Bone marrow stromal cell transplantation for ischemic stroke – its multi-functional feature

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In this article, the author reviews recent advancements of basic research on bone marrow stromal cell (BMSC) transplantation for ischemic stroke. The BMSCs are easily isolated from the patients themselves and transplanted into them without any ethical and immunological problem. Animal experiments have shown that BMSC transplantation significantly enhance the recovery of motor and/or cognitive function in various types of neurological disorders such as ischemic stroke. The transplanted BMSCs aggressively migrate toward the damaged tissue and proliferate in the host brain. The BMSCs significantly improve the neuronal receptor function and local glucose metabolism in the peri-infarct area when transplanted into the infarct brain. Recent studies strongly suggest that the BMSCs contain heterogeneous subpopulations and contribute to functional recovery through multiple mechanisms, including neuroprotection, inflammatory modulation, cell fusion, and neural differentiation. The author describes the importance to establish BMSC transplantation as a therapeutic entity that is scientifically proven.

Key words: bone marrow stromal cell, transplantation, cell therapy, ischemic stroke

INTRODUCTION

There are few drugs to effectively protect or repair the central nervous system (CNS) in clinical situation in spite of the huge efforts to develop them for longer than 50 years (Savitz and Fisher 2007). As the results, cell therapy has recently been expected as the alternative treatment strategy to enhance functional recovery after various kinds of neurological disorders, including ischemic stroke and spinal cord injury. Previously, a variety of cells have been studied as the candidates of donor cells for this purpose. These include embryonic stem (ES) cells, neural stem cells, induced pluripotent stem (iPS) cells, umbilical cord blood cells, and bone marrow stromal cells (BMSCs) (Jablonska and Lukomska 2011). Of these, the BMSCs may have the most enormous therapeutic potential among them, because they can be obtained from the patients themselves and easily expanded without posing any ethical and immunological prob-

lems. The BMSCs are non-hematopoietic cells and are also known as mesenchymal stromal cells (MSCs). For the decades, numerous numbers of studies have indicated that the transplanted BMSCs significantly enhance functional recovery after the insults in animal models of various neurological disorders. For example, the BMSCs significantly enhance the recovery of motor function when transplanted into the animal models of cerebral infarct, SCI, and TBI (Bliss et al. 2007, Parr et al. 2007). More interestingly, the BMSCs have the potential to ameliorate cognitive dysfunction under certain conditions. Thus, Wu and coauthors (2007) directly transplanted the BMSC into the hippocampus and found significant improvement of cognitive function in Alzheimer' disease model of rats. Maruichi and others (2009) stereotactically transplanted the BMSC into the mice subjected to diffuse axonal injury, and concluded that BMSC transplantation significantly enhance the recovery of cognitive function on Morris Water Maze test. Furthermore, Shichinohe and colleagues (2010) have also demonstrated that the BMSC significantly ameliorate white matter damage and improve cognitive function in chronic cerebral ischemia model of rats.

Based on these preclinical results, some of preliminary clinical testing has already been conducted to evaluate the safety and therapeutic effects of BMSC transplantation for the patients with both acute and chronic neurological disorders (Bang et al. 2005, Lee et al. 2008, Zhang et al. 2008, Pal et al. 2009, Lee et al. 2010, Mazzini et al. 2010, Saito et al. 2012). However, it should be reminded that a variety of questions or problems still remains to be solved in order to establish BMSC transplantation as scientifically proven entity in clinical situation (Abe et al. 2012). This article reviews recent knowledge on basic aspects of BMSC transplantation for ischemic stroke.

Mechanisms of CNS protection and repair by BMSCs

Recent studies have shed light on the mechanisms through which the BMSCs enhance functional recovery after cerebral infarct. Thus, Shichinohe and coworkers (2006) reported that BMSC transplantation significantly improved the binding potential for ^{125}I-iomazenil, a specific ligand for γ-aminobutyric acid (GABA) receptor, in the peri-infarct area. Mori and colleagues (2005) also showed that the engrafted BMSCs also improve glucose metabolism in response to sensory stimuli when transplanted into the rat cold injury model. Very recent study has demonstrated that the BMSCs may enhance functional recovery by promoting the recovery of local glucose metabolism in the peri-infarct area when directly transplanted into the infarct brain (Miyamoto et al. 2012).

Furthermore, biological or molecular roles of the BMSCs in the CNS have recently been elucidated. As first reported by Friedenstein and coauthors (1976), the BMSCs can be isolated using their biological properties to adhere to tissue culture surfaces. The adherent cells are well known to differentiate into osteoblast, chondrocytes, adipocytes, cardiomyocytes, and neural cells (Friedenstein et al. 1976, Prockop et al. 2003). However, they are morphologically heterogeneous. Therefore, it is quite natural to hypothesize that the BMSCs are the mixture of biologically various subpopulations of cells and contribute to enhance functional recovery through multiple mechanisms. In fact, our recent study has proven it (see below) (Hokari et al. 2008).

Migration and proliferation of BMSCs

The transplanted BMSC are known to aggressively migrate towards the lesion, although the underlying mechanisms are not clarified. Recent studies have shown that some chemokine such as monocyte chemoattractant protein-1 (MCP-1) and stromal cell-derived factor (SDF)-1α are expressed around the damaged CNS tissue and play an important role in the migration of the transplanted cells (Wang et al. 2002, Askari et al. 2003). Recently, CXCR4, a specific receptor for SDF-1α, are believed to play an important role in their migration in the CNS (Shichinohe et al. 2007). Son and coworkers (2006) also reported that SDF-1/CXCR4 and HGF/c-Met axes were involved in the recruitment of BMSC to the damaged tissue. It may be quite valuable to elucidate the temporal profile of these chemokines around damaged CNS tissue to determine the optimal timing of BMSC transplantation.

There are few studies whether the engrafted BMSCs retain their proliferative activity in the host brain or not. Therefore, we labeled the GFP-expressing BMSCs with a superparamagnetic iron oxide (SPIO) agent and transplanted into the ipsilateral striatum of the mice infarct brain. Fluorescence immunohistochemistry revealed that many of the GFP-positive cells were widely distributed in the peri-infarct area and partially expressed MAP2 and NeuN at 3 months after transplantation. However, only a small number of SPIO-positive cells could be detected on Turnbull blue staining. Surprisingly, the ratio of the SPIO- to GFP-positive cells was less than 3%. The results strongly suggested that the BMSCs actively proliferate, toward the lesion, and partially express the neuronal phenotype in the host brain during 3 months after transplantation (Yano et al. 2005).

Nursing effects of BMSCs

The BMSCs may produce some neuroprotective or neurotrophic factors and support the survival of the host neural cells (Zhong et al. 2003). This hypothesis is readily reasonable because the BMSC *per se* support the homing and proliferation of the hematopoietic cells in the bone marrow by producing a variety of cytokines such as stromal cell-derived factor-1α (SDF-1α) (Kortesidis et al. 2005). Indeed, the conditioned medium of BMSCs significantly promote neurite outgrowth from the dorsal root ganglion (Neuhuber

et al. 2005). Recent study has clearly shown that the BMSCs release soluble neuroprotective factors, including nerve growth factor (NGF), hepatocyte growth factor (HGF) and brain-derived neurotrophic factor (BDNF), and significantly ameliorate gluta-mate-induced damage of neurons (Hokari et al. 2008). Furthermore, the BMSC-conditioned medium activates phosphorylation of mitogen-activated protein kinase/extracellular signal-regulated protein kinase and/or phosphoinositide 3-kinase/serine/threonine kinase (PI3K/Akt) in primary culture of rat dorsal root ganglion (DRG) neurons (Gu et al. 2009). The BMSCs markedly promote the neurite extension from the neurons in the organotypic slice of the brain and spinal cord (Kamei et al. 2007, Shichinohe et al. 2008). Hofstetter and colleagues (2002) transplanted the BMSC into the injured cord and found that the engrafted BMSC were tightly associated with longi-tudinally arranged immature astrocytes and formed bundles bridging the epicenter of the injury. Very recently, He and coauthors (2011) have reported that the BMSC significantly increase the expression of bFGF, BDNF, and vascular endothelial growth factor (VEGF) in the ischemic brain. These findings strong-ly suggest that the BMSCs trigger endogenous signal-ing pathways of survival and repair in neurons by secreting soluble neurotrophic factors (Gornicka-Pawlak et al. 2011).

Very recent studies have demonstrated the alterna-tive pathways through which the BMSC may protect the neurons. Thus, Scheibe and others (2012) investi-gated the mechanism through which the BMSCs pro-tect the neurons against oxygen-glucose deprivation model *in vitro*. They found that the BMSCs released plasminogen activator inhibitor (PAI)-1 and signifi-cantly improved neuronal survival by increasing the phosphorylation of STAT3 and Akt in the neurons (Scheibe et al. 2012). Nowadays, the neurovascular units (NVUs) are known quite important to maintain-ing the homeostasis in the CNS. The NVUs consist of endothelial cells, astrocytes, and neurons. The BMSCs also protect the neurovascular integrity between base-ment membrane and astrocyte end-feet and ameliorate brain damage in stroke-prone spontaneous hyperten-sive rats (SHR-SP) (Ito et al. 2012). Alternatively, it is well known that the BMSCs release the angiogenic factors such as VEGF and contribute to increase the vessel density in the ischemic organs (Hoffmann et al. 2010).

Immunomodulatory effects of BMSCs

Both neutrophils and macrophages are well known to play an important role in the early inflammation after cerebral infarct (Barone and Feuerstein 1999). Indeed, their inflammatory response may be an essen-tial process to clear cellular debris and initiate the healing pathways. Simultaneously, however, these inflammatory reactions may also give rise to cytotoxic damage to the surviving neurons, astrocytes, and endothelial cells in the peri-infarct area (Barone and Feuerstein 1999).

On the other hands, the BMSCs have currently been investigated as donor cells for novel cell therapy to pre-vent and to treat clinical disease associated with aberrant immune response. Preclinical studies strongly suggest that the BMSCs may protect against infectious challenge either by direct effects on the pathogen or through indi-rect effects on the host. In the host, the BMSCs may attenuate pro-inflammatory cytokine and chemokine induction, reduce pro-inflammatory cell migration into sites of injury and infection, and induce immunoregula-tory soluble and cellular factors to preserve organ func-tion (Auletta et al. 2012). Based on these observations, the BMSCs have been expected as immunomodulators in tissue repair, autoimmune disease, and graft versus host disease (GVHD) (Mundra et al. 2012).

Interestingly, large numbers of mature neutrophils are retained near the BMSCs in the bone marrow, suggest-ing that the BMSCs protect these neutrophil pools from apoptosis and also prevent their inappropriate activation and release of granules to prevent accidental damage to the bone marrow (Bianco and Gehron Robey 2000, Raffaghello et al. 2008). The BMSCs also reduce their migration and release of reactive oxygen species (ROS) (van den Akker et al. 2013). Likewise, the BMSCs trig-ger the macrophage to go towards the anti-inflammatory phenotype and also reduce their release of pro-inflam-matory cytokines such as interleukin (IL)-1, IL-6, and interferon (TNF)-γ, while markedly increase their anti-inflammatory cytokines such as IL-10 (van den Akker et al. 2013). Therefore, the transplanted BMSCs may prevent excessive inflammatory response and prevent further tissue damage in the peri-infarct area.

Cell fusion of BMSCs

Several studies have demonstrated that the BMSCs fuse with the host cells when they are transplanted into

various kinds of organs or are co-cultured with donor cells (Terada et al. 2002, Alvarez-Dolado et al. 2003, Spees et al. 2003, Vassilopoulos et al. 2003). Spees and coauthors (2003) co-cultured the BMSCs with heat-shocked human small airway epithelial cells, and found that about 25% of them fused with epithelial cells (Spees et al. 2003). We have found similar results when the Yang and colleagues (2012) recently reported that the BMSCs fuse with the hydrogen peroxide-treated cardiomyocytes and significantly ameliorate their apoptosis *in vitro*. They also found that the BMSCs highly fused with the cardiomyocytes when injected to the mice subjected to myocardial infarction (Yang et al. 2012). However, the function of the resulting hybrid cells should be further investigated to explore their roles in tissue protection (Curril et al. 2010). Very recently, Islam and coworkers (2012) reported that the BMSCs transfer their mitochondria to the pulmonary alveolar epithelia through gap junction channels. The mitochondrial transfer increased alveolar ATP concentration and protected them against acute lung injury (Islam et al. 2012).

Neural differentiation of BMSCs

The BMSC *per se* are believed to differentiate into neural cells in the host's brain. This theory is based on the findings that BMSC simulate neuronal morphology and express the proteins specific for neurons *in vitro* (Sanchez-Ramos et al. 2000, Woodbury et al. 2000) or *in vivo* (Azizi et al. 1998, Kopen et al. 1999). Although the hypothesis is quite attractive, there still remain several questions. Actually, several studies posed a question about their *in vitro* differentiation into neurons (Lu et al. 2004, Neuhuber et al. 2004). Recent studies have shown that the BMSCs can alter their
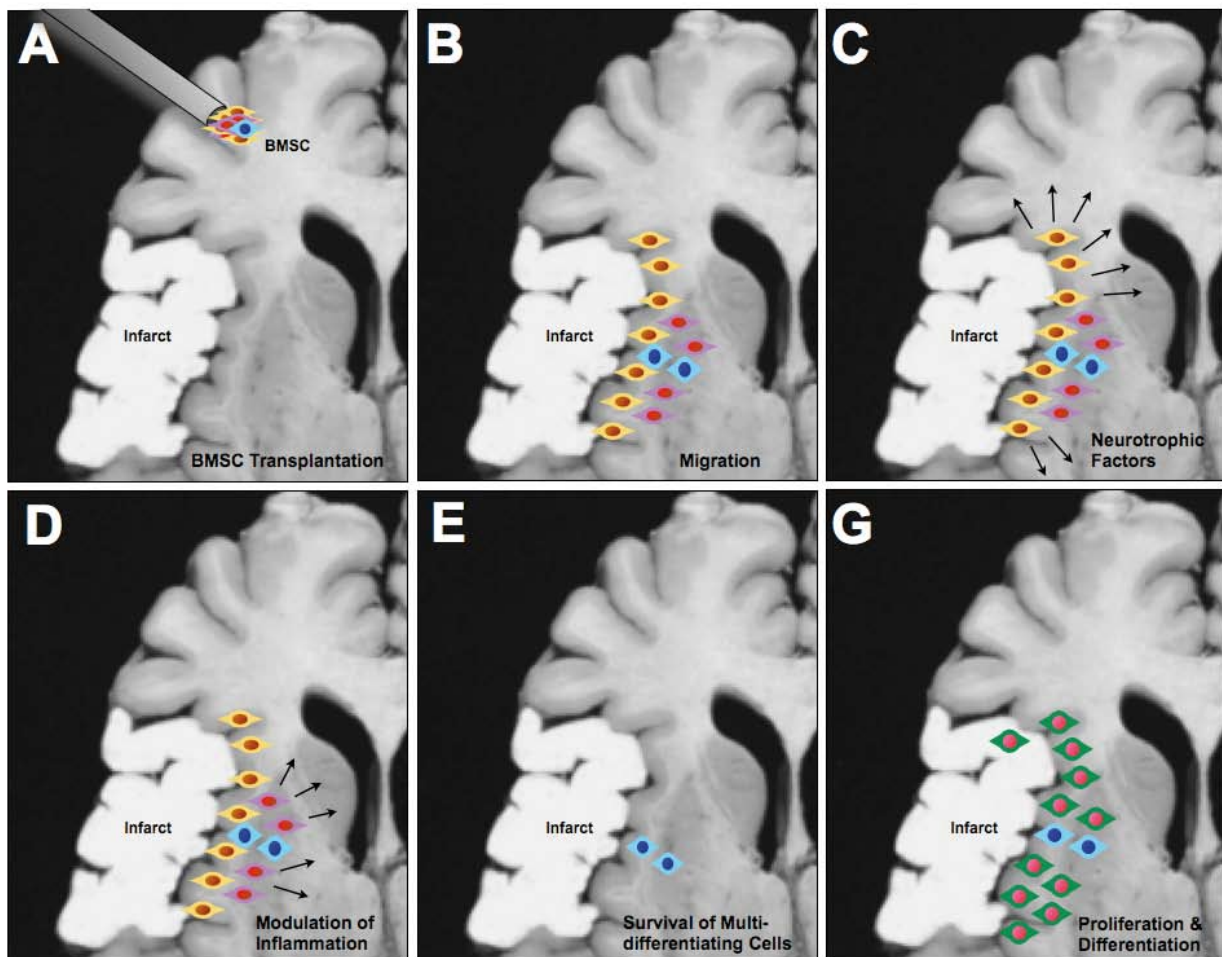

Fig. 1. Possible mechanism of functional recovery after ischemic stroke by bone marrow stromal cell (BMSC) transplantation

gene expression profile in response to exogenous stimuli and increase the genes related to the neural cells (Bossolasco et al. 2005, Hermann et al. 2006, Yamaguchi et al. 2006). Using microarray analysis, Yamaguchi and others (2006) showed that the BMSCs significantly reduce their genes related to mesenchymal cells and increased the neuron-related genes, when chemically treated with basic fibroblast growth factor (bFGF), retinoic acid (RA), and dimethyl sulfoxide (DMSO).

The BMSCs can acquire the neuronal phenotype under more physiological conditions. Thus, Sanchez-Ramos and colleagues (2000) showed that a small fraction of BMSCs cultured in epidermal growth factor (EGF) or retinoic acid/BDNF expressed nestin, NeuN, or GFAP, and that the proportion of NeuN-expressing cells increased when BMSC were co-cultured with fetal mouse midbrain neurons. Subsequently, Spees and others (2003) co-cultured the BMSCs with heat-shocked small airway epithelial cells without any chemical agents, and found that BMSC rapidly differentiated into epithelial-like cells and repaired epithelial monolayer. Wislet-Gendebien and coauthors (2005) also co-cultured the BMSCs with cerebellar granule cells and assessed their fates. They found that the nestin-expressing BMSCs express other neuronal markers and that BMSC-derived neuron-like cells fire single-action potentials in response to neurotransmitters such as glutamate. Hokari and colleagues (2008) also demonstrated that a certain subpopulation of the BMSCs morphologically simulated the neuron and expressed the neuron-specific proteins without any evidence of cell fusion, when co-cultured with the neurons. These findings strongly suggest that at least a certain subpopulation of the BMSCs have the potential to alter their gene expression profile and to differentiate into the neural cells in response to the surrounding environment. In fact, the local environment may be the predominant determinant of the phenotypic fate of engrafted BMSCs in the host brain. Thus, the majority of them express the neuronal markers such as NeuN, MAP2, and Tuj-1 in the neocortex, while they express astrocytic phenotype in the corpus callosum or spinal cord (Lee et al. 2003, 2004, Shichinohe et al. 2007, Maruichi et al. 2009, Kawabori et al. 2012). The findings correlate very well with previous results. Shihabuddin and coworkers (2000) reported that adult spinal cord neural stem cells differentiated into neurons after transplantation into dentate gyrus of hip-pocampus, but were unable to exhibit neurogenic potential when transplanted back into the adult spinal cord. Johansson and others (1999) also showed that neural progenitor cells start to proliferate, but differentiate into astrocytes after spinal cord injury. More importantly, the findings indicate that only the subgroup of BMSCs with potential of neural differentiation can survive in the host brain for a long time (>4 weeks).

More interestingly, recent study has shown that the engrafted BMSCs express γ-aminobutyric acid (GABA) receptor and improve the binding potential for ^{125}I-iomazenil in the peri-infarct area (Shichinohe et al. 2006). Using micro-PET/CT apparatus, Miyamoto and colleagues (2013) serially quantified local glucose metabolism in the rat subjected to cerebral infarct and found that BMSC transplantation significantly enhance the recovery of glucose metabolism in the peri-infarct area. Alternatively, Chiba and coauthors (2009) have recently found that the BMSCs acquire neuronal phenotype and build synaptic connection with the corticospinal tract, when transplanted into the injured spinal cord of rats. *In vitro* studies have also indicated that the BMSCs exhibit electrical functions simulating those of neurons (Kohyama et al. 2001, Jiang et al. 2003, Jin et al. 2003), although this is still controversial (Hofstetter et al. 2002).

Very recently, Wakao and coworkers (2011) successfully isolated stress-tolerant adult human stem cells from cultured skin fibroblasts or BMSCs. These cells can self-renew, express a set of genes associated with pluripotency, and differentiate into endodermal, ectodermal, and mesodermal cells both *in vitro* and *in vivo*. When transplanted into immunodeficient mice by local or intravenous injection, they were integrated into damaged skin, muscle, or liver and differentiated into cytokeratin 14-, dystrophin-, or albumin-positive cells in the respective tissues. Furthermore, they can be efficiently isolated as SSEA-3-positive cells. Unlike authentic ES cells, their proliferation activity is not very high and they do not form teratomas in immunodeficient mouse testes. The findings are quite attractive, because non-tumorigenic stem cells with the ability to generate the multiple cell types of the three germ layers can be obtained through easily accessible adult human mesenchymal cells without introducing exogenous genes (Kuroda et al. 2010). These cells were named as multilineage-differentiating stress enduring (Muse) cells. Furthermore, they have proven that Muse

cells are a primary source of induced pluripotent stem (iPS) cells in human fibroblasts (Wakao et al. 2011). There results strongly suggest that a certain subpopulation of BMSCs may have the biological properties of neural differentiation and contribute to regenerate the infarct brain (Fig. 1).

CONCLUSION

Recent studies have gradually clarified the biological feature of BMSCs as the donor cells for ischemic stroke. The author emphasizes that it would be essential to fully explore it to apply BMSC transplantation into clinical situation.

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