

Differentiation to Neural-Like Cells – A Potential Application of Mesenchymal Stem Cells Based on Traditional Chinese Medicine Theories

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ABSTRACT

Many neurological disorders, such as stroke, Parkinson's disease and multiple sclerosis, etc., are caused by the loss of neurons and glial cells. Both *in vivo* and *in vitro*, mesenchymal stem cells (MSCs) have their potential to differentiate themselves to nerve cells, which provides a new train of thought for the treatment of nervous system diseases. Compared with neural stem cells (NSCs), the advantages of MSCs, such as being obtained conveniently, easy separation, culture and proliferation *in vitro*, no rejection for self out, etc., provide a much wider space to treat nervous system diseases. Studies on the combination of Chinese Herbal Medicine (CHM) and MSCs have revealed that CHM and some effective components could activate endogenous stem cells and induce stem cells to differentiate into neural-like cells *in vitro* and promote angiogenesis. Traditional Chinese Medicine (TCM) has formed a unique theoretical system after development for thousands of years. Guided by TCM theories, researches on MSCs differentiation into neural-like cells directional induced by CHM create a new method for the application of CHM in the field of MSCs. This review aims to summarize the latest research findings on MSCs differentiation into neural-like cells directional induced by CHM from the viewpoint of syndrome differentiation and treatment.

Keywords: Syndrome differentiation and treatment, Chinese herbal medicine, Mesenchymal stem cells, Neural regeneration.

INTRODUCTION

In the field of stem cell research, TCM has a unique theoretical system and application form. It has been reported that CHM and some effective components have a promoting effect on the proliferation and differentiation of stem cells. Compared with other revulsants or methods, CHM has many characteristics, such as safety and comprehensive clinical application. TCM treatment emphasizes the concept of holism, syndrome differentiation and treatment. CHM can directly act on stem cells in the body and promote the proliferation and differentiation. It can also influence the microenvironment, promote their survival and function, adjust the body's immune function, reduce or eliminate the immune rejection.

Nowadays, many studies have shown that CHM can effectively induce MSCs from different sources to differentiate to neural-like cells [1-3]. Guided by TCM theories (the concept of holism, syndrome differentiation

and treatment, et al.), the study of the mechanisms of proliferation, differentiation and resistance to damage of MSCs can bring great development for MSCs' application in nervous system diseases.

Characteristics of MSCs Differentiation into Neural-like Cells

MSCs are the kind of adult stem cells that exist in a variety

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of organizations, such as bone marrow, umbilical cord blood and umbilical cord, the placenta tissue, amniotic fluid and adipose tissue [4-6]. They can differentiate into ectomesenchymal series cells including ossification, cartilage and fat cells [7-9], or non-mesoderm lineages such as Schwann-like cells which play roles for the development, myelination and regeneration in the peripheral nervous system [10-12]. MSCs were originally found in the bone marrow [13]. Bone marrow is the main source.

MSCs can not only promote other cells to differentiate into neural-like cells, but also differentiate into neural-like cells under certain conditions. MSCs are capable to secrete cytokines, chemokines and growth factors, which play important roles in creating favorable microenvironments for proliferation of neural cells at the injury site, hence enhance angiogenesis, synaptogenesis and neurogenesis in the damaged brain tissue [14,15]. The microenvironment provided by bone marrow mesenchymal stem cells (BMSCs) is beneficial for NSCs to differentiate selectively into neuronal and astrocytic phenotype cells. BMSCs not only induce neuronal differentiation of NSCs but also enhance the survival of neurons. Soluble factors secreted by BMSCs are responsible for their effect on the neuronal differentiation of NSCs [16]. BMSCs have capacity to cross the blood-brain barrier and migrate into injured tissues systematically [17], and then they differentiate into mesenchymal lineage cells, including neurons and non-neuronal cells in the brain [18]. Hoda Jahani et al. [19] indicated that MSCs cultured on nanofibrous scaffold had potential differentiation to neuronal cells, electrospun scaffolds, particularly scaffolds with random nanofibers indicating to have potential in promoting the differentiation of mesenchymal cells.

Studies indicate that cells derived from bone marrow survive, proliferate, migrate and can differentiate into glial and neuronal phenotypes. Compared with NSCs, MSCs are easy to isolate from the small aspirates of bone marrow that can be obtained under local anesthesia, capable of rapid proliferation in culture, amenable to survive and integrate in the host brain, and immunologically inert [20]. All the benefits provide a wider application for MSCs in nerve tissue repairment.

The Mechanism of the Differentiation of MSCs into Neural-like Cells Based on TCM Theories

After 5000 years of development, TCM has the complete theory system and rich clinical practice experiences. The concept of holism, syndrome differentiation and treatment are the two basic features of TCM. Essential qi theory, yin-yang theory, five phase theory, zang-fu theory, et al. constitute its main theories (Table 1).

In essential qi theory, essence means the fundamental substance that builds up the physical structure and maintains body function, especially reproductive essence stored in the kidney. Shen [21] pointed that kidney essence can

correspond to embryonic stem cells and the kinds of tissues and organs differentiated from ESCs. Zhang et al. [22] elaborated essence theory of TCM from the perspective of stem cells.

Table1. The basic features and basic theories of TCM.

Basic features	Definition/Description
concept of holism	one of the philosophical ideas regarding the human body as an organic whole, which is integrated with the external environment
syndrome differentiation and treatment	diagnosis of the pattern/syndrome, through comprehensive analysis of symptoms and signs, which has implications for determining the cause, nature and location of the illness and the patient's physical condition, and their treatment
BASIC THEORIES	
Essential Qi Theory	one of the basic theories in traditional Chinese medicine about qi, the essential part of which constitutes the body and maintain the activities of life, visceral function and metabolism
Yin-yang Theory	an ancient Chinese philosophical concept, dealing with two opposite aspects of matters in nature which are interrelated with each other. Its principle is widely applied to traditional Chinese medicine
Five Phase Theory	one of the philosophical theories of medical practice in ancient China, concerning the composition and evolution of the physical universe, epitomized by the nature and the inhibition-generation relationships of the five phases, wood, fire, earth, metal and water, serving as the guiding ideology and methodology of physiology, pathology, clinical diagnosis and treatment, also known as five elements theory
Zang-fu Theory	a collective term for internal organs, also called zang-organs and fu-organs

They considered that the totipotent stem cells (TSCs) contain all the congenital essence. By comparing the functions of TSCs and other founded adult stem cells with the functions of proliferation, growth and development, generation marrow and blood of the essence,

we found that the basic attributes of essence and stem cells were analogous (**Figure 1**).

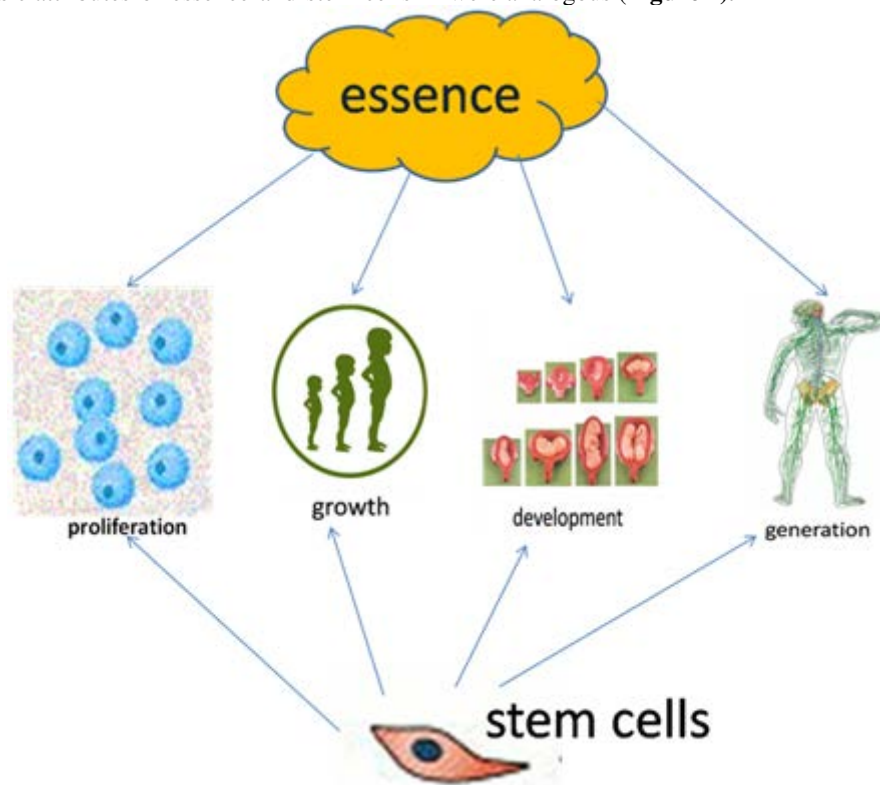


Figure 1. The basic attributes of essence and stem cells are analogous. The essence for an individual's birth comes from both father and mother. It is the material foundation for a human embryo, viscera, tissues and organs based on TCM theories. The fertilized egg, which is composed of a sperm and an egg, is a totipotent stem cell. The process of stem cells differentiation is from totipotent stem cells to embryonic stem cells, and then to the stem cells of various tissues and organs, and finally into thesocytes. So based on these understandings, we deem the basic attributes of essence and stem cells are analogous.

In the field of TCM, qi refers both to the refined nutritive substance that flows within the human body as well as to its functional activities. We consider that the nutritive substance that qi mains is likely to cell health factors. The functional activities now have been fully understudied via acupuncture, qigong and kongfu. Blood is described as the red fluid circulating through the blood vessels, which can nourish and moisten the whole body. Essence and blood can promote mutually and transformate into each other. While the interaction between essence and blood is achieved by the functional activities of qi (**Figure 2**).

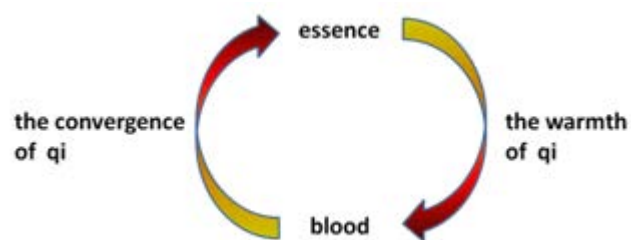


Figure 2. Essence and blood share a common source. Essence especially means the reproductive essence stored in

the kidney. Blood is mainly stored in the liver. In the five phase theory, the kidney belongs to water, while the liver to wood. So on the relationship of mother and child, the kidney (water) generates the liver (wood). In addition, the warmth of kidney qi promotes the liver stronger. On the other hand, the blood stored in the liver is derived into the kidney to generate essence duo to the convergence of qi.

Brain is one of the extraordinary organs enclosed within the skull where the marrow converges, and the spirit, mental activities as well as thinking take place. Marrow is defined as an extraordinary organ including bone marrow and spinal marrow, both of which are nourished by the kidney essence. *Su Wen* points out that "The marrow is belongs to the brain". *Ling Shu* says "At the beginning of one generation, the essence generates firstly. After the generation of the essence, the brain is produced". *Yixue Zhongzhong Canxi Lu* summarizes the theories as that "The brain is the sea of marrow, which is generated by the Yin qi and Yang qi of the kidney". So, it is clear that kidney essence is the foundation of the brain's generation (**Figure 3**). Therefore, essence, qi, blood, marrow and brain form an interactive system. Kidney

essence, qi and blood are the material basis of brain's growth and development. MSCs are mainly derived from bone marrow; they not only have the characteristics of essence, marrow and blood, but also have the functions

of transformation into various substances. So it can be concluded that the application of MSCs in nervous system diseases would play an unexpected role.

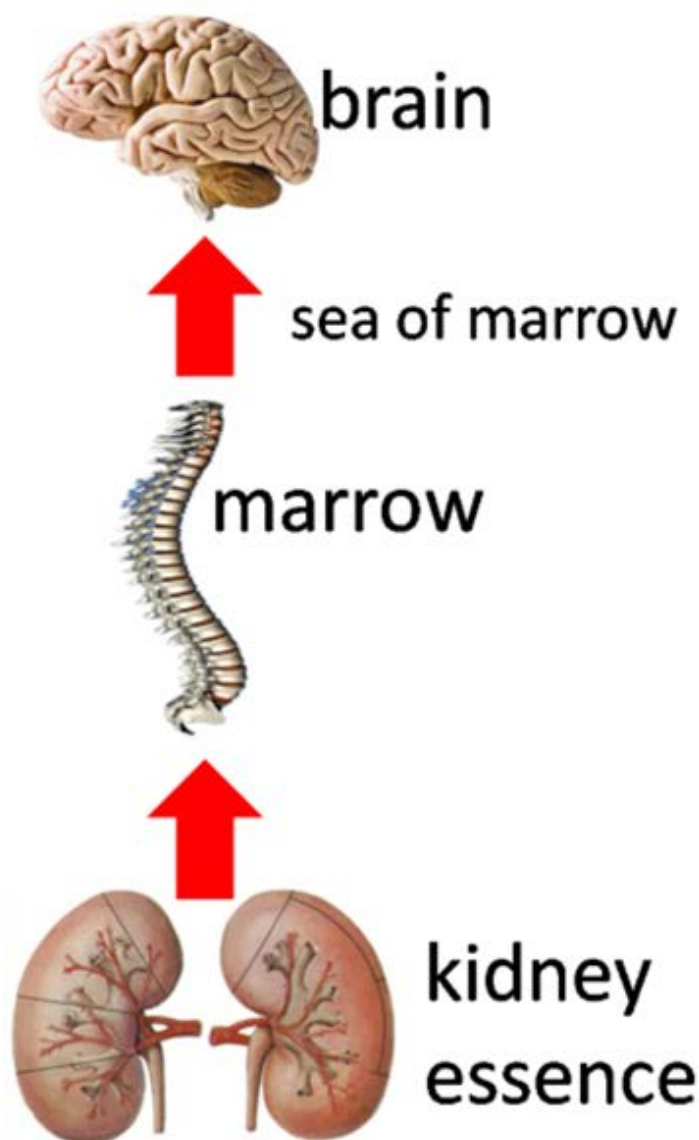


Figure 3. The generation of the brain. The kidney essence is the foundation of the brain's generation.

If the balance of Yin and Yang in zang-fu is broken, the flow of qi and blood would be in disorder, the brain would be damaged, nerve cells would be necrosis or apoptosis, the regulatory mechanism of proliferation, migration and differentiation of stem cells would also be damaged. Therefore, the regulatory mechanism of activation and repairment of MSCs is closely related to essence, marrow, qi and blood, and depends on the coordination of them.

The Therapy Methods and Using of Medication Based on the TCM Theories inducing Mscs Differentiated into Neural-Like Cells

Traditional Chinese Medicine attaches importance to the holistic concept, emphasizing the unity of the whole and local, macro and micro environments. Also it emphasizes the theory of syndrome differentiation and treatment. The major TCM theories and Chinese herbs inducing MSCs differentiated into neural-like cells are summarized in **Table 2**.

Table 2. Summary of major TCM theories and Chinese Herbal Medicines for MSCs differentiation into neural-like cells in experimental designs *in vivo* and *in vitro*.

TCM theories	Chinese Herbal Medicines	MSCs source	methods of administration	Intervention program	Resulting marker expression
Tonify Qi and Reinforce the Healthy Qi	Astragaloside IV[23]	C57/BL6 mouse	added in medium	5mg/d, 16 h at 37°C	TLR4, MMP-2, NF-kB p65, TNF- α , MCP-1
	Astragalus mongholicus[24]	SD rats	added in medium	125 μ g/ mL, 3 h at 37°C	Nestin, NSE, GFAP, Ngn-1, Wnt-1
	Astragalus[25]	SD rats	added in medium	1/10, 1/20, 1/40, 1/80, 1/100, 10ml per ramus	NF, NSE, nestin, Cyclin D1, c-myc, WIF1, GSK-3 β and β -catenin
	Ginsenoside Rg1[26]	SD rats	intraperitoneal injection	5mg/kg/d, for 4 weeks	escape latency(s) of Morris watermaze, NGF mRNA
	Shenqi Fuzheng injection[27]	Adult ribs	added in medium	4 \times 10 ⁻⁴ g/ ml, 30min	NSE, NF, GFAP
Tonify Qi and Activate Blood Circulation	Angelica sinensis and Hedysarum polybotry[28]	BMSC-D1, Serial number: CR L-10915, USA ATCC	added in medium	6, 12g / L, 1ml, 5h at 37°C	NSE, nestin, NFP, MAP2, GFAP
	Buyang Huanwu Tang[29]	SD rats	intra-gastric perfusion	15 ml/kg/d, 10d	VEGF, Ki-67
	Naomai Yihao Capsule[30]	SD rats	intra-gastric perfusion	1.5 g/ kg/d, 3 d, 7 d, 14 d	behavior rating scale, CD31
	Yiqihuoxue recipe[31]	SD rats	added in medium	35 g/L, 5h	Nestin, NSE, GFAP
Activate Blood and Resolve Stasis	Danshensu[33]	Adult bone marrow	added in medium	10 μ g/ ml, 24h	NSE, NF-M, nestin, GFAP
	Salvianolate[34]	neonatal umbilical	added in medium	0.01/0.1/1/10/100 /1000 μ g/ ml, 48h	Ach, Nsetin, NSE, ChAT

	extract of Ginkgo biloba [35]	cord human adipose tissue	added in medium	40 mg /L、 80 mg /L、 120 mg /L、 3、 7、 10、 14 d	Nestin, NSE, GFAP
	ginkgolide B[36]	Wistar rats	added in medium	20mg/L、 40 mg /L、 80 mg /L,7d	NSE, GFAP, Oligo4
	Total saponins of Panax notoginseng[37]	SD rats	added in medium	0 µg/ml, 0.03 µg/ml, 0.1 µg/ml, 0.3 µg/ml, 1 µg/ml, 3 µg/ml, 10 µg/ml, 30 µg/ml, 100 µg/ml, 300 µg/ml,48h	VEGF-A, Flt-1, Kdr
	Sodium Ferulate[38]	SD rats	intraperitoneal injection	60 mg/kg, 3d	Glut1, Tuj1, GFAP
	Ligustrazine[39]	SD rats	released from the chitosan microspheres	23.67% drug loading capacity per nerve guide conduit, 7d,14d	NSE, MAP2
Tonify the Kidney to Supply Essence	Tortoise plastron[40]	SD rats	added in medium	1.25mg/ml、 2mg /ml,24h	NSE, GFAP, Nestin
	Plastrum Testudinis[41]	SD rats	intra-gastric perfusion	4mg/d,7d	Brdu, Brdu /NF
	Rehmannia glutinosa polysaccharide[42,43,44]	SD rats	added in medium	50、 100、 200µg /ml,24h	NSE, GFAP, Nestin, βIII-tubulin, Notch1, Jagged1, NICD,
	Lycium barbarum polysaccharide[45]	SD rats	added in medium	1 g/l, 8h,24h	Nestin, NF, GFAP
	Sanjia Fumai	SD rats	intra-gastric	4mg/d, 7d	neuron like cells

	Tang[46]		perfusion		
Open the Orifices to Induce Resuscitation	musk polypeptide[47,48]	Fetal rat brain	added in medium	100-150 mg/L, 7d	Nestin, NSE, NF, GFAP
	Gastrodia elata[49]	SD rats	added in medium	100µl gastrodia elata /ml, 24h	NSE, GFAP, Nestin
	Niupo Zhibao Weiwang[50,51]	SD rats	intra-gastric perfusion	4mg/d, 7d	BrdU, NF
Induce Resuscitation by Funblocking Therapy	Rhubarb aglycone[52,53]	SD rats	intra-gastric perfusion	12.96mg/(kg/d), 7d, 14d, 28d	NSE, GFAP, NGF, GDNF, MMP-9, TIMP-1
	Baicalin[54,55]	Umbilical cord blood	added in medium	100µmol/L, 4weeks	NSE, MAP-2, GFAP, CD29, CD34 ⁺ , CD83, CD45
	Berberine[56]	SD rats	added in medium	0.00025 - 2.5 g/L, 8h	NSE, NF, GFAP

TLR4=Toll-like receptor 4; MMP-2=matrix metalloproteinase-2; TNF- α =tumor necrosis factor; MCP-1=monocyte chemotactic protein-1; NSE=neuron specific enolase; GFAP=glial fibrillary acidic protein; Ngn-1=neurogenin-1; NF=neurofilament; GSK-3 β =glycogen synthase kinase-3 β ; NGF mRNA=nerve growth factor mRNA; NFP=neurofilament protein; MAP2=microtubule-associated protein-2; VEGF=vascular endothelial growth factor; NF-M= neurofilament protein; Ach= acetylcholine; ChAT=choline acetyltransferase; Flt-1=FMS-like tyrosine kinase 1; Kdr=kinase insert domain receptor; Glut1= Glucose transporter 1; Tuj1=neuron-specific class III beta-tubulin; GDNF=glialcelline-derived neurotrophic factor; MMP-9= matrix metalloproteinase-9; TIMP-1=tissue inhibitor of metalloproteinase-1.

Tonify Qi and reinforce the healthy Qi

The ancient Chinese sages said: "When there is sufficient health qi inside, the pathogenic qi have no way to invade the healthy body". Tonifying qi herbs can stimulate the healthy qi in patients; sufficient healthy qi provides a guarantee for the transformation of spirit. *Astragaloside IV* (AS-IV) is the representative of qi invigorating drugs, and has been widely used for the treatment of nervous system diseases in China. AS-IV attenuated TLR4 expression through the NF- κ B signaling pathway in MSCs so as to promote the proliferation of MSCs [23]. Further studies showed that MSCs can differentiate into neurocyte-like and gliocyte-like cells *in vitro*. Wnt-1 gene and Ngn-1 gene played important regulatory roles during the differentiation of the rat bone marrow-derived mesenchymal stem cells to neurocyte-like

cells [24]. *Astragalus injection* can induce the differentiation of MSCs into neuron-like cells and the process of differentiation might be mediated by activation of Wnt signaling pathways [25]. Wu et al. [26] demonstrated that *Ginsenoside Rg1* could strengthen the spatial learning memory ability in dementia rats after transplanted with BMSCs. Possibly, its mechanism might be related with the up-regulating mRNA expression of nerve growth factor (NGF) in basal forebrain after BMSCs transplantation. It has been proved that *Shenqi Fuzheng injection* can induce hMSCs to differentiate into neurons *in vivo* in rats with middle cerebral artery occlusion (mcao).

Immunohistochemical staining showed that *Shenqi Fuzheng injection* significantly increased the differentiation of hMSCs to human neuron specific enolase (NSE),

neurofilament(NF) and glial fibrillary acid protein(GFAP) [27].

Tonify Qi and activate blood circulation

“The YuanQi was deficiency, will not reach the blood vessels, eventually lead to the stagnation of blood and the formation of blood stasis.” The method of tonifying qi and activating blood circulation has been commonly used in clinic for treatment of nervous system diseases such as central infarction, cerebral hemorrhage and so on. Recently, scholars have studied Chinese medicine and the extracts in inducing the differentiation of bone marrow MSCs to neurocyte-like cells and acquired some outcomes. Nie et al. [28] observed and evaluated the effect of transdifferentiation of MSCs into nerve cells by ultrafiltration membrane extract mixture from *Angelica sinensis* and *Hedysarum polybotrys*. Results demonstrated that BMSCs changed neural-morphologically after induction. The expression levels of NSE, nestin, NFP, MAP2, GFAP were highest in the positive control group ($P < 0.05$), followed by the ultrafiltration membrane extract mixture group ($P < 0.05$). *Buyang Huanwu Tang* combined with MSCs transplantation could repair the injured blood vessels and lesion tissues, the mechanism study showed that VEGF and Ki-67 expressions were significantly up-regulated in the MSCs group and the combination group, with significant differences as compared with the model group and the sham operation group ($P < 0.05$), and with the most strongest effect in the combination group [29]. *Naomai Yihao Capsule* has the function of tonifying qi, activating blood circulation, and resolving phlegm so as to regulate the "sea of blood in brain". The observation of *Naomai Yihao Capsule* combined with BMSCs transplantation showed that *Naomai Yihao Capsule* could promote the angiogenesis and neurological impairment recovery by increasing the expression of CD31 in the brain tissue in focal cerebral ischemia rats which were administered with BMSCs transplantation, and the effect was reinforced with the extension of treatment time [30]. Zhang et al. [31] induced bone marrow MSCs using *Yiqihuoxue recipe* and found that *Yiqihuoxue recipe* could express NSE, a marker of neurons, GFAP, a marker of glial cells, and nestin, a marker of neural stem cells. These evidences indicate that *Yiqihuoxue recipe* can induce the differentiation of bone marrow MSCs *in vitro*.

Activate blood and resolve stasis

At the acute phase of cerebral injury, the functions of qi and blood are abnormal, blood stasis appear, which will lead to the necrosis or apoptosis of neurons. The regulatory mechanism of proliferation, immigration, and differentiation of neural stem cells would also be damaged. If blood stasis has not been removed, there would no generation of new blood. Experiments have proved that some Chinese herbs are anti-thrombolysis, they play an important role in differentiating MSCs into nerve cells, so as to improve microcirculation in central nervous system that might

improve, repair and rehabilitate from stroke and brain injury [32]. After induction by *Danshensu*, MSCs exhibited the typical form of perikaryon with pyknotic cell body and prominence projected like that of neuron. These cells were positively expressed in NSE, NF-M and nestin, and negatively expressed in GFAP [33]. *Salvianolate* inhibited the proliferation of human umbilical cord mesenchymal stem cells(hUCMSCs) under high concentrations. Cells showed a neuron-like morphology when treated with brain derived neurotrophic factor (BDNF) or salvianolate combined BDNF. The contents of Ach and positive expression rates of Nestin, NES and choline acetyltransferase in high-dose and middle-dose combination groups were significantly higher than those in low-dose combination group and BDNF group ($P < 0.05$). There was no significant difference between the high-dose and middle-dose combination groups ($P > 0.05$) [34]. Extract of *Ginkgo biloba*(EGb761) increased the human adipose-derived stem cells (hADSCs) proliferation, especially on 3 d ($P < 0.05$). EGb761 induced hADSCs to neural differentiation, not to glial cell differentiation [35]. The percentages of NSE-positive neuron-like cells in the different concentrations of *ginkgolide B* were higher than the percentage in the control group. However, there were no significant differences between the different concentrations [36]. Zheng et al. [37] studied the effects of total saponins of *Panax notoginseng* (tPNS) on angiogenesis in rat bone marrow mesenchymal stem cells (rBMSCs). The study showed that tPNS (100 $\mu\text{g/ml}$) significantly enhanced the mRNA expression level of VEGF-A and Kdr compared to the control group, while they had no obvious effect on the expression of Flt-1. tPNS (1 $\mu\text{g/ml}$ and 100 $\mu\text{g/ml}$) significantly increased capillary network forming of rBMSCs after endothelial differentiation in Matrigel *in vitro*. tPNS (50 $\mu\text{g/kg}$, 100 $\mu\text{g/kg}$ and 150 $\mu\text{g/kg}$) also significantly increased angiogenesis induced by the combination with implantation of rBMSCs and Matrigel *in vivo*. *Sodium Ferulate* (SF), as the main active constituent of *Chuanxiong*, combined with BMSCs administration could facilitate BMSCs migration into the ischemic brain by up-regulation of stromal cell-derived factor-1 alpha (SDF-1 α)/chemokine (CXC motif) receptor-4 axis after stroke. The combination treatment of SF and BMSCs could not only promote expression of Glucose transporter 1(Glut1) and Neuron-specific class III beta-tubulin (Tuj1) in the periinfarct area, but also improve BMSCs expression of Glut1, GFAP and Tuj1. Moreover, it showed that combination treatment could enhance the endogenous expression of Tuj-1 in ischemic boundary zone [38]. A novel tissue inducible nerve guide conduit, chitosan microspheres, has better biological compatibility and tissue inducible function. The *ligustrazine* released from the chitosan microspheres could promote MSCs to express NSE and MAP2, the relevant marker molecule of nerve cells [39].

Tonify the kidney to supply essence

The MSCs from bone marrow and NSCs from central nervous system both belong to congenital essence. They can transform into each other. Kidney-tonifying and essence-replenishing method may play an important role to promote MSCs differentiation into NSCs. *Plastrum Testudinis* (PT), as an important CHM to tonify the Kidney, was proved to induce MSCs to differentiate into NSCs *in vitro*, but not into neuron like cells or astrocytes [40]. Other study found that after induced adult rats MSCs 12 h with PT, the positive expression of neuron like cells NF reached the peak [41]. *Rehmannia glutinosa polysaccharide* (RGP) is one of the effective components of CHM *Rehmanniae*, with the effect of tonifying the kidney to supply essence. The detection of immunocytochemical stain and RT-PCR method showed that neural cell markers were not expressed in the control group, but expressed in the other groups. Positive cells rate of nestin and NSE in the RGP induction group was higher than the β -mercaptoethanol(BMT) induction group and the BDNF induction group ($P < 0.05$) and positive cells rate of GFAP lower ($P < 0.05$), but there was no difference between the BMT induction group and the BDNF induction group in nestin, NSE and GFAP positive cells rate. The all cells were Notch 1 protein positive in RGP induction group, which were reduced gradually over time, according to immunocytochemistry. Western blot results showed that the contents of NICD was up-regulated 24 h after RGP induction and decreased gradually, and even fell below the baseline level and significantly lower than control group at 5th day ($P < 0.05$) [42-44]. *Lycium barbarum polysaccharide* also has inductive effect on differentiation of BMSCs into neurons of adult rats *in vitro*. After induction for 4 hours, some BMSCs showed processes extended obviously. Twenty four hours later, the differentiated cells showed significant processes, and the processes were connected to each other, showing typical neural cell morphology. In these cells, the expression of microfilament and nestin was positive, but GFAP was negative [45]. Kuang et al. [46] observed the ability of *Sanjia Fumai Tang* medicated serum in inducing the differentiation of MSCs into neurons *in vitro* in adult rats. The results found that with the extension of the induction time, the cell morphology of MSCs changed obviously, the cell size decreased, the cytoplasm contracted to the nucleus, the morphology changed into circular, forming a network like structure and neuron like cells increased. But the same change did not be observed in the control group. After 12 hours' induction, neuron cell's positive rate reached its peak, and there were still neuron cells survived after 7 days, and the longest survival time was the *Sanjia Fumai Tang* group.

Open the orifices to induce resuscitation

The method of opening the orifices to induce resuscitation for the treatment of cerebral infarction has a long history, and the curative effect is exact. Both the method and activated NSCs which are usually in a resting state have

particular but similar effect on promoting the nerve regeneration. Xiao et al. [47,48] directly induced 5-10 generation MSCs with culture medium containing *musk polypeptide in vitro*, the results showed that the cells changed into neuron like cells, immunohistochemical sample also showed that the neuronal cell NSE and NF induced, nest protein expressed positively, GFAP expressed negatively. Neuron like cell count analysis found that the percentage of NSE and NF-H positive cells were higher after induced by *musk polypeptide*. As inducers, *Gastrodia elata* similarly could induce most of MSCs to differentiate into neuron-like cells, revealing cytodendrite. By immunochemical staining, cells showed positive NSE, nestin, and negative of GFAP [49]. *Niupo Zhibao Weiwan*(NZW) was varied from *Zhibaodan*. The study found that NZW medicated serum could enhance the expression of Brdu and NF in MSCs transplantation region, and the enhancement effect can last 6 weeks [50,51].

Induce resuscitation by Fu-unblocking therapy

The method of inducing resuscitation by Fu-unblocking therapy can not only modulate the function of stomach, spleen, lung, liver and other viscera, but also improve permeability of the blood-brain barrier, relieve cerebral edema, and promote nerve regeneration. *Rhubarb aglycone* can decrease the degradation of basal lamina Col IV and the permeability of brain micrangium in cerebral ischemic rats with BMSCs transplantation by means of regulating the balance of matrix metalloproteinase-9 (MMP-9), increasing the expression of tissue inhibitor of metalloproteinase-1 (TIMP-1) [52]. *Rhubarb aglycone* also can advance the time of protecting neurocytes after BMSCs transplantation. The mechanism may be related to the fact that it can up-regulate the expressions of NGF and glial cell-line derived neurotrophic factor (GDNF) in earlier phase and increase NGF expression in metaphase and anaphase [53]. 100 $\mu\text{mol/L}$ *baicalin* can promote amplification of cord blood MSCs *in vitro*. After culture cultured for 4 weeks, the expression of neuron specific enolase and microtubule associated protein 2 were lower in the blank control group and β -mercaptoethanol group compared to the *baicalin* group ($P < 0.01$), and no significant difference was found in the co-culture group ($P > 0.05$). All these indicated that *baicalin* also can induce the differentiation of cord blood MSCs into neuron-like cells [54,55]. *Berberine* was also approved with the same effect of inducing adult rat MSCs to differentiate into neuron-like cells *in vitro* [56].

Views and Prospects

At present, researchers have carried out many theoretical discussions and experimental studies in inducing the differentiation of mesenchymal stem cells into neural-like cells via the use of TCM thoughts from different angles, such as the composition of CHM monomer, single CHM and effective component, and have achieved encouraging results. However, in the process of these current studies, the

focus was usually partial to some effective components of the composition, single herb or the compound, which led to the neglect of the concept of holism of TCM theory. So the theory of medicinal properties and pharmacodynamics of CHM should be combined with the basic theories of TCM. Only by means of getting research ideas from the viewpoint of the holistic concept of TCM theory and combining with modern research methods, could the development of modernization of TCM be better.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

AUTHOR CONTRIBUTIONS

Lijun Qiao drafted the manuscript. Lijun Qiao, Aili Lu, Mei Feng, Caiwen Qian and Lingbo Hou retrieved the literature together. Jun Zhang, Tongxiang Lin, Yuanqi Zhao reviewed this manuscript. All authors approved the final version of this paper.

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