 300 years. The formula was first introduced in a renowned TCM textbook named *Yilin Gaicuo (Correction on Errors in Medical Books)* by Dr. Wang Qing-ren in the Qing Dynasty. According to Dr. Wang's opinion, paralysis caused by ischemic stroke is similar to the loss of '*five of ten*' due to *qi* deficiency and blood stasis. Thus, boosting the *qi* and restoring the blood circulation are the key points to treat this clinical pattern of ischemic stroke. BYHWD consists of 7 items including *Astragalus membranaceus, Angelica sinensis, Paeonia lactiflora, Ligusticum chuanxiong, Carthamus tinctorius, Prunus persica* and *Lumbricus* at a ratio of 120:4.5:3:3:3:3:3. *A. membranaceus* is the dominating herb in this formula, which accounts for about 85% of the whole decoction. It functions as the 'king' component in the formula to invigorate *qi* and enhance *yang-qi*. *A. sinensis*, *P. lactiflora*, *L. chuanxiong*, *C. tinctorius* and *P. persica* are 'minister' and 'assistant' components used to promote blood circulation and replenish blood. *Lumbricus* is used as a 'guiding' component to smooth the movement of *qi* and blood in the channels. Combining these 7 items together, BYHWD can enhance the effect of boosting *qi* and removing the blood stasis. Several clinical trials have been conducted to evaluate the efficacy of BYHWD for post-stroke disability. BYHWD revealed to promote neurogenesis, reduce infarction volume and improve neurological functions in post-stroke animal models and human subjects [71–75]. Herein, we summarized the current research progress in the pharmacological activities of BYHWD and its ingredients and reviewed its potentials for post-stroke rehabilitation.

Neuroprotective Effects and Its Molecular Targets

Reactive oxygen species are important pathological factors in cerebral ischemia/reperfusion injury. Our previous studies showed that BYHWD inhibited the neuronal nitric oxide synthase activity and protected neurons from oxidative injury in a permanent focal cerebral ischemia model [76]. Both in vitro and in vivo studies revealed that BYHWD had anti-apoptotic effects against cerebral hypoxic/ischemia reperfusion injury. The in vitro studies showed that BYHWD protected cortical neurons from hypoxia-induced apoptotic cell death through scavenging NO and superoxide (O_2^-), downregulating the expressions of p53 and p21 genes and upregulating the expression of bcl-2 [77, 78]. The in vivo studies showed that oral administration of BYHWD reduced the number of TUNEL-positive neurons by attenuating the expression of caspase-3 p20, a product of catalytically active caspase-3 in the hippocampal CA1 region [79]. BYHWD obviously inhibited the expression of HSP70 mRNA but had no effect on the HSP70 protein [80]. BYHWD revealed to modulate pro-inflammatory mediators including IL-1β and TNF-α in an experimental cerebral ischemia stroke model [81]. The active fractions and compounds, including alkaloid, glycoside, polysaccharide and aglycone, were found to inhibit inflammatory cytokines, alleviate the inflammatory reactions and downregulate the expression of caspase-1 in cerebral ischemia/reperfusion injury. The alkaloid, glycoside and aglycone fractions inhibited the expression of caspase-3 in the hippocampus, cortex and medulla and protected neurons from cerebral ischemia injury [82]. Ca^{2+} overload and excitatory amino acid are important mediators of neurotoxicity in cerebral ischemia/reperfusion injury. BYHWD showed to reduce intracellular Ca^{2+} concentration in neural progenitor cells [83] and regulate the metabolism of endothelin and calcitonin gene-related peptide in patients with early cerebral infarction [74]. A recent study reported that BYHWD treatment significantly decreased the level of excitatory amino acids and increased inhibitory amino acids in cerebrospinal fluid extracted from the rats subjected to cerebral ischemia/reperfusion injury [84].

In addition, the effects of BYHWD on the antithrombotic functions were also studied by using human umbilical vein perfusion. BYHWD reduced the von Willebrand factor release and inhibited the conversion of fibrinogen to fibrin catalyzed by thrombin [85]. Administration of BYHWD increased the blood flow in the hippocampal region after occlusion and inhibited the hypoperfusion after reperfusion in ischemia/reperfusion rats [86]. In addition, BYHWD reduced the platelet activator factor in the arterial blood after thrombosis [87]. Therefore, the neuroprotective mechanisms of BYHWD are related to regulate multiple cellular signaling pathways.

Neurogenesis-Promoting Effects and Molecular Targets

Our early studies revealed that BYHWD containing serum significantly promoted the proliferation of neurons under both normal and hypoxic conditions [88]. Further studies found that BYHWD increased the 5-bromo-2-deoxyuridine-positive neural progenitor cells in the rat hippocampus and SVZ after ischemic stroke, suggesting that BYHWD could improve the neural progenitor proliferation [72]. Furthermore, BYHWD treatment also stimulated the differentiation of neural progenitor cells as evidenced by increasing the neurofilament-positive cells and glial fibrillary acidic protein-positive cells in cultured neural progenitor cells [83]. A recent study reported that BYHWD treatment significantly increased 5-bromo-2-deoxyuridine-positive cells in the SVZ, subgranular zone and corpus striatum of the infarcted brain by upregulating the expression of migration activators including stromal cell-derived factor 1, CXC chemokine receptor 4, VEGF, Reelin and BDNF in the ipsilateral infarct area after middle cerebral artery occlusion cerebral ischemia. In addition, BYHWD treatment was able to promote the neuronal differentiation, which closely related to the migratory process of neural progenitor cells in middle cerebral artery occlusion rats. These results suggest that

BYHWD might promote the migration of neural progenitor cells to the ischemic brain area [89].

VEGF and its receptor Flk1 are important neurotrophic, neuroprotective and neuroproliferative factors. Our study showed that BYHWD upregulated the expressions of VEGF and Flk1 at the SVZ and cortex in the post-ischemic brains [72]. Further studies revealed that BYHWD treatment increased the expression of VEGF in the serum of stroke patients [90]. Moreover, the combination of mesenchymal stem cell transplantation and BYHWD treatment repaired the injured blood vessels and lesion tissues by inducing the expression of VEGF and Ki-67 [91].

Moreover, a genomic assay was applied to explore the regulation of BYHWD on overall genomic profiles in the post-stroke brains. Treatment of BYHWD remarkably led to upregulate 25 genes but downregulate 6 genes in the brain tissues of a rat model of experimental cerebral ischemia [92]. A recent study revealed that BYHWD treatment upregulated the expressions of 93 genes but downregulated 284 genes in a cerebral ischemia mouse model. Among the 93 genes, 6 are associated with neurogenesis and 9 are related to nervous system development. In addition, BYHWD also showed to regulate the genes related to anti-inflammation (14 genes), anti-apoptosis (15 genes), anti-angiogenesis (11 genes) and anti-coagulation (7 genes) [73].

Identification of Active Compounds and Their Metabolites

About 54 main chemical constituents in BYHWD have been identified pharmacologically. They belong to C-glycosyl quinochalcones, flavonoid O-glycosides, isoflavones, monoterpene glycosides, saponins, organic acids and amino acids [93]. To understand the active compounds contributing to the bioactivities of BYHWD, rapid resolution liquid chromatography was coupled with quadrupole time-of-flight tandem mass spectrometry to identify the absorbed components and metabolites in rat urine after oral administration of BYHWD. A total of 50 compounds were detected in rat urine samples within 20 min, including 12 parent compounds and 38 metabolites. Except for 3 prototype components (hydroxysafflor yellow A, paeoniflorin and amygdalin), the identified metabolites mainly came from *radix astragali*, *radix angelicae sinensis*, and *rhizoma chuanxiong*. The results indicated that glucuronidation and sulfation were the major metabolic pathways of isoflavonoids, while glutathione conjugation, glucuronidation and sulfation were the main metabolic pathways of phthalides [69].

A recent study further identified the active compounds of BYHWD by comparing the high-performance liquid chromatography of a drug-containing urine sample with that of a drug-free sample. A total of 17 characteristic compounds were isolated from the methanol extract of a drug-containing urine sample by column chromatography. Their structures, including 11 isoflavanoids, 2 pterocarpanoids and 4 isoflavonoids, were identified by spectroscopic means. Of the 17 compounds, 8 were new compounds. Based on the possible relationship and metabolic pathways of the 17 compounds in vivo, 3R-7,2′-dihydroxy-3′,4′-dimethoxyisoflavan(isomucronulatol), 6aR,11aR-3-hydroxy-9,10-dimethoxypterocarpan (methylnissolin, astrapterocarpan), 7,3′-dihydroxy-4′-methoxyisoflavone (calycosin) and 7-hydroxy-4′-methoxyisoflavone (formononetin) were the most important absorptive original isoflavonoid constituents of BYHWD in vivo, which underwent reactions of glucuronidation, hydroxylation, demethylation and reduction [70].

Other Representative TCM Formulas

MLC901 is a simplified formula from the China State FDA-registered botanical drug MLC601, originally developed from a TCM formula named *danqi piantang jiaonang* [94], containing *radix astragali*, *radix salvia miltiorrhizae*, *radix paeoniae rubra*, *rhizoma chuanxiong*, *radix angelicae sinensis*, *C. tinctorius*, *P. persica, radix polygalae*, and *Rhizoma acori tatari-*

nowii. Previously randomized double-blind, controlled trials showed that *danqi piantang jiaonang* could improve the recovery of neurological functions in stroke patients. MLC901 was reported to increase the proliferation of human embryonic stem cell-derived progenitors [95]. The molecular targets and its active compounds contributing to neurogenesis-promoting activities remain to be addressed. *Fuzhi san* is a herbal formula used for improving learning and memory. This TCM formula includes *Panax ginseng*, *Scutellaria baicalensis*, *Acorus tala-rinowi*, and *Glycyrrhiza uralens*is. A recent study revealed that *fuzhi san* increased the proliferation of neural progenitor cells and the survival of newborn cells in the hippocampal dentate gyrus of SAMP-8 aging mice and improved learning and memory activities [96]. *Liuwei dihuang tang* is a classic TCM formula with the herbs of *rehmanniae radix*, *dioscorae radix*, *corni fructus*, *alimatis rhizoma*, *moutan cortex radicis*, *hoelen*, *maximowicziae fructus* and *cervi cornu.* The aqueous extract of *liuwei dihuang tang* revealed to induce the proliferation of rat neural stem cells and increased spatial learning ability by the radial-arm maze test [97]. In addition, *kami-ondam-tang (jiawei-wen-dan-tang)* is also a potential herbal formula for promoting neurogenesis. The formula consists of the following herbal items: *pinelliae rhizoma, bambusae caulis, aurantii immaturus fructus, poria, citri reticulatae pericarpium, glycyrrhizae radix, polygalae radix, scrophulariae radix, ginseng radix, rehmanniae radix, zizyphi spinosae semen, jujubae fructus* and *zingiberis rhizoma*. *Kami-ondam-tang* treatment increased the doublecortin-positive cells in the hippocampus area and increased step-through latency in the passive avoidance task in mice. The molecular mechanisms are associated with promoting the expressions of pAkt, BDNF and pCREB [98].

Representative Active Compounds from Chinese Herbal Medicine for Promoting Neurogenesis

EGb761 is a standard extract of *Ginkgo biloba,* a medicinal herb widely used for the treatments of stroke. *Bilobalide* is a unique ingredient of *Ginkgo biloba,* whereas quercetin is its representative antioxidant component. EGb761 and its active compounds *bilobalide* and quercetin have the ability to promote proliferation of progenitor cells in the rat hippocampus and embryonic brains through activating the CREB signaling pathway [99, 100]. *Salvia miltiorrhiza* is a commonly used herb for the treatment of stroke in TCM clinical practices. Salvianolic acid B is one of the active compounds from *S. miltiorrhiza*. Salvianolic acid B promoted the self-renewal of neural progenitor cells and induced the proliferation of rat embryonic neural stem cells through modulating the PI3K/Akt signaling pathway [101]. In an ischemic stroke rat model, even delayed treatment with salvianolic acid B showed to improve cognitive impairment in post-ischemic rat brain [102]. Baicalin is a flavonoid compound isolated from *S. baicalensis G*. Baicalin was previously reported to promote the differentiation of human umbilical cord blood mesenchymal stem cells and rat bone marrow stromal cells into neurons [103, 104]. Recently, we found that baicalin downregulated p-stat3 and Hes1, and upregulated NeuroD1 and Mash1 (Asc1) in cultured embryonic NSCs. Furthermore, baicalin promoted the neural differentiation but inhibited glial formation, indicating that it could regulate cell fate decision in embryonic NSCs [105]. Tenuigenin and the *polygala tenuifolia* root extract are isolated and extracted from *Polygala tenuifolia*, a medicinal herb used for improving insomnia and memory. Recent studies showed that they improved NSC proliferation and neuronal differentiation [106, 107]. Other active compounds from herbal medicine can also be found in the literature, including ginsenosides Rg5 [108], and panax notoginseng saponins [109], wolfberry polysaccharides [110] or epimedium flavonoids [111].

Conclusion and Perspectives

With the development of stem cell biology, targeting adult neurogenesis becomes an important therapeutic strategy for brain repair in post-stroke treatment. Drug discovery from these natural extractions have been paid more attention nowadays with the globally increased popularity of and expenditures on herbal therapies recently. TCM herbs offer a great and unique source of both single compounds and complex combined compounds for drug screening. US and Chinese scientists have jointly established a library of 202 authenticated medicinal plant and fungal species and about 10,000 standard fractions from these materials. More and more compounds and extracts from herbal medicine have been reported to have the potential to induce neurogenesis in the literature. The current progress already opens a door to explore the active compounds from commonly used TCM herbs for stroke treatment and seek their molecular targets. Those discoveries are useful not only for elucidating the scientific basis of herbal medicine for stroke treatment but providing a cue for drug development. However, we must remark that many studies in the literature only reported the phenomenon instead of mechanistic studies. Studies rarely used pharmacological intervention or genetic tools to build the internal relationship between the molecular targets and neurogenesis-promoting effects. How those compounds regulate the signaling and molecule targets, and whether they directly bind those proteins or indirectly affect their activities and expressions remains to be addressed. Overall, Chinese herbal medicine is an important resource for drug discovery and valuable therapeutic strategy to promote adult neurogenesis. Although the precise mechanisms and molecular targets are still unclear, current studies indeed bring novel insights into drug discovery and advancements in fighting post-stroke disability.

Last but not least, we should remark that adult neurogenesis is involved in multiple signal networks; however, much of those are still unknown. The complex networks for controlling neurogenesis make drug development extremely difficult. It is hard to adjust overall abnormal network systems by simply targeting several signaling pathways and molecules. Thus, there is currently no FDA-approved drug for the neuroprotection and neurogenesis. TCM formulas may provide an alternative strategy for this purpose since they generally target multiple signaling pathways. Understanding the synergic effects of different compounds and fractions of herbal formula will be the future direction. With the development of proteomics, metabonomics and bioinformatics, we are at the gate to open the complex and magic world. We believe that the combination of stem cell biology and modern Chinese medicine will lead to the development of novel therapeutic strategies for post-stroke treatment.

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Disclosure Statement

The authors declare that they have no conflicts of interest.

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