Review Article

Active Compounds and Molecular Targets of Chinese Herbal Medicine for Neurogenesis in Stroke Treatment: Implication for Cross Talk between Traditional Chinese Medicine and Biomedical Sciences

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Abstract

Neural stem/progenitor cells (NSCs) could be attractive therapeutic targets for promoting adult neurogenesis, brain plasticity, and repair in stroke and neurodegenerative diseases, raising great potentials for regeneration therapy. In adult ischemic brains, NSCs have limited capacities of growth, differentiation, and generating new neurons for repairing the damaged central nervous system. However, the spontaneous brain repair seems to be insufficient to recover neurological deficits in most stroke cases. To overcome those problems, pharmacological manipulations targeting on endogenous NSCs or transplanted stem cells could be a promoting strategy for regeneration therapy. Chinese herbal medicine has great potentials for developing novel therapeutic strategies for adult neurogenesis and brain repair in poststroke treatment. Chinese herbal medicine has a long history for poststroke treatment. Recent studies create great opportunity for drug discovery for promoting neurogenesis and improving the recovery of neurological functions in poststroke treatment. Many active compounds or extracts from medicinal herbs have shown promising effects on regulating proliferation, self-renewal and differentiation of NSCs, and promoting neural network formation as well as neurological functional recovery with *in vitro* and *in vivo* experimental evidence. Therefore, targeting neural stem/progenitor cells can be an important opportunity for the studies of Traditional Chinese Medicine in regeneration medicine. Due to the complex interactions of herbal ingredients in network regulation, huge challenge remains to be resolved for further study.

Keywords: Chinese herbal medicine, neurogenesis, stroke

Introduction

Stroke is the second leading disease of mortality and a leading cause of adult disability according to the WHO report.[1,2] In China, the age-adjusted stroke prevalence ranges from 259.86 to 719 per 100,000 people per year.[3] Ischemic stroke caused by vessel occlusions accounts for 85% of all stroke patients.[4] With rapid degeneration of brain structure following the functional neuronal loss, ischemic stroke patients suffer from several neurological dysfunctions, inducing paralysis, loss of speech, loss of vision, and trouble in balance or coordination and coma.

Under ischemic stroke conditions, the core of ischemic brain tissue undergoes necrotic cell death within a few minutes after cerebral ischemia. Early restoration of blood flow by thrombolytic therapy is an essential strategy to reduce morbidity and mortality in these patients. Paradoxically, recanalization for

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reperfusion, such as thrombolytic treatment with recombinant tissue-plasminogen activator (rt-PA), evokes additional brain injury to ischemic penumbra, a region bordering the ischemic infarct core. Cerebral ischemia-reperfusion (I/R) injury exacerbates neuronal cell death and blood-brain barrier (BBB) damage, and increases the severity of morbidity in surviving victims. As the only FDA approved thrombolytic reagent, rt-PA is an effective drug for recanalization of the ischemic brains but has restrictive therapeutic time within 4.5 h after onset of ischemic stroke. Although early thrombolytic therapy could decrease morbidity and mortality, most of the patients could not catch up the golden window. Treated beyond the 4.5 h, ischemic stroke patients had the tenfold increases of the risk of hemorrhagic transformation (HT) and the increased morbidity and mortality.[5,6] Limited therapy is available for poststroke rehabilitation and improving recovery of neurological deficits, leading most stroke victims long-term disability.

Recent progress in regeneration therapy brings new hopes for the treatment of poststroke disability as well as other neurodegenerative diseases. Endogenous adult neurogenesis is an important direction for regeneration therapy.[7,8] Adult spontaneous neurogenesis mainly occurs in the subgranular zone in the dentate gyrus (DG) of the hippocampus (HIP) and subventricular zone (SVZ) adjacent to the lateral ventricle (LV).[9,10] Enhanced neurogenesis has been reported in different experimental systems including ischemic brains of neonatal mice, adult rats, and aged humans *in vivo*.[11-14] Nevertheless, the spontaneous neurogenesis fails to recover neurological function in ischemic stroke patients. Recent progress in clinical trials indicates that stem/progenitor cell transplantation could be an attractive brain repair strategy for poststroke treatment.[15-18] Development of stem cell therapy strategies for regeneration medicine is timely important for stroke treatment.

Traditional Chinese Medicine (TCM) has been used for centuries in China, Korea, Japan, as well as other Asian countries for the treatment of the diseases characteristically clinical symptoms and statuses similar to cerebrovascular diseases including dizziness, paralysis, loss of speech, loss of vision, coma and dementia. With over 2000-year practice, TCM accumulates many medicinal herbs and TCM formulas and histological documents about the theory and practices of TCM for stroke treatment. Last decades, large effects were made to understand the therapeutic principles of Chinese herbal medicine and acupuncture and explore the applications of TCM formula, single herbal extractions, and pure compounds for regenerative therapy. In the present study, we focus on the pure compounds and/or extracts of commonly used Chinese medicinal herbs for promoting neurogenesis and analyze the future development of Chinese Medicine targeting stem cell therapy for brain repair in poststroke treatment.

Traditional Chinese Medicine Theory for Poststroke Treatment

With its long history, TCM accumulates huge histological documents for understanding the pathological basis of stroke and therapeutic approaches. Stroke is defined as "*Wind Stroke*" in TCM. "*Wind Stroke*" refers to the syndrome conditions characterized by a sudden collapse, loss of consciousness, deviation of the tongue and mouth, hemiplegia, slurred speech, or only deviation of the tongue and mouth and hemiplegia without collapses. The pathogens and therapeutic approaches of "*Wind Stroke*" date back to the Han Dynasty described in "Treatise on Cold Damage (Shang Han Lun)" and "Synopsis of the Golden Chamber (Jin Kui Yao Lue)." The pathological basis of "*Wind Stroke*" was attributed to the "*Wind Stroke Attacking due to Collateral Deficiency.*" "*Wind Stroke*" is considered as the consequences of *Zang-fu and Meridian* dysfunctions and *Qi-Blood* disruption due to the accumulations of abnormal diet and lifestyles, psychological stress, and mental disorders, etc., The pathogens of the "*Wind Stroke*" are complex. The core pathological basis of the "*Wind Stroke*" can be attributed to the dysfunctions of *Zhang-fu and Meridian*, which eventually leads to cerebral thrombosis or cerebral hemorrhage. The deficiency of *Qi-Blood* and imbalance of *Yin-Yang* are the basis of attacking of "*Wind Stroke.*" The pathological factors or statuses, including *Qi Stagnation*, *Blood Stasis*, *Wind Invasion, Liver Fire, Phlegm Accumulation, Deficiency of Qi-Blood and Yin-Yang*, contribute to the neurological dysfunctions and cognitive impairments in the "*Wind Stroke*" patients. According to TCM theory, "*Wind Stroke*" has several different clinical patterns catalyzed "*Meridian stroke,*" "*Zhang-fu stroke*," and sequela. "*Meridian stroke*" is defined as the clinical pattern with only deviation of the tongue and mouth and hemiplegia without collapses and loss consciousness, whereas "*Zhang-fu stroke*" would be accompanied by obnubilation. Thus, the pathology of the "*Wind Stroke"* is correlated with the dysfunctions of *Zang-fu and Meridian* and the disruption of *Qi-blood and Yin-Yang*. Restoring the dynamic balances of *Qi-blood* and *Yin-yang* is an essential principle for stroke treatment. With 1000-year accumulation, many medicinal herbs and classical TCM formulas have potentials for the treatment of poststroke disability. The accumulated knowledge and histological documents from direct experience in human subjects are important sources for regeneration medicine. Recent years, large efforts have been made to explore the scientific basis of Chinese medicinal herbs for poststroke treatment. With the progress of stem cell biology and regeneration medicine, many frontier technologies and methods have been developed, greatly facilitating the studies of the therapeutic principles of Chinese herbal medicine for poststroke treatment. Several years ago, we reviewed the progress of Chinese herbal medicine for promising neurogenesis.[19-21] Recently, more and more research articles have been published, bringing better understanding the therapeutic principles and active compounds of medicinal herbs for promoting neurogenesis in poststroke treatment. In this mini-review, we specially focus on the relationship between the traditional therapeutic functions of Chinese medicinal herbs and the bioactivities of those herbs and their active compounds for promoting adult neurogenesis. We hope to provide cross talk between TCM and biomedicine for regeneration therapy in poststroke treatment.

Table 1: Herbs and their active compounds promoting neurogenesis

TCM categories	Plant	Active compounds	Experimental model	Treatment time point and path	Period of treatment	Dosage	Targets	Major results	References
Resolving blood stasis	*S. miltiorrhiza* (Danshen)	Whole herb extract	iPSCs; Rat MCAO stroke model	Treatment in medium; IP (for *in vivo*)	3 or 7 days	5 μg/ml (*in vitro*); 100 mg/kg (*in vivo*)	NA	Increased grafted MAP2+ cells; induced functional recovery	[22]
		Sal B	iPSCs with or without H_2O_2 treatment	Treatment in medium	24 h	5, 20, and 40 mg/ml	BDNF	Increased BM-NSCs proliferation, differentiation; reduced apoptosis	[24]
			iPSCs	Treatment in medium	7 days	5-100 μM	PI3K/AKT/ GSK3β/β-catenin	Increased cell viability and neuronal differentiation	[26]
		TIIA	Embryonic NSCs; C17.2 cell; PC12 cell	Treatment in medium	1-7 days	3 nM-3 μM	p-cav-1, p-MAPK, BDNF, NG	Induced neuronal differentiation	[25]
	Ligusticum walliichi (chuangxiong)	TMP	NPCs; Rat MCAO model	Treatment in medium; not mentioned for *in vivo*	7 and 21 days	2, 10, 50 μg/ml (*in vitro*); 20,40 mg/kg (*in vivo*)	SDF-1, PI3K/Akt, PKC, and ERK	Induced NPC migration	[27]
		TMP	SH-SY5Y cell	Treatment in medium	5 days	40, 80 μM	TopoIIβ, Ac-H3, Ac-H4	Inhibited cell proliferation and induced neuronal differentiation	[28]
		Whole herb extract	NSCs; chronic mild stress induced depression	Treatment in medium; orally (for *in vivo*)	48 h (*in vitro*); 14 days (*in vivo*)	0.04-40 μg/ml (*in vitro*); 150 mg/kg (*in vivo*)	Corticosterone	Induced hippocampal precursor cell proliferation	[39]
	Radix angelica Sinensis (Dangui)	Whole herb extract	Permanent bilateral common carotid artery occlusion				BDNF, CREB, GAD65	Induced neurogenesis and improved cognitive function	[29]
	P. notoginseng (Sanqi)	*P. notoginseng* saponins	Embryonic cortical NSCs	Treatment in medium	4 days	15.4 mg/mL	bFGF, BDNF	Promote NSC survival, self-renewal, proliferation, and differentiation	[30]
		Total saponins of *P. notoginseng*	Global cerebral ischemia and reperfusion	30 min after ischemia, once a day	7 or 14 days	75 mg/kg	cAMP	Promoted differentiation of immature neuroblasts	[31]
		GinsenosideRg1	D-Galactose -induced aging	I.P	28 days	20 mg/kg	GSH-Px, SOD, IL-1β, IL-6, TNF-a, p53, $p21^{Cip1/Waf1}$ and $p19^{Arf}$	Improved hippocampal neurogenesis and cognitive capacity, reduced senescence and protected NSCs/NPCs, reduced astrocytes activation	[32]
			Embryonic cortical NSCs with OGD treatment	Treatment in medium	6 h	0.32 μg/ml	NA	Promoted proliferation and glial-like-directed differentiation	[33]

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TCM categories	Plant	Active compounds	Experimental model	Treatment time point and path	Period of treatment	Dosage	Targets	Major results	References
		Ginsenoside-Rb1	NPCs with (t-BHP) -induced oxidative injury	Treatment in medium	24 h	10 μM	Nrf2/HO-1	Reduced NPCs cytotoxicity and apoptosis	[34,35]
Removing phlegm accumulation	*G. biloba*	EGb761	Vascular dementia	Orally, per day	15 days, 1, 2, 4 months	50 mg/kg	NA	Enhanced proliferation of neural stem cells; improved learning and memory	[36]
	P. tenuifolia Willd (Yuanzh)	Tenuigenin	Hippocampal NSCs	Treatment in medium	6 days	1, 2, 4 μg/ml	NA	Increased NSCs proliferation and differentiation	[37]
	Rhizoma Acori tatarinowii (RAT, Shichangpu)	RAT extract	Hippocampal NPCs; adult and aged mice; transgenic AD model mice	Orally	28 days	200 mg/20 g	ERK	Enhanced NPC proliferation and neurogenesis	[38]
		Asarones (α and β)	Hippocampal NPCs; adult and aged mice; transgenic AD model mice	Orally	28 days	α-asarone 10 mg/kg; β-asarone 30 mg/kg	ERK	Promoted hippocampal NPC proliferation and neurogenesis; improved recognition memory	[38]
Removing internal heat	*Radix phellodendri*	Whole herb extract	NSCs; chronic mild stress-induced depression	Treatment in medium; orally (for *in vivo*)	48 h (*in vitro*); 14 days (*in vivo*)	0.04-40 μg/ml (*in vitro*); 90 mg/kg (*in vivo*)	Corticosterone	Induced hippocampal precursor cell proliferation	[39]
	Radix scutellaria (huangqin)	Whole herb extract	NSCs; chronic mild stress-induced depression	Treatment in medium; orally (for *in vivo*)	48 h (*in vitro*); 14 days (*in vivo*)	0.04-40 μg/ml (*in vitro*); 90 mg/kg (*in vivo*)	Corticosterone	Induced hippocampal precursor cell proliferation	[39]
		Baicalin	Cortex NPCs	Treatment in medium	2 h and 7 days	2 μM, 20 μM	bHLH family; stat3	Promoted neuronal differentiation	[42]
			MSCs	Treatment in medium	6 days	200-400 μM	NA	Induced neuronal differentiation	[43]
			APPL2 Tg mice and chronic corticosterone -induced depression mouse model	Orally	7 days	3.35, 6.7 mg/kg	APPL2; glucocorticoid receptor	Improved neurogenesis; attenuated depressive- and anxiety-like behaviors; and improved olfactory function	[44]
Tonic medicinal herbs	*Radix astragali* (Astragalus membranaceous Fisch; Huangqi)	Whole herb extract	Cortical neurons; Cognitive defect induced by amyloid peptide Abeta (25-35)	Treatment in medium; Orally for *in vivo*	3 days (*in vitro*); 15 days (*in vivo*)	100 μg/ml (*in vitro*); 1 g/kg (*in vivo*)	NA	Promoted axonal regeneration, reconstruction of neuronal synapses, neuronal death; improved memory	[50]

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TCM categories	Plant	Active compounds	Experimental model	Treatment time point and path	Period of treatment	Dosage	Targets	Major results	References
		AS-IV	Mice	IP	2 weeks	25 mg/kg	CXCL1/CXCR2	Increased hippocampal neurogenesis	[51]
			Embryonic NSCs	Treatment in medium	7 or 14 days	5, 20 μg/ml	Shh, Nurr1, Pt × 3	Promoted proliferation and differentiation into dopamine neurons	[52]
		Astragalus polysaccharide	Embryonic NSCs	Treatment in medium	7 or 14 days	0.02, 0.05 μg/ml	Shh, Nurr1, Pt × 3	Promoted proliferation and differentiation into dopamine neurons	[52]
		Astraisoflavan	Embryonic NSCs	Treatment in medium	7 or 14 days	10, 20, 50 ug/ml	Shh, Nurr1, Pt × 3	Promoted proliferation and differentiation into dopamine neurons	[52]
		AS-VI	NSCs; RAT MCAO	Treatment in medium; IV for *in vivo*	7 days	2 μg/kg EGFR	EGFR/MAPK	Promoted neuron proliferation and improved neurological function	[53]
	Herba epimedii	Icariin	Aging RAT	Orally	3 months	0.02 g/kg	NA	Activated quiescent NSCs and improved cognitive function	[54]
			Embryonic NSCs	Treatment in medium	3 days	10-200 nM	ERK	Promoted cell self-renewal	[55]
		Extract	Mice AD	Orally for *in vivo*	90 days (*in vivo*)	200 mg/kg	FGFR1, ERK, AKT	Promoted cognitive function and neural progenitor proliferation	[56]
	G. lucidum, lingzhi	Polysaccharides	NPC; mice AD	Treatment in medium; orally for *in vivo*	90 days (*in vivo*)	10-300 μg/ml (*in vitro*); 30 mg/kg (*in vivo*)	FGFR1, ERK, AKT	Promoted cognitive function and neural progenitor proliferation	[56]
	Cuscuta japonica Choisy (CJ, tushizi in Chinese)	Water extract	Mice	Orally	21 days	50, 100 mg/kg/day	NA	Stimulated neuronal cell proliferation, differentiation, and maturation; Improved the cognitive function	[57]
Wind-dispelling Herbs	*Angelicae Pubescentis Radix, named Duhuo*	Osthole	Bone marrow-derived-NSCs (BM-NSCs)	Treatment in medium	24 hours	10, 50, and 100 μM	Bcl-2, Bax, PI3K, Akt	Protected BM-NSCs against oxidative stress injury	[58]

iPSCs: Induced pluripotent stem cells, MCAO: Middle cerebral artery occlusion, NSCs: Neural stem cells, NA: Not available, BDNF: Brain-derived neurotrophic factor, BM-NSCs: Bone marrow-derived NSCs, PI3K: Phosphatidylinositol 3-kinase, MAPK: Mitogen-activated protein kinase, SDF-1: Stromal cell-derived factor 1, PKC: Protein kinase C, ERK: Extracellular signal-regulated kinase, TopoIIβ: Topoisomerase Iiβ, AC: Acetylated histone, cAMP: Cyclic adenosine monophosphate, CREB: cAMP response element-binding protein, GAD65: Glutamic acid decarboxylase 65, GSH-Px: Glutathione peroxidase, SOD: Superoxide dismutase, IL: Interleukin, bFGF: Basic fibroblast growth factor, NPCs: Neural progenitor cells, OGD: Oxygen and glucose deprivation, t-BHP: tert-Butylhydroperoxide, AD: Alzheimer's disease, MSCs: Marrow stromal cells, CXCL1: Chemokine (C-X-C motif) ligand 1, CXCR2: C-X-C motif chemokine receptor 2, Shh: Sonic hedgehog, Nurr1: Nuclear hormone 1, Ptx: Pituitary homeobox 3, IV: Intravenous, EGFR: Epidermal growth factor receptor, FGFR1: Fibroblast growth factor receptor 1, TIIA: Tanshinone II A, Sal B: Salvianolic acid B, TMP: Tetramethylpyrazine, *S. miltiorrhiza*: *Salvia miltiorrhiza*, *P. notoginseng*: *Panax notoginseng*, *G. biloba*: *Ginkgo biloba*, *P. tenuifolia*: *Polygala tenuifolia*, RAT: *Rhizoma Acori tatarinowii*, IP: Intraperitoneal, GSK3β: Glycogen synthase kinase 3 beta, TNF: Tumor necrosis factor, NGF: Nerve growth factor, TCM: Traditional Chinese Medicine

Current Progress of Chinese Herbal Medicine for Promoting Neurogenesis and their Molecular Targets for Poststroke Treatment

Recent years, extensive efforts have been made to verify the therapeutic outcome of TCM approaches and explore the underlying mechanisms and molecular targets for their neuroprotection and neurogenesis-promoting effects. Many TCM formulae, single herbs or pure compounds revealed their bioactivities to promote neuronal survival and neurite growth and facilitate neurological functional recovery via targeting different cellular signaling pathways in different experimental systems. Interestingly, those herbs and TCM formulae are generally in the catalog lists of the medicinal herbs with the properties of *Removing Blood-stagnation and/or Phlegm Accumulation to Improve Qi-Blood Movement in Meridian, or Tonifying Zang-fu Functions for Resolving Qi-Blood and Yin-Yang Deficiency* according to classical TCM theory. The cross talking between TCM and modern biomedical sciences provide a unique opportunity for better understanding the ancient therapeutic arts and brings novel ideas for drug discovery in regeneration therapy. In this session, we reviewed the update progress on the studies of the active compounds, herbal extracts, and TCM formulas with potentials for promoting neurogenesis and their relevant molecular targets and mechanisms [Table 1].

Neurogenesis-promoting effects of Medicinal Herbs used for Resolving Blood Stasis

According to TCM theory, the *Blood Stasis* is a common syndrome pattern in ischemic stroke patients, and subsequently, the therapeutic strategy for promoting blood circulation is widely used to treat the disability of ischemic stroke patients in TCM practice. Many studies suggest that the neurogenesis-promoting effects of medicinal herbs with the properties of improving blood circulation could be important sources for poststroke treatment.

Salvia miltiorrhiza, named Danshen in Chinese, is commonly used for stroke with anti-oxidative, anti-inflammatory, and anti-apoptotic properties. *S. miltiorrhiza* could improve the differentiation of induced pluripotent stem cell (iPSCs) into neurons *in vitro* and promote the survival, integration, and differentiation of the iPSCs-derived neural stem cell after their transplantation into the ischemic brain tissues. *S. miltiorrhiza* treatment effectively promoted the survival of grafted MAP2(+) cells in the ischemic brains after cells transplantation at 7 days and enhanced functional recovery at 7 and 14 days.[22] *S. miltiorrhiza* also promoted the proliferation and differentiation of bone marrow-derived neural stem cells (BM-NSCs) into NF-M(+) neurons and NG2(+) oligodendrocyte precursors but reduced the formation of GFAP(+) astrocytes. Salvianolic acid B (Sal B) had neuroprotective effects on BM-NSCs upon exposure to H_2O_2.[23] Sal B is a representative active compound from Danshen with antioxidant, anti-inflammatory, antiapoptotic, and neuroprotective properties. Sal B revealed its neuroprotective effects on protecting iPSCs-derived NSCs against H_2O_2-induced neural injury.[24] Tanshinone II A (TIIA) is another representative bioactive compound from Danshen. In our previous study, TIIA dose-dependently promoted neuronal differentiation in three different stem cells, including immortalized C17.2 NSCs, rat embryonic cortical NSCs, and rat PC12 pheochromocytoma cells. The underlying mechanisms could be related to regulating mitogen-activated protein kinase (MAPK) 42/44 mediated brain-derived neurotrophic factor (BDNF) and NGF signals in a caveolae-dependent manner.[25] Similarly, Sal B was reported to promote neural differentiation of iPSCs via regulating phosphatidylinositol 3-kinase (PI3K)/AKT/G SK3 β/β-catenin pathway.[26] Those studies suggest that *S. miltiorrhiza* and its active compounds have the potentials for inducing adult neurogenesis from different sources of stem cells for regeneration therapy.

Ligusticum walliichi, named Chuanxiong in Chinese, is an important medicinal herb with functions of promoting blood circulation and widely used for the treatment of ischemic stroke. Tetramethylpyrazine (TMP) is an active compound isolated from *Chuanxiong*. A recent study showed that TMP promoted the migration of neural progenitor cells (NPCs) toward the ischemic region in the middle cerebral artery occlusion rat model via inducing the expression and secretion of stromal cell-derived factor 1, a chemokine for guiding NPCs trafficking. TMP treatment rapidly activated PI3K/Akt, protein kinase C, and extracellular signal-regulated kinase (ERK), but not Pyk2, in NPCs.[27] Another *in vitro* study revealed that TMP promoted SH-SY5Y cells to differentiate toward postmitotic neurons by the epigenetic regulation of topoisomerase Iiβ (TopoIIβ), a nuclear enzyme for neuronal development.[28] TMP could be a promising compound for inducing the growth, development, and migration of endogenous NSCs for brain repair. Those studies provide a cue for better understanding the therapeutic principles of *Chuanxiong* and exploring its representative active ingredients for poststroke treatment.

Radix Angelica Sinensis (RAS, Dangui in Chinese) is another representative Chinese herbal medicine, with the properties of promoting blood circulation to dispel blood stasis and used for ischemic stroke treatment. RAS has potentially beneficial effects for the patients suffering from cognitive impairment associated with chronic cerebral hypoperfusion. RAS treatment enhanced adult HIP neurogenesis and improved the cognitive functions after chronic cerebral hypoperfusion through inducing BDNF and phosphorylated cyclic adenosine monophosphate (cAMP)-responsive element binding protein as well as increasing γ-aminobutyric acid expression.[29]

Panax notoginseng, named Sanqi in Chinese, is another typical medicinal herb to promoting blood circulation for removing blood stasis. A recent study investigated the effects of *P. notoginseng* saponins (PNS) on the proliferation, differentiation, and self-renewal of rat embryonic NSCs. PNS

promoted rat embryonic cortical NSCs survival, self-renewal, proliferation, and differentiation through promoting autocrine or paracrine of neurotrophic factors such as basic fibroblast growth factor and BDNF in rat embryonic NSCs.[30] Total saponins of *P. notoginseng* promoted differentiation of doublecortin(+) cells expressing immature neuroblasts in the olfactory bulb (OB) via activating cAMP response element binding protein in postischemia/reperfusion brains.[31] Ginsenoside Rg1 is a representative active ingredient of *Panax ginseng*. Ginsenoside Rg1 revealed its neuroprotective effects on the HIP in the D-gal (D-galactose) induced aging rat model. Ginsenoside Rg1 treatment improved cognitive functions, protected NSCs/NPCs, and promoted neurogenesis. The underlying mechanisms could be related to enhancing the antioxidant and anti-inflammatory capacity in the HIP.[32] Ginsenoside Rg1 treatment also revealed to promote the proliferation and glial-like-directed differentiation of cortical NSCs.[33]

Oxidative stress and redox signaling play important roles in regulating neurogenesis. The antioxidant property of the active compounds could be important for promoting neurogenesis. A recent study compared the neuroprotective effects of the different ginsenosides, including Rb1, Rd, Rg1, and Re on the cultured NPCs in the tert-Butylhydroperoxide (t-BHP)-induced oxidative stress model. Only Rb1 treatment attenuated tBHP toxicity in the NPCs by modulating the nuclear factor (erythroidderived 2) like 2/heme oxygenase1 pathway[34] as well as activating Nrf2 pathway.[35] Therefore, the neuroprotective and neurogenic effects of PNS and its active compounds Rg1 and Rb1 are valuable for further investigations to confirm their activities in regeneration therapy.

Neurogenesis-promoting effects of Medicinal Herbs for Removing Phlegm Accumulation

According to TCM theory, Phlegm accumulation is one of the critical pathological factors contributing to the cognitive deficit and neurological dysfunctions in different types of *Wind Stroke* including "*Meridian stroke*," "*Zhang-fu stroke,*" and sequela. *Removing Phlegm Therapy* is an important therapeutic strategy to improve the recovery of cognitive and other neurological functions. *Ginkgo biloba* is a typical medicinal herb for removing phlegm accumulation in TCM practice. EGb761 is a standard extract from *G. biloba* as functional health supplement for improving memory loss and cognitive impairments in stroke, senile dementia, and Parkinson's disease patients. EGb761 revealed to enhance NSCs proliferation in the SVZ and DG and promoted learning and memory in rats with vascular dementia.[36] *Polygala tenuifolia Willd* (PTW), named Yuanzhi in Chinese, is a representative medicinal herb with the properties of removing phlegm and promoting resuscitation of cognitive impairment according to the TCM concept. PTW has been commonly used for improving cognitive impairment for postischemic stroke in TCM practice. Tenuigenin, an active ingredient of PTW, showed to promote the proliferation and differentiation of hippocampal NSCs *in vitro*.[37] Interestingly, PTW is simultaneously used with *Rhizoma Acori tatarinowii* (RAT, Shichangpu in Chinese) as a paired herbal group for removing phlegm and promoting resuscitation of cognitive impairment clinically. The neurogenesis-promoting effects of the RAT's active components have been reported as well. Oral administration of RAT extract and its active constituent asarones enhanced NPCs proliferation and neurogenesis in the hippocampi of adult and aged mice as well as transgenic Alzheimer's disease (AD) model mice. RAT and asarones activated ERK signaling cascades for inducing neurogenesis.[38] Those studies provide promising experimental evidence to elucidate the active compounds of PTW and RAT and their potential molecular targets for the improvement of brain repairs in poststroke treatment. However, experimental evidence is still lack to elucidate whether the paired herbal group of PTW and RAT would have better synergic effects than the individual herbal item on inducing neurogenesis and promoting the recoveries of neurological dysfunctions and cognitive impairments in poststroke treatment.

Neurogenesis-promoting effects of Medicinal Herbs for Removing Internal Heat for Detoxification

Herbal items with the properties of removing internal heat for detoxification are commonly used at the acute and subacute statuses in both ischemic stroke and hemorrhagic stroke for anti-inflammation and improving cognitive functions in TCM practice. Whether the herbs for removing internal heat and detoxification have neurogenetic promotive effects is an interesting question. *Radix scutellaria (Huangqin)* and *Radix phellodendri (Huangbo)* are two representative herbs with the functions for removing internal heat and detoxification. Oral administration of those herbs reversed the elevation of plasma corticosterone levels and body weight loss and depressive behaviors in chronic mild stress-induced depression-like mouse model, and the anti-depressive mechanisms could be related to the inhibition of hypothalamic-pituitary-adrenal (HPA) axis hyperactivity and the increasing of hippocampal precursor cell proliferation.[39] Our previous study revealed that Baicalin can directly scavenge superoxide and react with peroxynitrite, inhibited the formation of 3-nitrotyrosine, reduced infarct size, and attenuated apoptotic cell death in cerebral I/R injury.[40] Our recent study further revealed that baicalin protected the blood–brain-barrier (BBB) integrity and prevented HT and neurotoxicity in postischemic brain injury with the delayed t-PA treatment.[41] On the other hand, we found that baicalin-regulated cell fate decision in embryonic NPCs by promoting neural differentiation but inhibiting glial formation in cultured embryonic NPCs. The underlying mechanisms are related to regulating the signal transducer and activator of transcription 3 and basic helix-loop-helix signal pathways.[42] Other independent group also proved that baicalin stimulated the differentiation of human umbilical cord blood mesenchymal stem cells and rat BM stromal cells into neurons.[43] Furthermore, our recent study found that baicalin could modulate APPL2/glucocorticoid receptor signaling cascades, promote neurogenesis in the HIP and SVZ-OB, and subsequently attenuating emotional and

olfactory dysfunctions in a chronic corticosterone-induced depression mouse model.[44] Thus, *R. scutellaria (Huangqin)* and its active compounds like baicalin have great potentials as neurogenic promoters for brain repair in the treatment of poststroke disability and other neurological disorders such as chronic stress-induced depressions.

Neurogenesis-promoting effects of Tonic Medicinal Herbs

Tonifying Qi-Blood and Yin-Yang Strategy is an essential TCM therapeutic principle for poststroke treatment. Many investigations have been conducted to explore the potential therapeutic values of the herbs and their active compounds for brain repair of poststroke disability. *Radix astragali* (Astragalus menbranaceus Fisch; Huangqi) is the most common used Qi-tonifying Chinese medicinal herb for the treatments of stroke and neurodegenerative diseases. *Astragalosides* are bioactive components isolated from *R. astragali*. Astragaloside IV (AS-IV) is one of the representative compounds of *Astragalosides,* and its neuroprotective effects have been extensively studied. AS-IV revealed its neuroprotective effects against experimental cerebral ischemic injury with different molecular mechanisms. The underlying mechanisms of AS-IV for protecting ischemic brain injury are multiple aspects including antioxidative/nitration stress, anti-inflammation and anti-apoptosis,[45] prevented neutrophils accumulation and inhibited the expression of intercellular adhesion molecule-1,[46] reduced the frequencies of synchronized spontaneous Ca^{2+} oscillations, and spontaneous excitatory postsynaptic currents in hippocampal neurons.[47] AS-IV treatment protected the BBB integrity by reserving the expression of tight junction proteins such as occludin and zonae occlude-1 in ischemia-reperfused rat brains.[48] A recent study reported that As-IV could activate Nrf2 signaling pathway and subsequently protected the integrity of BBB in LPS-induced mice.[49] In addition to the neuroprotection, AS-IV treatment also enhanced the axonal regeneration and reconstruction of neuronal synapses and prevented Aβ (25–35)-induced neuronal death.[50] AS-IV promoted neurogenesis in the mouse hippocampal DGs, and the underlying mechanisms could be related to modulating the homeostasis of the chemokine (C-X-C motif) ligand 1/C-X-C Motif Chemokine Receptor signaling pathway.[51] Except for AS-IV, Astragalus polysaccharides and Astraisoflavan, the other active ingredients of *R. astragali,* showed their neurogenesis-promoting effects on promoting the proliferation of NSCs and guiding the committed differentiation of NSCs into dopamine neurons through modulating Sonic hedgehog, orphan nuclear hormone 1, and pituitary homeobox 3 -signaling pathways.[52] Our recent study revealed that Astragaloside VI (AS-VI) had even better neurogenesis-promoting effects than other Astragalosides. AS-VI effectively activated epidermal growth factor receptor/MAPK signaling cascades, promoted proliferation and differentiation of NSCs in transient cerebral ischemic brains and improved the neurological functions in postischemic stroke rats. AS-VI has great potentials to be a drug candidate for regeneration therapy.[53] Those studies not only bring novel insights into the therapeutic principles of *R. astragali* but also provide the cue for drug discovery in the treatment of poststroke disability.

Herba epimedii is a famous Chinese herbal medicine with the "*Yang-tonic*" function. Icariin (ICA) is a representative compound isolated from *H. epimedii*. ICA was previously reported to improve cognitive deficits and activate quiescent NSCs in aging rats.[54] ICA dose-dependently promoted neurosphere formation when the NSCs cultured with the growth protocol. ICA treatment-induced NSCs proliferation but had no effect on NSCs differentiation in differentiation protocol. ICA could activate MAPK pathway for inducing neurogenesis.[55]

Ganoderma lucidum, Linzhi in Chinese, is commonly used food supplement as well as tonic medicinal herb. Oral administration of the polysaccharides and water extract of *G. lucidum* promoted NPCs proliferation, enhanced neurogenesis, and alleviated cognitive deficits in transgenic AD mice. *G. lucidum* polysaccharides (GLP) also promoted self-renewal of NPCs via activating fibroblast growth factor receptor 1 and its downstream ERK and AKT cascades.[56] Whether GLP has similar effects on promoting neurogenesis is an interesting topic for further investigation.

Cuscuta japonica Choisy (CJ, tushizi in Chinese) is also an important component in TCM remedies with the tonic functions for treatment of *Kidney-Qi* and *Kidney-Essence Deficiency Syndrome* in poststroke disability according to the TCM theory. A recent study investigated the effects of CJ on promoting adult hippocampal neurogenesis and memory function in mice. CJ water extract dose-dependently promoted the proliferation, differentiation, and maturation of NSCs for neurogenesis and improved the cognitive functions in the mice.[57]

Neurogenesis-promoting effects of Wind-dispelling Herbs

According to the TCM therapy, "*External Wind Invasion into Meridian and Channel*" and "*Internal Wind-like Pathological Factors due to Dysfunction of Zang-fu*" are two major pathological statuses in "*Wind Stroke*." The theory is commonly used for the explanations of the symptoms of neurological deficits such as acute sudden collapse, loss of consciousness, deviation of the tongue and mouth, hemiplegia, and slurred speech. The medicinal herbs with the wind-dispelling properties have been used for the treatment of "*Wind Stroke*" for 1000 years. Interestingly, the neurogenesis-promoting effects of wind-dispelling herbs and their active ingredients have been reported as well. *Angelicae Pubescentis Radix,* named Duhuo in Chinese, is a representative herb used in many famous TCM formulas for "Wind Stroke". Osthole (Ost) is a principal bioactive component of *Radix Angelicae Pubescentis*. A previous study indicates that Ost protects bone marrow-derived-NSCs (BM-NSCs) against oxidative stress injury, and it can be used to improve the inflammatory environment of neurodegenerative diseases so and promote the survival rate of transplanted NSCs.[58] The study provides a novel idea to use Chinese medicinal herbs for stem cell transplantation in poststroke treatment.

Discussion

In summary, current studies extensively investigated the effects of Chinese herbal medicine on promoting adult neurogenesis and recovering neurological functions in different *in vitro* and *in vivo* systems. The experimental systems cover almost all stages of the neurogenic process such as proliferation, self-renewal, differentiation, precursors growth, development, and migration of the different types of stem cells. The cell types include BM derived-neural stem cells, pluripotent stem cells, immortalized C17.2 NSCs, embryonic cortical NSCs, and PC12 cells. The experiments for targeting neurogenesis not only work with the *in vitro* cultured stem cells but also different animal models for endogenous neurogenesis and iPSCs transplantation. Many critical neurogenic cellular signaling pathways are included in these studies. Importantly, we should emphasize that most of the reported medicinal extracts or pure compounds are obtained from the parent herbs in the catalogs of "*Improving Blood Circulation to Resolve Blood Stasis,*" "*Removing Phlegm Accumulation for Qi Movement in Meridians and Channels*" "*Removing Internal Heat for Detoxification,*" "*Dispelling Wind for Regulating Qi-Blood Movement in Meridians and Channels,"* and "*Tonifying Qi-Blood and Zang-fu Functions*" in TCM. Those herbs are generally used for revolving different pathological statues and the different stages of "Wind Stroke" according to classic TCM concepts. Thus, the research progress provides very useful information for bridging the cross talk between TCM and modern conventional medicine in regeneration medicine. In addition, these studies also provide important cues for seeking potential drug candidates for brain repair in different experimental systems.

Nevertheless, it is still premature to draw the conclusion about the scientific basis of those medicinal herbs for poststroke treatments and the related drug discovery. There are many challenging questions remained for future studies: (1) Roles of active compounds and their derivatives for parent medicinal herbs: Indeed, many active compounds or extracts showed their neurogenesis-promoting effects in the literature. It is unclear whether those active compounds are the major compounds contributing to the neurogenic bioactivities of their parent medicinal herbs. Most of the results were only obtained from the *in vitro*-cultured cell systems or the *in vivo* animal models by injection of the compounds. Seldom study is conducted to investigate the relationship between the neurogenic effects and the bioavailability of the captioned compounds. For example, tetramethylpyrazine-induced neurogenesis in the postischemic brains, but the bioavailability of this compound was not investigated in those studies.[59,60] However, evidence from other studies does support that tetramethylpyrazine could pass through the BBB, with the elimination half-life in rat blood and brain of 82.1 and 184.6 min, respectively.[61-63] Ginsenoside Rg1 treatment increased neurogenesis in DG and HIP in both normal and ischemic brains, but the bioavailability was not addressed in those study.[64-66] Interestingly, evidence from another study showed that Ginsenoside Rg1 could be detected in brain extracellular fluid (bECF) and cerebrospinal fluid (CSF), and distributed to the medial prefrontal cortex, HIP, and LV after subcutaneous injection.[67] Such study indicates that Ginsenoside Rg1 could be delivered to the brain to exert its pharmacological effects on neurogenesis. Similarly, Ginsenoside Rb1 could also pass through the BBB, partially via the GLUT1 transporter.[68] α–Asarone passed through BBB after taken into the circulatory system or by traveling from the olfactory epithelium into the brain tissue via the OB.[69] β–Asarone could also distribute into the brain after intragastric administration.[70] Baicalin could penetrate BBB at the dosage of 24 mg/kg with the time to maximum at 30 min in the CSF.[71] Sal B showed direct stimulation effects on neurogenesis *in vivo* and *in vitro*, but the bioavailability information was not provided in the same study.[72] Meanwhile, other studies suggest that Sal B had low bioavailability and low penetration through the BBB.[73,74] Therefore, the neurogenesis effects of Sal B could be possibly indirect. Similarly, AS-IV had limited distribution in the brain tissue, suggesting that it had difficulty in penetrating the BBB.[75] ICA also showed low distribution to the brain tissue.[76] The relationship of their neurogenesis effects and bioavailability needs further investigation. The pharmacokinetics and pharmacodynamics of those compounds in animal models are critical parameters for evaluating the values of drug discovery. The structure and function relationships of the active compounds and their derivatives are also important for further investigations. (2) Molecular targets of active compounds and their derivatives for neurogenesis promotion: many studies investigated the different cellular signal pathways for exploring the underlying mechanisms for neurogenesis in their reports. However, whether those signaling pathways are direct or indirect targets is unknown yet. Many compounds appear to target several molecular targets simultaneously in literature. We recently proposed the one-compound-multitarget concept for the combination prospects of natural compounds with thrombolytic therapy in acute ischemic stroke.[77] Neurogenic effects of the active compounds for promoting neurogenesis should share the same situation. For data interpretation, the pharmacological actions of those compounds could be due to direct interactions, or simply because of the indirect responses of the molecules to the regulation of upstream signaling and/or the synergic effects of the network signaling systems. The changes of the cellular signaling and molecule targets could come from the direct binding of the compounds to the proteins or signaling molecules or only indirectly affected by other factors. (3) Network regulations of medicinal herbs for neurogenesis: Notably, medicinal herbs are generally used as a TCM formula according to the principle of "King-Minister-Assistance-Guider" in the TCM clinical practice. Given that single herb alone already contains 1000 bioactive compounds, these active compounds could yield complex interactions and have synergic effects on modulating

adult neurogenesis. In a TCM formula, the active compounds from different medicinal herbal components would yield much more complex interactions and network regulations than the single herb. The development of advanced chemical analytic technology and high throughput gene profile platforms provide a unique opportunity to explore the complex synergic effects of different active compounds in TCM formulas. In recent studies, many TCM formulas already revealed their promoting effects on promoting neurogenesis and recovering neurological deficits in the poststroke study. The progress of regeneration medicine and multiple advanced analytic technologies such as proteomics, metabolomics, and bioinformatics greatly facilitate us to explore the complex and magic world of TCM formulas. With the complex synergic effects and interactions, we will write a separate review article to discuss this important topic in the future.

Conclusion

The combination of stem cell biology and modern Chinese medicine create a novel opportunity for cross talk of TCM and Western medicine in regeneration medicine, leading novel therapeutic strategies for poststroke treatment.

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Conflicts of interest

There are no conflicts of interest.

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