

Bone marrow mesenchymal stem cell therapy in ischemic stroke: mechanisms of action and treatment optimization strategies

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Abstract

Animal and clinical studies have confirmed the therapeutic effect of bone marrow mesenchymal stem cells on cerebral ischemia, but their mechanisms of action remain poorly understood. Here, we summarize the transplantation approaches, directional migration, differentiation, replacement, neural circuit reconstruction, angiogenesis, neurotrophic factor secretion, apoptosis, immunomodulation, multiple mechanisms of action, and optimization strategies for bone marrow mesenchymal stem cells in the treatment of ischemic stroke. We also explore the safety of bone marrow mesenchymal stem cell transplantation and conclude that bone marrow mesenchymal stem cell transplantation is an important direction for future treatment of cerebral ischemia. Determining the optimal timing and dose for the transplantation are important directions for future research.

Key Words: nerve regeneration; ischemia/reperfusion injury; animal model; mechanisms of action; clinical application; research progress; genetic modification; angiogenesis; replacement therapy; neural regeneration

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Introduction

Ischemic stroke is currently one of the main causes of death in adults worldwide, with an especially high incidence and ischemic stroke-related disability rate in the elderly (Donnan et al., 2008; Johnston et al., 2009). Because of the short therapeutic time window in ischemic stroke, many patients with ischemic stroke may suffer from severe long-term disability, even if they receive timely interventional and thrombolytic therapies (Pandya et al., 2011). However, nerve regeneration appears several days or weeks after ischemic stroke, offering potential for a second therapeutic time window (Gopurappilly et al., 2011). New treatment approaches to alleviate disability after stroke may be considered during this second window, including stem cell transplantation.

Bone marrow mesenchymal stem cells (BMSCs) have self-renewal potential. These cells express markers for mesenchymal or endothelial cells (CD105, CD73, and CD90) as well as adhesion molecules (CD106, CD166 and CD29) (Javazon et al., 2004; Dominici et al., 2006), but do not express hematopoietic stem cell markers (CD11, CD14, CD34, CD45, CD79, CD19 and HLA-DR). BMSCs can differentiate not only into mesodermal cells but also into endodermal and ectodermal cells (Sanchez-Ramos et al., 2000; Phinney and Prockop, 2007; Uccelli et al., 2008). Sufficient evidence has shown that BMSCs affect the pathological processes

underlying ischemic stroke through multiple mechanisms of action, including inducing angiogenesis, secreting neurotrophic factor, inhibiting apoptosis, and modulating the immune system (Li et al., 2008; Tate et al., 2010; Liu et al., 2012; Jellema et al., 2013; Zhao et al., 2013; Mitkari et al., 2014). Thus, BMSCs have great potential in the treatment of stroke (Li et al., 2008; Tate et al., 2010; Liu et al., 2012; Jellema et al., 2013; Zhao et al., 2013; Mitkari et al., 2014). In addition, BMSCs are generally derived from autologous tissue, precluding ethical controversy. BMSCs are easily cultured *in vitro*, have weak immunogenicity and good safety, and have been considered ideal seed cells in the treatment of ischemic stroke (Guo et al., 2013; Ishizaka et al., 2013; Kawabori et al., 2013; Hess et al., 2014; Ha et al., 2015).

Considering the potential multiple mechanisms of action of BMSCs following transplantation, we sought to analyze the various transplantation approaches, differences in mechanisms of action, and effectiveness and safety of BMSCs in ischemic stroke therapy.

BMSC Transplantation Approaches

BMSC transplantation is conducted mainly using intracranial and intravascular deliveries (Guzman et al., 2008). The intracranial technique refers to stereotactic injection. Following direct injection into the corpus striatum, more BMSCs

are able to reach the targeted brain damage and the number of cells used is small and the onset time is short (Jin et al., 2005). Another intracranial delivery method is intraventricular injection. Its success depends on the migration of transplanted cells and their abilities to adapt to the cerebrospinal fluid and survive transiting the blood-brain barrier (Wang et al., 2013). Because intracranial transplantation is invasive, multiple injections in the infarct zone cause mechanical damage of local tissue and cells (Walczak et al., 2008).

Intravascular techniques include intravenous and intra-arterial approaches. Compared with intracranial delivery, intravascular injection more widely distributes transplanted cells through blood vessels and may be better for large-area brain damage (Bliss et al., 2010). A number of inflammatory cytokines are released after tissue damage (Tuttolomondo et al., 2008; Ahmadian Kia et al., 2011). BMSCs express a variety of chemokine receptors (Ponte et al., 2007) and are attracted to the area of injury or inflammation. After cerebral ischemia, intravenously transplanted BMSCs are targeted to the center of the ischemic area and peri-infarct zone. However, because of the large volume of BMSCs (Lee et al., 2009), most BMSCs may be captured in the pulmonary vascular system after intravenous infusion. Detante et al. (2009) verified that infused ^{99m}Tc -HMPAO-labeled BMSCs were transiently trapped in the rat lung in the first 2 hours after stroke before reaching the ischemic area. Thus, compared with intracranial injection, intravenous injection is relatively simple and less invasive (Wu et al., 2008), but the number of cells reaching the ischemic tissue is low. Intra-arterial transplantation can obtain better effects than intravenous transplantation. Intra-arterial transplantation diminishes the number of cells trapped in other tissues and delivers cells to the injury site in a short period. Injection through the internal carotid artery is a simple and effective way to transplant cells, as cells can distribute in the brain tissue where blood is supplied by the middle cerebral artery (Guo et al., 2013). Jiang et al. (2013) demonstrated that BMSCs reach the arterial end close to the injury site following intra-arterial transplantation, indicating that intra-arterial transplantation is a safe, feasible method for promoting the recovery of neurological function in patients with stroke.

Although the intra-arterial and intracranial approaches reduce the problems associated with delivery of BMSCs, the two methods are invasive. The safety or effectiveness of BMSC transplantation in stroke therapy should be determined. The optimal time window of transplantation is unclear, and it may be that sooner is better. In addition, the optimal injection dose will require further study (Keimpema et al., 2009; Komatsu et al., 2010; Ishizaka et al., 2013; Kawabori et al., 2013).

Mechanisms of Action for BMSCs

BMSCs participate in the treatment of cerebral ischemia through multiple mechanisms, including cell migration, angiogenesis, apoptosis inhibition, neurotrophic factor secretion, neural circuit reconstruction, and immunomodulation (Figure 1).

Directional migration of BMSCs

In vivo microscopy or autoradiography has revealed that

transplanted BMSCs mainly gather in the ischemic penumbra and the subventricular zone (Yilmaz et al., 2011; Park et al., 2014). Microglia and astrocytes in the infarct zone secrete stromal cell-derived factor 1 (SDF-1). BMSCs express chemokine receptor 4 (CXCR-4), the physiological receptor for SDF-1. The interaction of SDF-1 and CXCR-4 may cause BMSC migration into the infarct zone (Wang et al., 2008, 2012; Yu et al., 2012). A lack of CXCR-4 or SDF-1 α will significantly reduce the targeted migration of BMSCs (Shyu et al., 2008; Sun et al., 2009). Wang et al. (2014) determined that the synergistic effect of CXCR-4 and CXCR-7 expressed in BMSCs promotes BMSC migration, and concluded that the effect of CXCR-7 is better than that of CXCR-4. Zhang et al. (2015) confirmed that the chemotactic factor CX3CL1/fractalkine activates the Jak2-Stat5 α -ERK1/2 signaling pathway through CX3CR1, triggers integrin-dependent restructuring, and urges BMSC migration toward the ischemic tissue. These findings suggest that BMSC migration is the result of interactions among multiple factors. It remains poorly understood how BMSCs traverse the blood-brain barrier.

BMSC differentiation, replacement, and neural circuit reconstruction

In vitro study results have demonstrated that BMSCs can differentiate into neurons, glial cells, and endothelial cells (Woodbury et al., 2000; Phinney and Prockop, 2007). The markers for neurons and glial cells can be identified in the central nervous system (CNS) of animal models of ischemic stroke following BMSC transplantation (Eglitis et al., 1999; Li et al., 2000; Chen et al., 2001; Zhao et al., 2002; Skvortsova et al., 2008; Jiang et al., 2014). However, mesenchymal stem cells (MSCs) do not express the voltage-gated ion channels that are expressed in functional nerve cells (Hofstetter et al., 2002). The improvement in the behaviors of animals modeling ischemic stroke is likely based on the plasticity of nervous system as well as on activation and migration of endogenous neural stem cells (Ding et al., 2007; Song et al., 2013). Therefore, the possibility of MSCs directly differentiating into cells that replace the injured CNS cells after stroke is very small, and there is still a lack of definite evidence.

BMSCs enhance axonal plasticity and reconstruct neural circuits, which may be the basis for the recovery of neurological function after ischemic stroke (van Velthoven et al., 2012). After intravenous infusion of BMSCs, the numbers of axons and myelin sheaths increase in the rat corpus striatum, hippocampus, and corpus callosum. Axons in the ischemic zone grow along the extending direction of reactive astrocytes (Li et al., 2006; Shen et al., 2006; Liu et al., 2010; van Velthoven et al., 2012). BMSCs restore the connections of different brain regions through axonal sprouting, noticeably enhancing the survival of the motor cortex in the peri-infarct zone and contributing to functional recovery after stroke (Liu et al., 2010; van Velthoven et al., 2012; Song et al., 2013). BMSC transplantation repairs the neural network and reconstructs neural connections, and the recovery of the neural circuit may contribute to enhanced sensorimotor functions (Song et al., 2013). Nevertheless, the molecular mechanism of BMSC-induced synaptic plasticity remains unclear.

BMSCs enhance angiogenesis

Angiogenesis in the infarct and peri-infarct zones plays an important role in mediating neuronal survival and regeneration. BMSC transplantation enhances angiogenesis in the ischemic zone, increasing the number of new microvessels (Chen et al., 2003b) and ameliorating neurovascular injuries. BMSCs can also secrete vascular endothelial growth factor, basic fibroblast growth factor and placental growth factor (Wakabayashi et al., 2010; Vogelgesang and Dressel, 2011; Chuang et al., 2012). Liu et al. (2014) considered that mitochondrial transport through tunneling nanotubes may be the key mechanism used by BMSCs to protect mitochondrial function and promote angiogenesis. In addition to secreting bioactive molecules and promoting angiogenesis, BMSCs support the crosslinking of peripheral cells, astrocytes, and endothelial cells, maintain the integrity of the blood-brain barrier (Fisher, 2009), form a microenvironment supporting neurogenesis, and promote the recovery of neurological function (Honmou et al., 2012). Mitkari et al. (2014) verified that intra-arterial infusion of human BMSCs (hBMSCs) enhances microvascular regeneration in the infarct zone, but does not improve the behavioral ability of rats. BMSC transplantation can promote angiogenesis in the infarct area, thereby providing favorable conditions for nerve regeneration.

BMSCs facilitate neurotrophic factor secretion from neurons

In vitro test results show that BMSCs secrete 11 kinds of neurotrophic factors after coculture with cortical neurons under hypoxic conditions (Tate et al., 2010). To determine the effects of BMSC secretion on neurotrophic factors, rat BMSCs were cultured with complete medium in animal models of stroke; the complete medium enhanced connections between nerve cells and promoted functional recovery after stroke (Tsai et al., 2014). BMSCs play an active nutritional support role in the early stage of transplantation in rats with cerebral ischemia (Loseva et al., 2011). BMSCs also induce parenchymal cells in the CNS to secrete nerve growth factor, brain-derived neurotrophic factor (BDNF), glial cell line-derived neurotrophic factor (GDNF), epidermal growth factor, basic fibroblast growth factor, insulin-like growth factor 1, hepatocyte growth factor, and stem cell factor (Wakabayashi et al., 2010; Lin et al., 2011; Zhang et al., 2011; Ishizaka et al., 2013; Kaengkan et al., 2013; Song et al., 2013). These bioactive factors synergistically promote functional recovery after stroke. BMSCs positively regulate bone morphogenetic protein 2/4, and promote synaptic vesicle protein expression (Zhang et al., 2006). These factors accelerate the differentiation of astrocytes in the ischemic zone, elevate connexin 43 expression, promote small molecule exchange in the brain, and enhance synaptic efficacy (Xin et al., 2006). Bioactive molecules directly or indirectly produced by BMSCs accelerate neurogenesis, elevate white matter integrity, and induce synaptogenesis.

BMSCs suppress apoptosis

BMSC transplantation effectively inhibits apoptosis in the ischemic penumbra. Chen et al. (2003a) found that apopto-

sis is reduced and basic fibroblast growth factor expression is increased in rat models of stroke following BrdU-BMSC transplantation. The apoptotic response in astrocytes is reduced after BMSC transplantation (Leu et al., 2010; Darsalia et al., 2012; Jiang et al., 2014). A few apoptotic cells and many regulatory T lymphocytes are detected during intravenous infusion of hBMSCs (Li et al., 2002). MSCs diminish caspase-3 activity, reduce the Bax/Bcl-2 ratio (Leu et al., 2010; Li et al., 2012), decrease interleukin (IL)-1 β , IL-6, and tumor necrosis factor- α levels (Zhu et al., 2014), suppress apoptosis, and accelerate the proliferation of endogenous neural stem cells and glial cells (Mora-Lee et al., 2012) by activating an Akt-dependent anti-apoptotic cascade (Scheibe et al., 2012).

Immunomodulatory effects of BMSCs

BMSCs produce immunomodulatory effects, simultaneously weakening the innate and adaptive immune responses and mitigating the injury to the CNS. Ischemic stroke leads to a strong inflammatory response, resulting in leukocyte recruitment to the infarct zone (Iadecola and Anrather, 2011). *In vitro* test results have demonstrated that leukocyte proliferation is reduced and differentiation becomes abnormal after coculture with BMSCs (Bartholomew et al., 2002; Sato et al., 2007). The transforming growth factor beta secreted by MSCs diminishes monocyte chemoattractant protein-1 levels in the ischemic zone, reduces the number of circulating CD68⁺ immune cells in the infarct zone by traversing the damaged blood-brain barrier, and suppresses immune responses in the ischemic zone (Yoo et al., 2013). In addition, MSCs diminish IL-23/IL-17 expression (Ma et al., 2013), decrease IL-1 β , IL-6, and tumor necrosis factor- α levels (Zhu et al., 2014), and suppress the immune response by reducing STAT3 expression and phosphorylation in microglia (McGuckin et al., 2013). Liu et al. (2009) have confirmed that BMSCs increase IL-10 levels and decrease tumor necrosis factor- α expression to inhibit ischemic injury. Transplanted BMSCs inhibit T-cell proliferation, promote Treg cell expression, and nonspecifically suppress the production of CD4⁺ and CD8⁺ T cells (Di Nicola et al., 2002; Meisel et al., 2004; Aggarwal and Pittenger, 2005; Nasef et al., 2007). Moreover, MSCs can suppress the inflammatory reaction by down-regulating macrophages, B cells, natural killer cells, and antigen-presenting cells (Beyth et al., 2005; Corcione et al., 2006; Krampera et al., 2006; Maggini et al., 2010; Marigo and Dazzi, 2011; Ribeiro et al., 2011). Although intravenously infused MSCs are captured in the lung, and intra-arterially infused MSCs gather in the spleen, MSCs still have immunomodulatory effects on the brain (Li and Chopp, 2009; Ankrum and Karp, 2010; Oh et al., 2010). These results indicate that transplanted MSCs have a long-term effect on immune function, but the immunomodulatory mechanisms remain poorly understood.

Modification of BMSCs

Genetic modification uses a variety of biotechnology and bioengineering tools and techniques to modify the genetic makeup of organisms. BMSCs may be used as genetic carriers, combining cell therapy and gene therapy by introducing target genes. Genetic modification has a unique advantage in

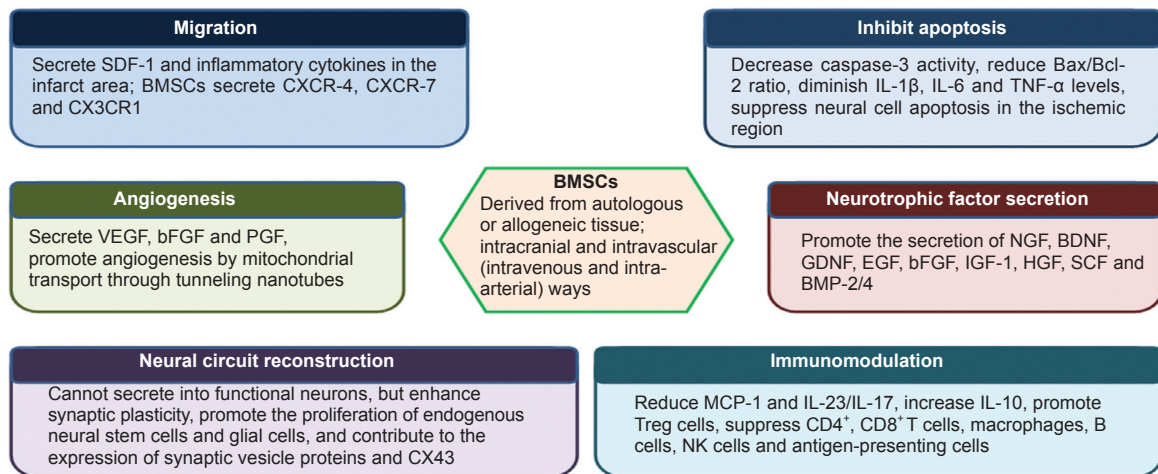


Figure 1 Mechanisms for the therapeutic effects of BMSC transplantation in cerebral ischemia.

Mechanisms of action are as follows: (1) BMSCs migrate to and survive in the ischemic hemisphere, creating a microenvironment conducive to cell survival and regeneration for the repair of injured nerve tissue. (2) BMSC transplantation lessens the apoptosis of neurons and glial cells in the infarct zone by immunomodulation. (3) BMSC transplantation contributes to the release of cytokines and neurotrophic factors, and provides nutritional support for neurons. (4) BMSC transplantation induces angiogenesis, improves cerebral blood circulation, and promotes nerve tissue repair. (5) BMSC transplantation likely stimulates axonal sprouting and myelin remodeling and promotes endogenous neurogenesis. SDF-1: stromal cell-derived factor 1; CXCR-4,7,1: chemokine receptor 4,7,1; CX-43: connexin 43; VEGF: vascular endothelial growth factor; bFGF: basic fibroblast growth factor; PGF: placental growth factor; NGF: nerve growth factor; BDNF: brain-derived neurotrophic factor; GDNF: glial cell line-derived neurotrophic factor; EGF: epidermal growth factor; IGF-1: insulin-like growth factor 1; HGF: hepatocyte growth factor; SCF: stem cell factor; BMP-2/4: bone morphogenetic protein 2/4; IL-1 β , 6, 10: interleukin-1 β , 6, 10; TNF- α : tumor necrosis factor- α .

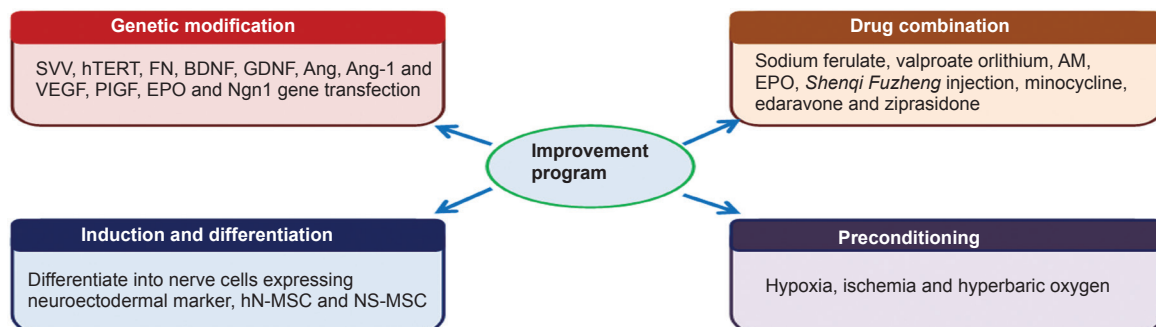


Figure 2 Modification of BMSCs.

BMSCs used as genetic carriers to introduce target genes have many advantages in promoting migration and survival, enhancing neurotrophic factor expression, accelerating angiogenesis in the ischemic zone, and resisting inflammation and apoptosis. The combination of BMSC transplantation and drug therapy is a simple, highly efficient, and feasible treatment option. The clear mechanism of action for the drug and the various mechanisms of action of BMSCs allow for synergistic therapeutic effects through changing the microenvironment of the ischemic zone and provide other beneficial modifications. Inducing BMSCs to differentiate into neural cells or dedifferentiate into primitive stem cells improves the efficiency of BMSCs in the treatment of stroke. Preconditioning, such as through hypoxia, enhances the survival of BMSCs. SVV: Survivin; hTERT: human telomerase reverse transcriptase; FN: fibronectin; Ang: angiogenin; VEGF: vascular endothelial growth factor; PIGF: placental growth factor; EPO: erythropoietin; Ngn1: neurogenin-1; AM: adrenomedullin; hN-MSC: neuronal cells differentiated from BMSCs; NS-MSC: neurosphere-like cell aggregates differentiated from BMSCs.

the transfection of an exogenous gene and can directionally increase the expression of beneficial biological factors. After transfection, BMSCs still retain their original therapeutic effects, including promoting migration and survival, increasing neurotrophic factor expression, accelerating angiogenesis in the ischemic zone, and resisting inflammation and apoptosis. Thus, BMSCs transfected with an exogenous gene offer a broad prospect in the treatment of cerebral infarction. In the clinic, fine manipulation for gene transfection and screening is necessary, which may delay the treatment process. By contrast, combining BMSCs and drug treatment is a simple, highly efficient, and feasible treatment method. Such

combinations generate not only the cumulative effect of dual therapy but also synergistic effects, and effectively promote the recovery of neurological function. However, this method needs suitable drug collocation, and the time window for administration warrants further investigation. Inducing BMSCs to differentiate into neural cells or dedifferentiate into primitive stem cells exerts the efficiency of BMSCs in the treatment of stroke. Preconditioning, such as hypoxia, enhances the ability of BMSCs to survive. In addition, preconditioning allows for a greater therapeutic effect by increasing cell number and the expression of some biological factors. Thus, preconditioning increases therapeutic efficacy

by changing the physical environment (Figure 2).

Genetic modification

Gene transfection-induced MSC differentiation into neural cells or gene transfection-induced endogenous neural stem cell proliferation and differentiation to reconstruct neural pathways increase the therapeutic value of BMSCs. *In vitro* and *in vivo* test results demonstrate that more survivin-modified BMSCs survive, infarct size becomes small after BMSC transplantation, and neurological function after stroke is noticeably restored (Liu et al., 2011a). After transplantation with human telomerase reverse transcriptase-MSCs, cell survival increases, infarct size is reduced, and behavioral function recovers (Honma et al., 2006). Fibronectin-modified MSCs increase therapeutic value by increasing stem cell survival and paracrine secretion of pro-survival or anti-inflammatory molecules (Garbayo et al., 2011).

BDNF preconditioning mitigates brain injury after focal cerebral ischemia. High BDNF expression in the ischemic zone may be able to achieve a better therapeutic effect. After BDNF-BMSC transplantation, more neuron-like cells are detected, fewer terminal deoxynucleotidyl transferase (TdT) dUTP nick-end labeling (TUNEL)-positive cells are evident in the peri-infarct zone, BDNF expression increases in the infarct zone, infarct size reduces, and neurological function is noticeably restored (Kurozumi et al., 2004; Hamada et al., 2005; Nomura et al., 2005; Huang et al., 2008). Three hours after middle cerebral artery occlusion (MCAO), rats were infused with GDNF-transfected hBMSCs. Their MRI and behavioral score results revealed a strong therapeutic effect of GDNF-hBMSCs (Horita et al., 2006). After intravenous infusion of BDNF-BMSCs and glial cell derived neurotrophic factor (GDNF)-BMSCs, ischemic injury was lessened and neurological function was significantly recovered in rats previously subjected to MCAO; however, the infusion of ciliary neurotrophic factor-overexpressing BMSCs or neurotrophin-3-BMSCs did not provide these positive effects (Kurozumi et al., 2005).

In rats undergoing permanent MCAO, intravenous administration of angiopoietin-1 gene-modified hMSCs improved angiogenesis at the lesion border and regional cerebral blood flow, reduced lesion volume, and improved functional recovery (Onda et al., 2008). Rats receiving angiopoietin-1-vascular endothelial growth factor-hMSCs presented the excellent structural-functional recovery (Toyama et al., 2009). Placental growth factor-hMSCs reduce lesion volume, induce angiogenesis, and elicit functional improvement (Liu et al., 2006). Transduction of the erythropoietin gene into MSCs induces secretion of various trophic factors, decreases infarct volume, and improves the recovery of neurological function (Cho et al., 2010).

Neurogenin-1-expressing MSCs express neuron-specific proteins, including NeuroD and voltage-gated Ca^{2+} and Na^{+} channels, neurofilament 200, microtubule-associated protein, and vesicular glutamate transporter 2. Moreover, neurogenin-1-MSCs functionally connect to host neurons and markedly ameliorate motor dysfunctions (Kim et al., 2008). A large fraction of the transplanted fibronectin-MSCs express β III-tubulin and promote neuronal differentiation (Garbayo et al., 2011). Flk-1⁺ hBMSCs enhance the prolif-

eration of neural stem cells or neural progenitor cells in the subventricular zone and hippocampus; many neural stem/progenitor cells migrated into the ischemic zone and differentiated into neural and glial cells, promoting the recovery of neurological function (Bao et al., 2011). BDNF-BMSCs induce cell proliferation in the regional ischemic zone, reduce infarct size, and markedly improve motor function. Epidermal growth factor-like domain 7-modified BMSCs improve motor function, but do not affect infarct size. Polysaccharopeptide-modified MSCs do not show therapeutic outcomes. Sonic hedgehog-modified BMSCs have a negative effect on functional recovery (van Velthoven et al., 2014). Taken together, these findings suggest that not all bioactive factor gene transfections achieve the desired results.

Drug combination

BMSC therapy combined with drug treatment for ischemic stroke is potentially a feasible and efficient therapeutic approach. Drugs and BMSCs exert synergistic effects through different paths, including accelerating stem cell migration and survival, resisting apoptosis, and promoting endogenous stem cell proliferation, neurotrophic factor secretion, and angiogenesis. Thus, their combination effectively contributes to the recovery of neurological function.

Sodium ferulate combined with BMSCs accelerates BMSC migration toward the ischemic zone in a rat model of MCAO by upregulating SDF-1 α and CXCR-4, promotes glucose metabolism by increasing glucose transporter 1 expression in the peri-infarct zone and BMSCs, and markedly reduces infarct size (Zhao et al., 2013). Valproate- or lithium-pretreated BMSCs enhance cell migration and targeting ability, and promote functional recovery; the mechanism is likely associated with valproate-induced CXCR-4 overexpression and lithium-induced matrix metalloproteinase-9 upregulation (Tsai et al., 2011). Adrenomedullin plus MSCs inhibits MSC apoptosis, induces angiogenesis, and improves neurological function (Hanabusa et al., 2005).

Cellular proliferation and neurogenesis were increased along the lateral ventricle wall, and neurological function was recovered after the combined administration of erythropoietin and MSCs, indicating that erythropoietin acts synergistically with MSCs to potentiate neurogenesis (Esneault et al., 2008). Chinese medicine administered with MSCs induces stem cells to differentiate into neuron-like cells, promotes angiogenesis, and accelerates the expression of neuron-specific enolase, neurofilament, and GFAP (Yao et al., 2005; Guan and Zhao, 2011). Treatment with minocycline combined with BMSCs increased the number of GFAP- and NeuN-positive cells (Bilen et al., 2013) and improved neurogenesis and functional recovery by accelerating the activation and proliferation of endogenous neural stem cells.

BMSCs and edaravone administration improved cerebral ischemia by reducing matrix metalloproteinase activation in a rat model of transient MCAO induced by tissue-type plasminogen activator (Tian et al., 2013). Their combination may indeed provide improved neurological function, but these results need further investigation. The combined administration of ziprasidone and neural progenitor cells reduces

the number of TUNEL-positive cells in the ischemic zone so as to enhance the anti-apoptotic effect. Their combination diminishes microglial aggregation in the ischemic zone, increases the number of neural progenitor cells, induces the expression of endogenous neurotrophic factor, such as BDNF, nerve growth factor and GDNF, and promotes the recovery of neurological function (Kaengkan et al., 2013). Thus, the combination of ziprasidone and BMSCs will likely resist apoptosis as well as contribute to stem cell survival and endogenous neurotrophic factor secretion.

Induction and differentiation

MSC differentiation into nerve cells or dedifferentiation into primitive stem cells may generate a better therapeutic effect than undifferentiated MSCs on ischemic stroke by improving cell survival, neurotrophic factor secretion, and neurogenesis. *In vitro* and *in vivo* test results demonstrate that dedifferentiated BMSCs have a high survival rate and great potential to differentiate into nerve cells (Liu et al., 2011b). Their increase in bcl-2 protein and microRNA-34a expression indicates good potential therapeutic effects on cerebral ischemia (Liu et al., 2011b). Human trabecular bone-derived MSCs were transfected with the notch intracellular domain to induce their differentiation into neuronal cells, which were then stereotactically transplanted into the local ischemic hemisphere of gerbils (Xu et al., 2010). The transplanted cells were distributed around the peri-infarct region 28 days later. The cell survival rate was high, with many cells positive for microtubule-associated protein 2, and the recovery of neurological function was good (Xu et al., 2010). MSCs coated with highly hydrophobic diphenylamino-s-triazine-bridged p-phenylene were efficiently converted into neurosphere-like cellular aggregates (Heo et al., 2013). The spherical cells were subsequently induced to differentiate into neural cells expressing neuroectodermal markers (Heo et al., 2013). These cells were intra-cerebrally administered to rats 48 hours after permanent MCAO (Heo et al., 2013). The results showed a marked attenuation of ischemic damage with significant functional recovery, and the effects were better than those of BMSCs alone (Heo et al., 2013).

Preconditioning

Hypoxia preconditioning improves BMSC survival, migration, and targeted migration. After intranasal delivery of BMSCs treated with hypoxia preconditioning in a mouse focal cerebral ischemia model, the expression of CXCR4, matrix metalloproteinase 2, and matrix metalloproteinase 9 increases, cell death decreases, infarct volume in the peri-infarct region is reduced, and neurological function is recovered (Wei et al., 2013). After preconditioned and non-preconditioned MSCs are exposed to 6 hours of lethal anoxia, the number of preconditioned cells is greater than that of non-preconditioned cells (Kim et al., 2012). Ischemia preconditioning induces activation of Akt/hypoxia-inducible factor-1 α (Kim et al., 2012). Both miR-107 and miR-210 participate independently *via* their respective putative target genes Pcdcl10 and Casp8ap2 (Kim et al., 2012). Lin et al. (2013) determined that hypoxia preconditioning upregulates hypoxia-inducible factor-1 α -activated Epac1 expres-

sion through Epac1-to-matrix metalloprotease signaling. Cell transplantation improves cerebral blood flow into the ischemic brain *via* induction of angiogenesis, which leads to recovery from stroke (Lin et al., 2013).

A significant reduction in T cells and MSCs and a significant increase in CD34⁺ and natural killer cells have been identified in poststroke Bone marrow-derived mononuclear cells (MNCs) compared with prestroke MNCs (Yang et al., 2012). Moreover, the concentrations of IL-10, IL-6, monocyte chemoattractant protein-1, vascular endothelial growth factor, and tumor necrosis factor- α are significantly increased in poststroke compared with prestroke MNCs (Yang et al., 2012). Poststroke MNCs in comparison with prestroke MNCs lead to greater recovery of neurological function and reduced lesion size (Yang et al., 2012). Therefore, the therapeutic effect of BMSCs from ischemic rats is likely higher than that of normal rats. Further comparative tests should be conducted to confirm this assertion, which is consistent with clinical study of autologous transplantation.

Hyperbaric oxygen promotes the proliferation and activation of BMSCs (Thom et al., 2006). Mobilization of BMSCs to an ischemic area is improved in long-term hyperbaric oxygen treatments, suggesting that the duration of therapy is crucial for promoting the homing of BMSCs to the ischemic brain by hyperbaric oxygen therapies (Lee et al., 2013). Hyperbaric oxygen also stimulates trophic factor expression and improves gliosis and neurogenesis (Lee et al., 2013).

In conclusion, hypoxia preconditioning of BMSCs, transplantation of BMSCs from ischemic rats, or hyperbaric oxygen preconditioning after transplantation enhances BMSC migration and survival, promotes angiogenesis, and effectively improves neurological function.

Effectiveness and safety of clinical trials

In a study of BMSC transplantation for ischemic stroke (Bang et al., 2005), ischemic stroke patients were randomly divided into experimental (BMSC transplantation) and control groups (no treatment). The study found that BMSCs markedly increased the modified Rankin score and Barthel index (Bang et al., 2005). No adverse effects, such as venous thromboembolism, abnormal cell proliferation, systemic cancer, systemic infection, or neurological decline, were identified after MSC transplantation (Bernardo et al., 2007; Bhasin et al., 2011; Hess et al., 2014). These findings provide support for the safety and poststroke function improvement of BMSC transplantation in ischemic stroke. Other studies have also been conducted, including clinical trials examining the safety and effectiveness of autologous and allogeneic BMSC transplantation, a method to shorten the cycle of BMSCs cultured *in vitro*, the optimum time after stroke for infusing BMSCs, the therapeutic effects of various doses, and protocols aimed at additional improvements (Keimpema et al., 2009; Komatsu et al., 2010; Ishizaka et al., 2013; Kawabori et al., 2013).

Summary

BMSCs migrate and survive in the ischemic hemisphere, creating a microenvironment conducive to survival and regeneration for the repair of injured nerve tissue. The

BMSC-induced anti-inflammatory response mitigates nerve edema. Immune modulation also relieves the apoptosis of nerve cells and glial cells in the infarct zone. BMSCs promote the release of cytokines and neurotrophic factors and provide nutritional support for the injured neurons in the ischemic penumbra. BMSCs induce angiogenesis, improve blood circulation in the brain, and contribute to nerve tissue repair. BMSCs may also stimulate axonal sprouting and myelin remodeling and promote endogenous neurogenesis. Although many achievements have been made in determining the therapeutic mechanisms of BMSCs, these mechanisms have not been fully clarified. In particular, additional research will be required to determine the molecular biological mechanisms of neural plasticity and angiogenesis.

BMSCs have been used as genetic carriers to combine cell therapy and gene therapy. The combination of BMSCs and drug treatment is a simple, highly efficient, and feasible treatment option that provides cumulative as well as synergistic effects. Inducing BMSCs to differentiate into neural cells or to dedifferentiate into primitive stem cells elicits the efficiency of BMSCs in the treatment of stroke. Preconditioning BMSCs, such as under hypoxic conditions, enhances the ability of BMSCs to survive. Preconditioning offers great therapeutic effects by increasing cell number and the expression of some biological factors. The evidence for the application of various optimized methods includes the cognition of the mechanism following cerebral ischemia/reperfusion injury and stem cell therapy in cerebral ischemia. Different optimizations amplify the role of BMSCs in various biological pathways and promote the therapeutic efficacy of BMSCs in stroke. Thus, the therapeutic advantages of transplanted BMSCs become more prominent. Future investigations should focus on genetic modifications and drug combinations as well as on optimal timing and doses for BMSC transplantation. Additionally, clinical trials are needed to determine the effectiveness and safety of genetically modified BMSCs. Overall, BMSC transplantation is an important direction for future treatment of ischemic stroke.

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