ACTA NEUROBIOLOGIAE EXPERIMENTALIS

Acta Neurobiol Exp 2007, 67: 187–195

Bone marrow stromal cells in traumatic brain injury (TBI) therapy: True perspective or false hope?

Malgorzata Opydo-Chanek

Department of Experimental Hematology, Institute of Zoology, Jagiellonian University, 6 Ingardena St., 30-060 Cracow, Poland, Email: opydo@zuk.iz.uj.edu.pl

Review

Abstract. Recent studies regard bone marrow stromal cells as a potential candidate for cellular therapy of traumatic brain injury and thus as an attractive alternative for embryonic and fetal stem cells. Numerous experiments indicate that bone marrow stromal cells play an important role in the repair of injured brain tissue and also support healing processes. Findings of *in vitro* and *in vivo* studies show that these cells have an ability to differentiate into cells of multiple tissues, including neurons and glial cells and to secrete an array of growth factors and cytokines, which have an influence on repair of damaged tissue. In addition, treatment of traumatic brain injury with bone marrow stromal cells promotes functional recovery of injured animals. Taking this into consideration, there is hope for using bone marrow stromal cells in brain injury therapy, which is very difficult because of specific events that occur in the pathological conditions. However, mechanisms responsible for the observed therapeutic potential of bone marrow stromal cells still remain unclear. The review presents achievements in studies on bone marrow stromal cells as a source of therapeutic benefits in treatment of traumatic brain injury and addresses the question of their possible future use in clinical trials.

Key words: bone marrow stromal cells, transplantation, traumatic brain injury, CNS

INTRODUCTION

The therapy of brain injury faces many difficulties as a result of the limited plasticity of the nervous system and complicated pathological processes present in the state of the brain disorder. Some of these processes are: myelin degeneration, axonal damage, death of neurons and glial cells, inflammation and blood-brain barrier breakdown. Apart from the early response of the brain to injury, there are also events detected some time after tissue damage, which cause additional difficulties for both physiological regeneration and medical treatment. These include secondary neuronal death, formation of glial scar and a microenvironment that inhibits axonal regrowth and repair of injured tissue.

The limited ability of the central nervous system (CNS) for self-renewal and all processes present during the pathological condition are important issues for the experimental approaches in CNS injury treatments. Recent studies have examined the administration of trophic factors: nerve growth factor (NGF) (Dixon et al. 1997, Zhou et al. 2003), fibroblast growth factor-2 (FGF-2) (Yoshimura et al. 2003), insulin-like growth factor-1 (IGF-1) (Guan et al. 2001, Saatman et al. 1997), which due to their propriety could create a pro-regenerative environment and also help to restore lost functions. Simultaneously, gene therapy is improving the possibility of a continuous and lengthy delivery of these supporting factors, which include the use of virus vectors (Andsberg et al. 2002) and transfected cells (Longhi et al. 2004, Philips et al. 2001). However, this strategy needs further studies on the optimal use of viruses and on the time regulation of factors administrated this way.

Considerable hope is vested in cellular therapy, in which transplanted cells would be the source of factors promoting regeneration and steering healing processes in the appropriate direction. It could be also the source of new cells, which will then replace the damaged ones. To achieve this aim, new experiments are being performed on fetal neural stem cells (Gao et al. 2006) and embryonic stem cells (Yoshizaki et al. 2004). The difficulties with isolation of these cells and ethical issues are the limiting factors in their use. The needs of multipotent cells have directed the attention of researchers to cells that are more easily accessed, with the potential to multiply and to differentiate into cells of distinct origin under the presence of appropriate stimulus. These are the bone marrow stem cells.

The bone marrow contains at least two populations of multipotent cells: hematopoietic stem cells (HSCs), which differentiate into blood cells and mesenchymal stem cells (also called bone marrow stromal cells, BMSCs, colony-forming-unit fibroblasts), non-hematopoietic, which residue in the bone marrow stromal system and have the ability to give rise to cells of mesenchymal lineage. There is evidence that under appropriate conditions BMSCs can differentiate into chondrocytes, osteocytes and adipocytes, both *in vitro* and *in vivo* (Gronthos et al. 2003). Moreover, bone marrow stromal cells show the potential to differentiate into hepatocytes (Miyazaki et al. 2004), endothelial cells (Spees et al. 2003), and nervous system cells (Hermann et al. 2004, Suzuki et al. 2004). Jiang and coauthors (2002) demonstrated multipotent adult progenitor cells (MAPC) within BMSCs culture that differentiated into endothelium, neural cells and epithelioid cells of the endoderm.

Furthermore, implantation of BMSCs to the place altered by the pathological processes promotes the improvement of the heart function (Olivares et al. 2004) and the repair of bones (Crevensten et al. 2004, Pereira et al. 1998).

In a physiological condition, bone marrow stem cells are characterized by their multipotency and self-renewal. Studies show that neural stem cells have the same properties and that is why they have become the subject of intensive studies (Bazan et al. 2004, Johanson et al. 1999, Kempermann et al. 2003). Their potential to differentiate is not only limited to neuroectodermal cells, but they can also differentiate into the blood system cells (Bjornson et al. 1999, Shih et al. 2001). Terskikh and colleagues (2001) found the presence of transcripts, which are expressed in both adult bone marrow stem cells and neural progenitor cells. Moreover, the hematopoietic growth factor – erythropoietin gene and its receptor are expressed in the human, monkey, and murine brain (Marti et al. 1996). The connection between expressions of different genes could be one of the factors responsible for the plasticity of stem cells, which is observed in *in vivo* and *in vitro* studies.

Taking into consideration the above-mentioned properties of bone marrow stromal cells, numerous studies have been performed to examine the influence of these cells on regeneration and functions restoration of damaged tissue after brain trauma.

IN VITRO AND *IN VIVO* IMPLICATION FOR BONE MARROW STROMAL CELLS USE IN TBI THERAPY

BMSCs isolation from bone marrow is classically performed by their adhesion to the culture plastic containers. BMSCs comprise a heterogeneous population of cells and in the culture at least three morphologically different types of cells can be seen: spindle-shaped cells, large flat cells and small round cells. They can divide rapidly and be induced to differentiate into multiple cell lineages under specific media containing growth factors and/or other substances like β-glycerophosphate and dexamethason in the case of osteogenic lineages stimulation (Banfi et al. 2000). Recently various methods of BMSCs culture have been developed to partially transdifferentiate human, rat or mouse adult BMSCs into neuron-like cells (Bossolasco et al. 2005, Croft and Przyborski 2004, Hermann et al. 2004, Kim et al. 2002, Sanchez-Ramos et al. 2000, Woodbury et al. 2000), enhancing their potentiality to be used in the treatment of a variety of neurological diseases, including TBI.

The use of medium composed of retinoic acid (RA), epidermal growth factor (EGF) and brain-derived neurotrophic factor (BDNF), was conducive to stromal cells differentiation into neural progenitor-like cells (expression of neuron-specific nuclear protein, NeuN, and neuronal progenitor marker, nestin) and glial-like cells (expression of glial acidic fibrillary protein-GFAP) in a small proportion (0.5–1%) after 2 weeks of culture. Simultaneously coculturing BMSCs with fetal mesencephalic cells increased the number of differentiated cells at least twofold (Sanchez-Ramos et al. 2000). In another study organotypic hippocampal slice was used as a microenvironment to demonstrate the capacity of BMSCs for morphological differentiation. Neuron-like differentiation occurred mostly within the hippocampal slice boundaries but the number of neuron-like cells (NeuN expression) decreased within the two weeks of culture (Abouelfetouh et al. 2004). Thus, the BMSCs contact with the host brain tissue and cells plays an important role in the differentiation of BMSCs, however differentiation potential of such expanded cells should be precisely evaluated as BMSCs lost their phenotypes of neuron-like cells with culture time.

The changes in morphology and phenotype of BMSCs were also observed after enrichment of culture medium with FGF and RA, and the simultaneous addition of fibronectin to the medium increased the amount of the differentiated cells (Kim et al. 2002).

The protocol, described by Woodbury and others (2000), induced the bone marrow stromal cells to exhibit a neuronal phenotype, expressing neuron-specific enolase (NSE), NeuN, neurofilament-M (NF-M) and tau. To induce neuronal differentiation, BMSCs were cultured in serum-free medium containing ß-mercaptoethanol or dimethylsulphoxide plus butylated hydroxyanisole. Nearly 80% of cultured cells expressed NSE and NF-M within hours. Similar results were documented in another study, in which BMSCs treated with antioxidants adopted a neural-like phenotype (Croft and Przyborski 2004). This type of differentiation protocol is however controversial as was shown by Paul Lu and coauthors (2004) who suggested that the observed chemical neuronal induction might be the result of cellular toxicity, cell shrinkage and changes in cytoskeleton and did not represent neuronal differentiation.

According to a study performed by Tondreau and others (2004) human bone marrow-derived mesenchymal stem cells express neuronal markers (nestin, neuron-specific class III beta-tubulin, Tuj-1, tyrosine hydroxylase, TH, microtubule-associated protein-2, MAP-2) and glial marker (GFAP) without any stimulation. The more mature neuronal and glial proteins were seen after five passages of BMSCs and specific neurogenic induction caused further differentiation of BMSCs into cells of neuronal origin by increasing the expression of TH, MAP-2 and GFAP and decreasing the expression of Tuj-1. Similar results were also shown by Bossolasco and colleagues (2005) who demonstrated *in vitro* neuro-glial potential of mesenchymal stem cells before and after treatment with different media. After five passages mesenchymal stem cells expressed NSE, βtubulin III, GAP 43, MAP2, GFAP by RT-PCR and NF-M, nestin, O4 by flow cytometry before any induction. However the expression of these genes at protein level was not detected by immunocytochemical analysis, what might be caused by too low level of protein expression or by the lack of specific additional signals required for that expression.

The transdifferentiation of bone marrow stromal cells was also demonstrated *in vivo*. Genetically or fluorescently labeled BMSCs migrated to the brain, engrafted the parenchyma and expressed the phenotype of neurons and astrocytes (Lee et al. 2004, Munoz-Elias et al. 2004, Zhao LR et al. 2002).

Although in the specific condition BMSCs seem to be able to display a morphology and protein expression profile characteristic for neural properties, there is still a question how closely they approach a mature, functional neural phenotype and integrate into functional neural circuitry, especially that other studies shown that phenotypic differentiation of transplanted bone marrow stromal cells is not the result of transdifferentiation but rather cell fusion (Terada et al. 2002).

Apart from cellular replacement as the potential therapeutic approach in BMSCs studies, the second possibility for therapeutic use of mesenchymal stem cells is connected with growth factors and cytokines production by these cells. BMSCs in themselves could be the source of neurotrophic factors in the damaged tissue, promoting the regeneration with a simultaneous inhibition of the processes which impede the repair. The BMSCs potential to produce NGF and glial cell line-derived neurotrophic factor (GDNF) was demonstrated in culture by using the RT-PCR technique and ELISA (Chen Q et al. 2005, Garcia et al. 2004, Ye et al. 2005). BMSCs cultured in DMEM medium secreted NGF, BDNF, GDNF, neurotrophin-3 (NT-3). However, during seven weeks of BMSCs culture, the most significant increase in the amounts of trophic factor was seen in the NGF level (Chen Q et al. 2005). According to Ye and coauthors (2005), the GDNF expression in the cells and culture medium increased gradually from 3 to 10 days of culture. Moreover, BMSCs express the genes for several neurotrophic factors including NGF-ß, BDNF, ciliary neurotrophic factor (CNTF), IGF-1, (Crigler et al. 2006, Yamaguchi et al. 2006), promote survival of neuroblastoma cells and neuritogenesis *in vitro* (Crigler et al. 2006), indicating their therapeutic role in the protection of the injured central nervous system. Isele and colleagues (2007) demonstrated that BMSCs protected neurons against apoptotic stress by stimulation of endogenous survival signaling pathways in neurons such as PI3-K/Akt and MAPK.

These data demonstrate that BMSCs have an ability to produce trophic factors and protect injured tissue in standard culture conditions. The additional factors in the injured brain might influence the properties of BMSCs, especially given that the increase in the amount of NGF was also shown in the cerebrospinal fluid of rats after intraventricular injection of BMSCs (Chen Q et al. 2005). Furthermore, the ability of bone marrow stromal cells to migrate and differentiate into CNS-origin cells gives new opportunities for their usage in the gene therapy. Curative proteins might be incorporated by these cells into the pathologically changed brain and provide the valuable factors for neuroprotection and neuroregeneration (Zhao LX et al. 2004).

THE BONE MARROW STROMAL CELLS IN CONTACT WITH TBI

Traumatic brain injury is followed by many changes, including contusion, hemorrhage, edema, axonal degeneration, anoxia and ischemia. In the site of the injury, the blood-brain barrier breakdown, disturbances in electrolytes homeostasis, apoptotic and necrotic death of neurons and oligodendrocytes and activation of astrocytes and microglia occur. The cells from the blood also infiltrate to the site of the injury. The inflowing macrophages together with the microglia initiate the inflammation (Tzeng and Wu 1999) and by cooperation with astrocytes and fibrocytes they contribute to glial scar formation (Fitch and Silver 1997). The scarring process depends on the relationship between those cells and on their function connected with cytokines production.

Apart from scar tissue, the secondary neuronal death occurs, which is also the obstacle preventing regeneration. Inflammation processes seem to be the factor responsible for neuronal death, during which toxic substances for neurons are produced (Holmin et al. 1997).

TBI therapy needs actions, which are directed towards: the reduction of secondary trauma results; the restoration of the tissue, which has suffered injury; and the neurological function improvement. Taking into consideration the fact that a number of processes coexist after trauma, treatment of only one pathological event may be insufficient to prevent other consequences of injury. Thus, the possibility of using mesenchymal stem cells attracts the attention of researchers engaged in TBI therapy, because these cells might have multiple potential therapeutic applications.

The latest studies show that bone marrow stromal cells, besides having the ability to replace damaged cells from the neuronal system, can also produce an array of trophic factors important for the repair processes under the influence of the injured tissue. Xiaoguang Chen and coauthors (2002) cultured human bone marrow stromal cells in the presence of injured brain tissue extract. They observed that the increase in the amount of BDNF, NGF, FGF, vascular endothelial growth factor (VEGF) and hepatocyte growth factor

(HGF) in the culture medium depended on the culture time and the time of brain tissue isolation. This correlation was also found in the pathological condition in vivo, in which the increase in the NGF and BDNF expression in the injured brain was observed after intravenous administration of stromal cells (Mahmood et al. 2004a) and after injection to Cistern Magnum (Hu et al. 2005). Thus, bone marrow stromal cells might be the new strategy of administration of factors essential for the repair.

On the other hand, bone marrow stromal cells may change their fate in the environment of neurotrophic factors. BMSCs cultured in the presence of BDNF and NGF resulted in more intensive migration and engraftment of the host's injured brain tissue, with a simultaneously observed improvement of functional recovery in comparison with the results derived after transplantation of stromal cells cultured in the standard condition (Mahmood et al. 2002). It was also shown after intraarterial transplantation of BMSCs and the addition of NGF and BDNF to the culture medium promoted the expression of neuronal protein MAP-2 (Lu D et al. 2001a). Thus, the environment of the culture or the damage tissue influences the transplanted stromal cells in two different ways: by induction of growth factors production and/or initiating their differentiation into neuronal or glial cells.

The differentiation of rat's bone marrow stromal cells was observed in the cortical compact injury (Lu D et al. 2001b) and fluid percussion model (Lu J et al. 2006) of TBI after intravenous injection of these cells. It was shown that a small number of transplanted cells expressed a neuronal marker NeuN (Lu D et al. 2001b), astrocytic marker GFAP (Lu D et al. 2001b, Lu J et al. 2006), MAP-2, oligodendrocyte marker CNPase and microglial marker OX-42 (Lu J et al. 2006). At the same time, the sensorimotor function was improved. However, no connection between differentiated graft cells and host cells was found, and the number of the differentiated cells was low. The researchers' hypothesis was that the functional outcome might be the result of the interaction between the injected cells and the host tissue, reflected in the production of growth factors and cytokines. It might be also the effect of enhanced endogenous cellular proliferation after BMSCs transplantation (Mahmood et al. 2004b).

Similar results have been obtained after intravenous transplantation of human bone marrow stromal cells to the rats' brain exposed to TBI (Mahmood et al. 2003). The engrafting of brain parenchyma by BMSCs was accompanied by the differentiation of some cells into neurons and astrocytes and also by neurological recovery. The absence of the graft rejection was the important issue. The graft-versus-host (GvH) disease symptoms were not also observed after the administration of rat's bone marrow cells suspension directly to the rat's cerebral cortex and striatum. What is more; the evidence of motor function recovery was demonstrated (Mahmood et al. 2001). It was not stated whether grafted bone marrow cells influenced the inflammation processes in the damaged tissue, as the increase of the proinflammatory cells number in the injured area might cause neurotoxic effects and disturbance in the CNS self-control of the inflammation, which refer to the local regulation of antigen expression, number of cytokines and ending of the inflammatory process (Bauer et al. 2001).

The experimental models of TBI cause the disturbance features approximate to symptoms observed in clinical TBI. Unfortunately, the clinical trials can give results which differ considerably from studies on the animal model, especially given that studies are made in standardized conditions. Moreover, the optimal therapeutical number of injected BMSCs have to be estimated in further pre-clinical trials. In addition, the time of transplantation after injury should also be considered. In two recent works Mahmood and colleagues (2005, 2006) have demonstrated long-term recovery after BMSCs transplantation. In the first one three treatment groups of male rats were injected intravenously with three different doses of human BMSCs (2×10^6, 4×10^6, 8×10^6) 1 day after brain injury. Statistically significant improvement in functional outcome was observed in all three treatment groups 3 months after injury and there were no differences between them. The difference between the groups was only seen in the number of engrafted cells (Mahmood et al. 2005). In the later work the same doses of rats' BMSCs were injected intravenously to female rats 1 week after brain injury. After three months functional outcome was significantly better in rats that received 4×10^6 and 8×10^6 whereas no improvement was seen in animals that received 2×10^6 BMSCs. Furthermore, the dose of 2×10^6 caused lower expression of BDNF in the injured brain in comparison with two other doses (Mahmood et al. 2006).

Moreover, the culture condition and the route of cells administration may also be significant. Dunyue

Lu and coauthors (2002) cocultured BMSCs with neurospheres prepared from fetal brain tissue. BMSCs united with embryonic cells, created long processes and at the same time, the growth of these cells in culture was increased. In the second phase of this study, cultured cells were transplanted directly to the injured site. These cells showed a better survival rate in comparison with the bone marrow stromal cells and neurospheres cultured alone. The decrease in the injury site dimension and neurological function outcome were also observed. Usage of new cell culture conditions and transplantation combined with additional pro-regenerative factors, including gene therapy, are the novel therapeutical strategies adopted in the studies of TBI treatment.

The influence of bone marrow stromal cells on secondary processes found after injury, such as secondary neuronal death and glial scar formation, seems to be the essential issue in further studies on BMSCs use in TBI therapy. Glial scar is considered to be the main obstacle to axonal regeneration. On the other hand it isolates the still-intact brain tissue from the secondary neuronal degeneration, and their components release factors that could promote neuronal survival and regeneration (Ribotta et al. 2004). From a therapeutical point of view, it would be important to find methods for such a modification and modulation of gliosis, that it retains the healing features along with retaining the possibility of axonal regeneration.

CONCLUSIONS

The perspective of using bone marrow stromal cells as a part of the therapy for TBI seems to be closer. Experimental studies indicate many opportunities for the application of these cells, depending on: time and localization of bone marrow cells administration; the source of transplanted bone marrow stromal cells (the type of transplantation); the conditions of BMSCs culture and transplantation to the injured tissue. The results of different preclinical studies demonstrate the ability of bone marrow stromal cells to migrate, engraft into the damaged brain tissue, and differentiate into residential-like cells. The neurological recovery is simultaneously observed. BMSCs seem to be also an important source for gene therapy, by which different compounds and factors, essential for the regeneration and repair of the injured tissue, can be administrated to the brain over long periods. All of these phenomena require, however, further studies on the conditions of use of BMSCs including, animal models of CNS disease, methods of analysis of obtained results and neurological outcome tests. The mechanism responsible for observed changes in cell phenotype and function and for behavioral recovery must be established and fully understood. Although the results of pre-clinical studies are optimistic, the question whether bone marrow stromal cells could help in TBI therapy is still open.

REFERENCES

Abouelfetouh A, Kondoh T, Ehara K, Kohmura E (2004) Morphological differentiation of bone marrow stromal cells into neuron-like cells after co-culture with hippocampal slice. Brain Res 1029: 114–119.

Andsberg G, Kokaia Z, Klein RL, Muzyczka N, Lindvall O, Mandel RJ (2002) Neuropathological and behavioral consequences of adeno-associated viral vector-mediated continuous intrastriatal neurotrophin delivery in a focal ischemia model in rats. Neurobiol Dis 9: 187–204.

Banfi A, Muraglia A, Dozin B, Mastrogiacomo M, Cancedda R, Quarto R (2000) Proliferation kinetics and differentiation potential of ex vivo expanded human bone marrow stromal cells: Implications for their use in cell therapy. Exp Hematol 28: 707–715.

Bauer J, Rauschka H, Lassmann H (2001) Inflammation in the nervous system: the human perspective. Glia 36: 235–243.

Bazan E, Alonso FJ, Redondo C, Lopez-Toledano MA, Alfaro JM, Reimers D, Herranz AS, Paino CL, Serrano AB, Cobacho N, Caso E, Lobo MV (2004) In vitro and in vivo characterization of neural stem cells. Histol Histopathol 9: 1261–1275.

Bjornson CR, Rietze RL, Reynolds BA, Magli MC, Vescovi AL (1999) Turning brain into blood: a hematopoietic fate adopted by adult neural stem cells in vivo. Science 283: 534–537.

Bossolasco P, Cova L, Calzarossa C, Rimoldi SG, Borsotti C, Lambertenghi Deliliers G, Silani V, Soligo D, Polli E (2005) Neuro-glial differentiation of human bone marrow stem cells in vitro. Exp Neurol 193: 312–325.

Chen Q, Long Y, Yuan X, Zou L, Sun J, Chen S, Perez-Polo JM, Yang K (2005) Protective effects of bone marrow stromal cells transplantation in injured rodent brain: synthesis of neurotrophic factors. J Neuosci Res 80: 611–619.

Chen X, Katakowski M, Li Y, Lu D, Wang L, Zhang L, Chen J, Xu Y, Gautam SC, Mahmood A, Chopp M (2002)

Human bone marrow stromal cultures conditioned by traumatic brain tissue extracts: growth factors production. J Neurosci Res 69: 687–691.

Crevensten G, Walsh AJ, Ananthakrishnan D, Page P, Wahba GM, Lotz JC, Berven S (2004) Intravertebral disc therapy for regeneration: Mesenchymal stem cell implantation in rat intravertebral discs. Ann Biomed Eng 32: 430–434.

Crigler L, Robey RC, Asawachaicharn A, Gaupp D, Phinnez DG (2006) Human mesenchymal stem cell subpopulations express a variety of neuro-regulatory molecules and promote neuronal cell survival and neuritogenesis. Exp Neurol 198: 54–64.

Croft AP, Przyborski SA (2004) Generation of neuroprogenitor-like cells from adult mammalian bone marrow stromal cells in vitro. Stem cells Dev 13: 409–420.

Dixon CE, Flinn P, Bao J, Venya R, Hayes RL (1997) Nerve growth factor attenuates cholinergic deficits following traumatic brain injury in rats. Exp Neurol 146: 479–490.

Fitch MT, Silver J (1997) Activated macrophages and the blood-brain barrier: inflammation after CNS injury leads to increase in putative inhibitory molecules. Exp Neurol 148: 587–603.

Gao J, Prough DS, McAdoo DJ, Grady JJ, Parsley MO, Ma L, Tarensenko YI, Wu P (2006) Transplantation of primed human fetal neural stem cells improves cognitive function in rats after traumatic brain injury. Exp Neurol 201: 281–292.

Garcia R, Aguiar J, Alberti E, de la Cuetara K, Pavon N (2004) Bone marrow stromal cells produce nerve growth factor and glial cell line-derived neurotrophic factor. Biochem Biophys Res Commun 316: 753–754.

Gronthos S, Zannettino AC, Hay SJ, Shi S, Graves SE, Kortesidis A, Simmons PJ (2003) Molecular and cellular characterization of highly purified stromal stem cells derived from human bone marrow. J Cell Sci 116: 1827–1835.

Guan J, Miller OT, Waugh KM, McCarthy DC, Gluckman PD (2001) Insulin-like growth factor-1 improves somatosensory function and reduces the extent of cortical infarction and ongoing neuronal loss after hypoxia-ischemia in rats. Neuroscience 105: 299–306.

Hermann A, Gastl R, Liebau S, Popa MO, Fiedler J, Boehm BO, Maisel M, Lerche H, Schwarz J, Brenner R, Storch A (2004) Efficient generation of neural stem cell-like cells from adult human bone marrow stromal cells. J Cell Sci 117: 4411–4422.

Holmin S, Schalling M, Hojeberg B, Nordqvist AC, Skeftruna AK, Mathiesen T (1997) Delayed cytokine expression in rat brain following experimental contusion. J Neurosurg 86: 493–504.

Hu DZ, Zhou LF, Zhu J, Mao Y, Wu XH (2005) Upregulated gene expression of local brain-derived neurotrophic factor and nerve growth factor after intracisternal administration of marrow stromal cells in rats with traumatic brain injury. Chin J Traumatol 8: 23–26.

Isele NB, Lee HS, Landshamer S, Straube A, Padovan CS, Plesnila N, Culmsee C (2007) Bone marrow stromal cells mediate protection through stimulation of PI3-K/Akt and MAPK signaling in neurons. Neurochem Int 50: 243–250.

Jiang Y, Jahagirdar BN, Reinhardt RL, Schwarty RE, Keene CD, Ortiz-Gonzalez XR, Reyes M, Lenvik T, Lund T, Blackstad M, Du J, Aldrich S, Lisberg A, Low WC, Largaespada DA, Verfaillie CM (2002) Pluripotency of mesenchymal stem cells derived from adult marrow. Nature 418: 41–49.

Johansson CB, Momma S, Clarke DL, Risling M, Lendahl U, Frisen J (1999) Identification of a neural stem cell in the adult mammalian central nervous system. Cell 96: 25–34.

Kempermann G, Gast D, Kronenberg G, Yamaguchi M, Gage FH (2003) Early determination and long-term persistence of adult-generated new neurons in the hippocampus of mice. Development 130:391–399.

Kim BJ, Seo JH, Bubien JK, Oh YS (2002) Differentiation of adult bone marrow stem cells into neuroprogenitor cells in vitro. Neuroreport 13: 1185–1188.

Lee JB, Kuroda S, Shichinohe H, Yano S, Kobayashi H, Hida K, Iwasaki Y (2004) A pre-clinical assessment model of rat autogeneic bone marrow stromal cell transplantation into the central nervous system. Brain Res Ptotoc 14: 37–44.

Longhi L, Watson DJ, Saatman KE, Thompson HJ, Zhang C, Fujimoto S, Royo N, Castelbuono D, Raghupathi R, Trojanowski JQ, Lee VM, Wolfe JH, Stocchetti N, McIntosh TK (2004) Ex vivo gene therapy using targeted engraftment of NGF-expressing human NT2N neurons attenuates cognitive deficits following traumatic brain injury in mice. J Neurotrauma 21: 1723–1736.

Lu D, Li Y, Wang L, Chen J, mahmood A, Chopp M (2001a) Intraarterial administration of marrow stromal cells in a rat model of traumatic brain injury. J Neurotrauma 18: 813–819.

Lu D, Mahmood A, Wang L, Li Y, Lu M, Chopp M (2001b) Adult bone marrow stromal cells administered intravenously to rats after traumatic brain injury migrate into brain and improve neurological outcome. Neuroreport 12: 559–563.

Lu D, Li Y, Mahmood A, Wang L, Rafiq T, Chopp M (2002) Neural and marrow-derived stromal cells sphere transplantation in rats model of traumatic brain injury. J Neurosurg 97: 935–940.

Lu J, Moochhala S, Moore XL, Ng CK, Tan MH, Lee LKH, He B, Wong MC, Ling EA (2006) Adult bone marrow cells differentiate into neural phenotypes and improve functional recovery in rats following traumatic brain injury. Neurosci Lett 398: 12–17.

Lu P, Blesch A, Tuszynski MH (2004) Induction of bone marrow stromal cells to neurons: differentiation, transdifferentiation, or artifact? J Neurosci Res 77: 174–191.

Mahmood A, Lu D, Yi L, Chen JL, Chopp M (2001) Intracranial bone marrow transplantation after traumatic brain injury improving functional outcome in adult rats. J Neurosurg 94: 589–595.

Mahmood A, Lu D, Wang L, Chopp M (2002) Intracerebral transplantation of marrow stromal cells cultured with neurotrophic factors promotes functional recovery in adult rats subjected to traumatic brain injury. J Neurotrauma 19: 1609–1617.

Mahmood A, Lu D, Lu M, Chopp M (2003) Treatment of traumatic brain injury in adult rats with intravenous administration of human bone marrow stromal cells. Neurosurgery 53: 697–703.

Mahmood A, Lu D, Chopp M (2004a) Intravenous administration of marrow stromal cells (MSCs) increases the expression of growth factors in rat brain after traumatic brain injury. J Neurotrauma 21: 33–39.

Mahmood A, Lu D, Chopp M (2004b) Marrow stromal cell transplantation after traumatic brain injury promotes cellular proliferation within the brain. Neurosurgery 55: 1185–1193.

Mahmood A, Lu D, Qu C, Goussev A, Chopp M (2005) Human marrow stromal cell treatment provides long-lasting benefit after traumatic brain injury in rats. Neurosurgery 57: 1026–1031.

Mahmood A, Lu D, Qu C, Goussev A, Chopp M (2006) Long-term recovery after bone marrow stromal cell treatment of traumatic brain injury in rats. J Neurosurg 104: 272–277.

Marti HH, Wenger RH, Rivas RA, Straumann U, Digicaylioglu M, Henn V, Yonekawa Y, Bauer C, Gassmann M (1996) Erythropoietin gene expression in human, monkey and murine brain. Eur J Neurosci 8: 666–676.

Miyazaki M, Masaka T, Akiyama I, Nakashima E, Sakaguchi M, Huh NH (2004) Propagation of adult rat bone marrow-derived hepatocyte-like cells by serial passages in vitro. Cell Transplant 13: 385–339.

Munoz-Elias G, Marcus AJ, Coyne TM, Woodbury D, Black IB (2004) Adult bone marrow stromal cells in the embryonic brain: engraftment, migration, differentiation and long-term survival. J Neurosci 24: 4585–4595.

Olivares EL, Ribeiro VP, Werneck de Castro JP, Ribeiro KC, Mattos EC, Goldenberg RC, Mill JG, Dohmann HF, dos Santos RR, de Carvalho AC, Masuda MO (2004) Bone marrow stromal cells improve cardiac performance in healed infracted rat hearts. Am J Physiol Heart Circ Physiol 287: H464–H470.

Pereira RF, O'Hara MD, Laptev AV, Halford KW (1998) Marrow stromal cells as a source of progenitor cells for nonhematopoietic tissues in transgenic mice with a phenotype of osteogenesis imperfecta. Proc Natl Acad Sci USA 95: 1142–1147.

Philips ME, Mattiasson G, Wieloch T, Bjorklund A, Johansson BB, Tomasevic G, Martinez-Serrano A, Lenzlinger PM, Sinson G, Grady MS, McIntosh TK (2001) Neuroprotective and behavioral efficacy of nerve growth factor-transfected hippocampal progenitor cell transplants after experimental traumatic brain injury. J Neurosurg 94: 765–774.

Ribotta MG, Menet V, Privat A (2004) Glial scar and axonal regeneration in the CNS: lessons from GFAP and vimentin transgenic mice. Acta Neurochir (Suppl) 89: 87–92.

Saatman KE, Contreras PC, Smith DH, Raghupathi R, McDermott KL, Fernandez SC, Sanderson KL, Voddi M, McIntosh TK (1997) Insulin-like growth factor-1 (IGF-1) improves both neurological motor and cognitive outcome following experimental brain injury. Exp Neurol 147: 418–427.

Sanchez-Ramos J, Song S, Cardozo-Pelaez F, Hazzi C, Stadefortd T, Willing A, Freeman TB, Saporta S, Janssen W, Patel N, Cooper DR, Sanberg PR (2000) Adult bone marrow stromal cells differentiate into neural cells in vitro. Exp Neurol 164: 247–256.

Shih CC, Weng Y, Mamelak A, LeBon T, Hu MC, Forman SJ (2001) Identification of the candidate human neurohematopoietic stem-cell population. Blood 98: 2412–2422.

Spees JL, Olson SD, Ylostalo J, Lynch PJ, Smith J, Perry A, Peister A, Wang MY, Prockop DJ (2003) Differentiation, cell fusion, and nuclear fusion during ex vivo repair of epithelium by human adult stem cells from bone marrow stroma. Proc Natl Acad Sci USA 100: 2397–2402.

Suzuki H, Taguchi T, Tanaka H, Kataoka H, Li Z, Muramatsu K, Gondo T, Kawai S (2004) Neurospheres induced from bone marrow stromal cells are multipotential for differentiation into neuron, astrocyte, and oligodendrocyte phenotypes. Biochem Biophys Res Commun 322: 918–922.

Terada N, Hamayaki T, Oka M, Hoki M, Mastalerz DM, Nakano Y, Meyer EM, Morel L, Petersen BE, Scott EW (2002) Bone marrow cells adopt the phenotype of other cells by spontaneous cell fusion. Nature 416: 542–545.

Terskikh AV, Easterday MC, Li L, Hood L, Kornblum HI, Geschwind DH, Weissman IL (2001) From hematopoiesis to neuropoiesis: Evidence of overlapping genetic programs. Proc Natl Acad Sci USA 98: 7934–7939.

Tondreau T, Lagneaux L, Dejeneffe M, Massy M, Mortier C, Delforge A, Bron D (2004) Bone marrow-derived mesenchymal stem cells already express specific neural proteins before any differentiation. Differentiation 72: 319–326.

Tzeng ST, Wu JP (1999) Responses of microglia and neural progenitors to mechanical brain injury. Neuroreport 10: 2287–2292.

Woodbury D, Schwarz EJ, Prockop DJ, Black B (2000) Adult rat and human bone marrow stromal cells differentiate into neurons. J Neurosci Res 61: 364–370.

Yamaguchi S, Kuroda S, Kobayashi H, Shichinohe H, Yano S, Hida K, Shinpo K, Kikuchi S, Iwasaki Y (2006) The effects of neuronal induction on gene expression profile in bone marrow stromal cells (BMSC)-a preliminary study using microarray analysis. Brain Res 1087: 15–27.

Ye M, Chen S, Wang X, Qi C, Lu G, Liang L, Xu J (2005) Glial cell line-derived neurotrophic factor in bone marrow stromal cells of rat. Neuroreport 16: 581–584.

Yoshimura S, Teramoto T, Whalen MJ, Irizarry MC, Takagi Y, Qiu J, Harada J, Waeber C, Breakefield XO, Moskowitz M (2003) FGF-2 regulates neurogenesis and degeneration in the dentate gyrus after traumatic brain injury. J Clin Invest 112: 1202–1210.

Yoshizaki T, Inaji M, Kouike H, Shimayaki T, Sawamoto K, Ando K, Date I, Kobayashi K, Suhara T, Uchiyama Z, Okano H (2004) Isolation and transplantation of dopaminergic neurons generated from mouse embryonic stem cells. Neurosci Lett 363: 33–37.

Zhao LR, Duan WM, Reyes M, Keene CD, Verfaillie CM, Low WC (2002) Human bone marrow stem cells exhibit neural phenotypes and ameliorate neurological deficits after grafting into the ischemic brain of rats. Exp Neurol 174: 11–20.

Zhao LX, Zhang J, Cao F, Meng L, Wang DM, Li YH, Nan X, Jiao WC, Zheng M, Xu XH, Pei XT (2004) Modification of the brain-derived neurotrophic factor gene: a portal to transform mesenchymal stem cells into advantageous engineering cells for neuroregeneration and neuroprotection. Exp Neurol 190: 396–406.

Zhou Z, Chen H, Zhang K, Yang H, Liu J, Huang Q (2003) Protective effect of nerve growth factor on neurons after traumatic brain injury. J Basic Clin Physiol Pharmacol 14: 217–224.

Received 19 January 2007, accepted 9 March 2007