Interaction between neural stem cells and bone marrow derived-mesenchymal stem cells during differentiation

RONG JU[1*], WEN ZENG[1*], RONG WU[2*] and ZHICHUN FENG[3]

[1]Department of Neonatology, Chengdu Women's and Children's Central Hospital, Chongqing Medical University, Chengdu, Sichuan 610091; [2]Neonatal Medical Center, Huaian Maternity and Child Healthcare Hospital Affiliated to Yangzhou University Medical Academy, Huaian, Jiangsu 223002; [3]Department of Neonatology, Bayi Children's Hospital Affiliated to General Hospital of Beijing Military Command, Beijing 100700, P.R. China

Received July 15, 2014; Accepted December 5, 2014

DOI: 10.3892/br.2014.405

Abstract. Due to their capacity to self-replicate or produce specific differentiated cell types, neural stem cells (NSCs) and bone marrow derived-mesenchymal stem cells (BMSCs) are potential sources for cell transplantation therapies, particularly for neural injury. However, the interaction between NSCs and BMSCs during differentiation has not yet been defined. The interaction is believed to improve the effectiveness and efficiency of cell therapy. In the present study, human NSCs and BMSCs were cultured and the Transwell co-culture system was used to observe the interplay between NSCs and BMSCs during differentiation. The results revealed that NSCs promoted BMSCs to differentiate into neurons and NSCs; whereas, BMSCs did not affect the differentiation of NSCs. Simultaneously, co-culture increased the concentration of brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF), which are secreted by NSCs and BMSCs. The present findings suggest that co-culture of NSCs and BMSCs can promote the differentiation and this process may be modulated by BDNF and NGF.

Introduction

In recent decades, stem cells have attracted increasing attention due to their capacity to self-replicate or produce specific differentiated cell types and their potential as cell therapies for human disease (1). Neural stem cells (NSCs) that reside within the central nervous system (CNS) can differentiate into neurons, astrocytes or oligodendrocytes under specific conditions, which can help to develop new therapies for CNS disease (2-4). Bone marrow derived-mesenchymal stem cells (BMSCs) are multipotent stem cells that are capable of differentiating into a variety of lineages (5-7). Through stress signals, BMSCs show significant tropism for the injured site to manage the regenerative process via direct or indirect interactions (8-10). BMSCs are easily obtained and cultured and they can avoid the immune rejection through autologous transplantation. Therefore, BMSCs have emerged as ideal cellular material for cell and gene therapies in regenerative medicine (11,12).

However, it requires more effort to achieve the aim of cell and gene therapies using NSCs or BMSCs clinically. One main unsolved problem is how to modulate NSCs or BMSCs to differentiate into the required specific types of cells, which is the premise of a safe and effective treatment. In addition, the interaction between them is not yet clear. In the present study, the Transwell co-culture system was used to mimic the *in vivo* environment and the differentiation of NSCs or BMSCs was observed to investigate the interaction between them.

Materials and methods

Cell culture

Preparation and culture of human embryo NSCs. Primary dissociated cell cultures were prepared from the periventricular region of the telencephalon of a 15 weeks gestation, as described previously (13). A suspension of primary neural cells at a density of $5x10^5$/ml was plated on uncoated tissue culture dishes (Corning Life Sciences, Glendale, AZ, USA) in the following growth medium: Dulbecco's modified Eagle's medium/F12 (1:1) supplemented with N2 (1:50), B27 (1:100) (Invitrogen, Life Technologies, Carlsbad, CA, USA), basic fibroblast growth factor (Millipore, Billerica, MA, USA), heparin and epidermal growth factor (Sigma-Aldrich, St. Louis, MO, USA). Medium was changed every 3-4 days. Cell aggregates were dissociated into passage culture with 0.25% trypsin/1 mmol/l EDTA (Sigma-Aldrich) when the neurospheres were >10 cell diameters in size and replated in growth medium at a density of $5x10^5$/ml. Cells from passages 3 to 7 were used.

Correspondence to: Professor Zhichun Feng, Department of Neonatology, Bayi Children's Hospital Affiliated to General Hospital of Beijing Military Command, 5 Nan Mencang, Beijing 100700, P.R. China
E-mail: zhjfengzc@126.com

*Contributed equally

Key words: neural stem cells, bone marrow derived-mesenchymal stem cells, co-culture, differentiation, brain-derived neurotrophic factor, nerve growth factor

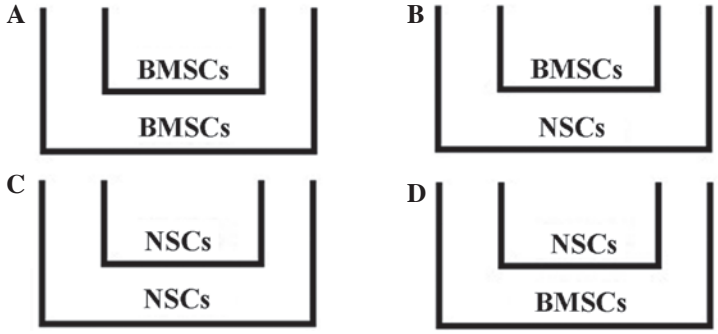

Figure 1. General scheme of the experimental groups. (A) Mono-culture of bone marrow derived-mesenchymal stem cells (BMSCs); (B) co-culture of neural stem cells (NSCs) and BMSCs, with BMSCs cultured in the Transwell chambers; (C) mono-culture of NSCs; and (D) co-culture of BMSCs and NSCs, with NSCs cultured in the Transwell chambers.

Preparation and culture of human bone marrow-derived BMSCs. Bone marrow cells were harvested from volunteer donors who had provided informed consent and the study was carried out according to local ethical guidelines. Briefly, primary BMSCs were generated as previously described (14). Cultures were maintained at 37˚C in a humidified atmosphere of 5% CO_2. Culture medium was replaced twice a week. BMSCs were harvested after reaching ≥90% confluence by digestion with 0.25% trypsin/1 mmol/l EDTA and replated into passage culture at a density of $1.0x10^6$/ml. All the BMSCs were cultured in <8 passages.

Transwell co-culture. To investigate the interaction of BMSCs and NSCs during differentiation, the Transwell chambers were used to co-culture BMSCs and NSCs and four experimental groups were established (Fig. 1). According to the experimental groups, BMSCs or NSCs were seeded onto the upper insert of a 6-well Transwell (with 0.4 μm pores; Millipore) at a density of $5x10^5$/ml placed above the NSCs or BMSCs, with the different culture medium. After 1, 4, 7, 10 and 14 days of culture, brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) were measured in the culture medium as described below. After 14-day culture, expression of neural markers was analyzed by immunofluorescence as described below.

Identifying the differentiation by immunofluorescence. For immunofluorescence, cells cultured in the Transwell chambers were fixed with 4% paraformaldehyde and permeabilized using 0.3% Triton X-100 in phosphate-buffered saline prior to blocking in 10% normal goat serum. Subsequently, the samples were incubated with primary antibodies at 4˚C overnight. Fluorochrome-conjugated species-specific secondary antibodies were used for immunodetection. Nuclei were stained with 4',6-diamidino-2-phenylindole (1 μg/ml; Sigma-Aldrich). The following antibodies and final dilutions were used: Rabbit anti-Nestin (1:250; cat. no. AB5922; Millipore), rabbit anti-microtubule-associated protein 2 (MAP-2) (1:200; cat. no. AB5622; Millipore) and goat anti-rabbit immunoglobulin G-cyanine 3 (1:1,000; cat. no. A10520; Invitrogen). The positive cells were counted per high-power field under a fluorescence microscope (Carl Zeiss Axiovert 200; Carl Zeiss Microscopy GmbH, Gottingen, Germany). Each sample was counted randomly in ten separated high-power fields.

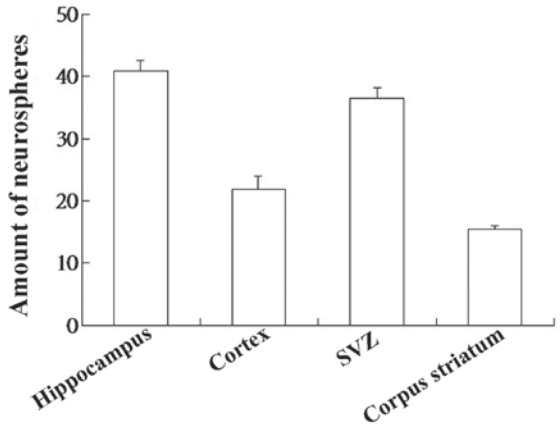

Figure 2. Distribution of neural stem cells in human embryo brain (n=5). SVZ, subventricular zone.

Enzyme-linked immunosorbent assay (ELISA). At the different time points of the co-culture, the media were collected and centrifuged at 10,000 x g at 4˚C for 5 min and the supernatants were stored at -20˚C. The immunoreactive levels of BDNF and NGF were measured by ELISA kits (Promega, Madison, WI, USA) according to the manufacturer's instructions. Absorbance at 450 nm was measured with a microplate reader (Bio-Rad, Hercules, CA, USA).

Statistical analysis. The results obtained were expressed as mean ± standard deviation of triplicate or quadruplicate cultures. One-way analysis of variance was performed for multiple comparisons. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Distribution of NSCs in human embryo brain. In the present study, the human embryo NSCs were cultured and distributions of NSCs varied in different regions of the human embryo brain. The number of NSCs in the hippocampus was higher than other regions ($P<0.05$; Fig. 2).

Interaction between NSCs and BMSCs during differentiation. Previous studies indicated that NSCs and BMSCs alleviated the damage of brain injury as they could differentiate into

Table I. Concentration of brain-derived neurotrophic factor at different time points in the four experimental groups.

Variables	7 days	10 days	14 days	F	P-value
Groups					
A	8.272±0.208	9.518±0.479	10.572±0.350	45.229	<0.001
B	9.004±0.286[a,b]	11.046±0.551[a,b]	12.282±0.698[a,b]	47.161	<0.001
C	8.248±0.274	9.610±0.642	10.726±0.253	41.890	<0.001
D	8.763±0.438[a,b]	10.702±0.514[a]	11.838±0.575[a,b]	47.033	<0.001
F	6.695	9.813	14.28		
P-value	0.003	0.001	0.000		

[a]Compared to group A, P<0.05; [b]compared to group B, P<0.05. Data are ng/l; n=5.

Table II. Concentration of nerve growth factor at different time points in the four experimental groups.

Variables	7 days	10 days	14 days	F	P-value
Groups					
A	9.808±0.329	12.146±0.865	14.852±0.502	86.139	<0.001
B	11.492±0.582[a,b]	14.168±0.322[a,b]	16.070±0.390[a,b]	133.383	<0.001
C	9.106±0.527	11.950±0.159	14.574±0.745	130.655	<0.001
D	10.850±0.454[a,b]	13.860±0.249[a,b]	15.828±0.451[a,b]	199.619	<0.001
F	24.262	27.895	9.143		
P-value	0.000	0.000	0.01		

[a]Compared to group A, P<0.05; [b]compared to group B, P<0.05. Data are ng/l; n=5.

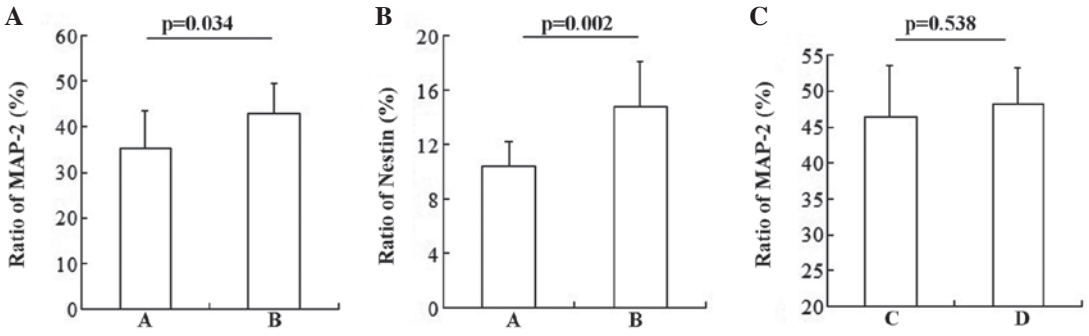

Figure 3. Differentiation of bone marrow derived-mesenchymal stem cells (BMSCs) and neural stem cells (NSCs) in different experimental groups (n=10). The ratio of (A) microtubule-associated protein 2 (MAP-2) and (B) Nestin positive cells that were differentiated from BMSCs in groups A and B; and (C) the ratio of MAP-2 positive cells that were differentiated from NSCs in groups C and D.

neural cells following transplantation (8,15,16). However, the interaction between them during differentiation remains unknown. To assess the interaction, the advantage of the Transwell co-culture system was used.

NSCs and BMSCs in the co-culture system were found to differentiate into neurons. The statistical result showed that NSCs promoted BMSCs to differentiate into neurons (Fig. 3A; group A vs. group B: 36.368±7.94 vs. 42.887±6.64%; P=0.034) and NSCs (Fig. 3B; group A vs. group B: 10.368±1.83 vs. 14.775±3.33%; P=0.002). However, BMSCs had no influence on the differentiation of NSCs (Fig. 3C; group C vs. group D: 46.377±7.15 vs. 48.116±5.07%; P=0.538).

Secretion of BDNF and NGF in co-culture. As known, NSCs and BMSCs secrete neurotrophic factors, including BDNF and NGF (17,18). The contribution of BDNF and NGF to a range of cell responses has been confirmed, including cell differentiation (19-21). Therefore, the level of BDNF and NGF in different experimental groups was detected. As the detection range of the ELISA kits is 7.8-500 ng/l, the concentration of BDNF and NGF at days 7, 10 and 14 was in the range (detection occurred at days 1, 4, 7, 10 and 14). The concentration of BDNF and NGF in the four groups increased with the prolonged time (P<0.05; Tables I and II). Compared to the mono-culture groups (groups A and C), a higher concentration

of BDNF and NGF was observed in the two co-culture groups (groups B and D) ($P<0.05$; Tables I and II). In addition, there was no significant difference in the concentration of BDNF and NGF between groups B and D ($P>0.05$).

Discussion

NSCs and BMSCs possess a significant expansion and differentiation potential, placing themselves as a potential source of material for cell transplantation therapies. Previous studies identified that transplanting BMSCs and NSCs decreased the apoptosis of neurons and improved the prognosis (10,15,22,23). However, the mechanisms are complicated and modulated by a variety of factors, such as cytokines, neurotrophic factors, inflammation and apoptosis (24-26). The interaction of NSCs with transplanted BMSCs and whether the interaction is beneficial to the treatment remains to be determined. Confirming these may aid in improving the understanding of the potential of cell therapy and improving the efficacy.

In the present study, the interaction between NSCs and BMSCs during differentiation was investigated and the following findings are considered: i) NSCs promoted BMSCs to differentiate into neurons and NSCs, although they do not come into direct contact; and ii) co-culture increased the level of BDNF and NGF. Similarly, certain studies also identified that NSCs induced the differentiation of BMSCs. Sanchez-Ramos *et al* (27) observed that the amount of neuron-specific nuclear protein-positive cells differentiated from BMSCs co-cultured with the brain tissue of fetal rat was approximately two-fold higher compared to the induced group of mono-cultured BMSCs.

In the present study, BMSCs did not affect the differentiation of NSCs; however, other studies exhibited conflicting results. Lou *et al* (28) reported that BMSCs induced NSCs to differentiate into more MAP-2 positive cells and Wang *et al* (29) also found that the interactions between MSCs and NSCs are involved in specifying neuronal fate.

Co-culture with Transwell chambers circumvents the contact of NSCs and BMSCs, however, the small soluble factors can pass through. Therefore, these small soluble factors may be involved in the interplay of NSCs and BMSCs during differentiation. NSCs and BMSCs can secrete BDNF and NGF, which play the crucial roles in cell differentiation and the concentration of the two neurotrophic factors was increased in the present study. Through binding to the tropomyosin-receptor-kinase (Trk) receptors (TrkA and TrkB), BDNF and NGF activated the downstream signaling. Previous studies indicated that BDNF and NGF modulated cell differentiation through the protein kinase B/mitogen-activated protein kinase pathway (21). BDNF has also been reported to possibly contribute to the differentiation of NSCs by triggering the Wnt/β-catenin signaling pathway (30). However, the mechanisms of BDNF and NGF in modulating the differentiation of NSCs and BMSCs requires further confirmation.

Taken together, the interaction of NSCs and BMSCs promoted BMSCs to differentiate into neurons and NSCs, which may be associated with BDNF and NGF. However, the underlying mechanism requires further clarification. To understand the interplay of NSCs and BMSCs during differentiation is likely to aid in obtaining new information for cell transplantation therapy in CNS disease.

References

1. Hess DC and Borlongan CV: Stem cells and neurological diseases. Cell Prolif 41 (Suppl 1): 94-114, 2008.
2. Song H, Stevens CF and Gage FH: Astroglia induce neurogenesis from adult neural stem cells. Nature 417: 39-44, 2002.
3. Johansson CB, Momma S, Clarke DL, Risling M, Lendahl U and Frisen J: Identification of a neural stem cell in the adult mammalian central nervous system. Cell 96: 25-34, 1999.
4. Temple S: The development of neural stem cells. Nature 414: 112-117, 2001.
5. Woodbury D, Schwarz EJ, Prockop DJ and Black IB: Adult rat and human bone marrow stromal cells differentiate into neurons. J Neurosci Res 61: 364-370, 2000.
6. Hermann A, Gastl R, Liebau S, *et al*: Efficient generation of neural stem cell-like cells from adult human bone marrow stromal cells. J Cell Sci 117: 4411-4422, 2004.
7. Lin W, Chen X, Wang X, Liu J and Gu X: Adult rat bone marrow stromal cells differentiate into Schwann cell-like cells in vitro. In Vitro Cell Dev Biol Anim 44: 31-40, 2008.
8. Zhao LR, Duan WM, Reyes M, Keene CD, Verfaillie CM and Low WC: 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, 2002.
9. Novikova LN, Brohlin M, Kingham PJ, Novikov LN and Wiberg M: Neuroprotective and growth-promoting effects of bone marrow stromal cells after cervical spinal cord injury in adult rats. Cytotherapy 13: 873-887, 2011.
10. Hawryluk GW, Mothe A, Wang J, Wang S, Tator C and Fehlings MG: An in vivo characterization of trophic factor production following neural precursor cell or bone marrow stromal cell transplantation for spinal cord injury. Stem Cells Dev 21: 2222-2238, 2012.
11. Hamada H, Kobune M, Nakamura K, *et al*: Mesenchymal stem cells (MSC) as therapeutic cytoreagents for gene therapy. Cancer Sci 96: 149-156, 2005.
12. El-Badri NS, Hakki A, Saporta S, *et al*: Cord blood mesenchymal stem cells: Potential use in neurological disorders. Stem Cells Dev 15: 497-506, 2006.
13. Flax JD, Aurora S, Yang C, *et al*: Engraftable human neural stem cells respond to development cues, replace neurons, and express foreign genes. Nat Biotechnol 16: 1033-1039, 1998.
14. Kafienah W, Mistry S, Williams C and Hollander AP: Nucleostemin is a marker of proliferating stromal stem cells in adult human bone marrow. Stem Cells 24: 1113-1120, 2006.
15. Chen JR, Cheng GY, Sheu CC, Tseng GF, Wang TJ and Huang YS: Transplanted bone marrow stromal cells migrate, differentiate and improve motor function in rats with experimentally induced cerebral stroke. J Anat 213: 249-258, 2008.
16. Chu K, Kim M, Park KI, *et al*: Human neural stem cells improve sensorimotor deficits in the adult rat brain with experimental focal ischemia. Brain Res 1016: 145-153, 2004.
17. Crigler L, Robey RC, Asawachaicharn A, Gaupp D and Phinney DG: Human mesenchymal stem cell subpopulations express a variety of neuro-regulatory molecules and promote neuronal cell survival and neuritogenesis. Exp Neurol 198: 54-64, 2006.
18. Llado J, Haenggeli C, Maragakis NJ, Snyder EY and Rothstein JD: Neural stem cells protect against glutamate-induced excitotoxicity and promote survival of injured motor neurons through the secretion of neurotrophic factors. Mol Cell Neurosci 27: 322-331, 2004.
19. Piirsoo M, Kaljas A, Tamm K and Timmusk T: Expression of NGF and GDNF family members and their receptors during peripheral nerve development and differentiation of Schwann cells in vitro. Neurosci Lett 469: 135-140, 2010.
20. Razavi S, Razavi MR, Kheirollahi-Kouhestani M, Mardani M and Mostafavi FS: Co-culture with neurotrophic factor secreting cells induced from adipose-derived stem cells: Promotes neurogenic differentiation. Biochem Biophys Res Commun 440: 381-387, 2013.
21. Yuan J, Huang G, Xiao Z, Lin L and Han T: Overexpression of β-NGF promotes differentiation of bone marrow mesenchymal stem cells into neurons through regulation of AKT and MAPK pathway. Mol Cell Biochem 383: 201-211, 2013.

22. Deng YB, Ye WB, Hu ZZ, *et al*: Intravenously administered BMSCs reduce neuronal apoptosis and promote neuronal proliferation through the release of VEGF after stroke in rats. Neurol Res 32: 148-156, 2010.
23. Shen CC, Lin CH, Yang YC, Chiao MT, Cheng WY and Ko JL: Intravenous implanted neural stem cells migrate to injury site, reduce infarct volume, and improve behavior after cerebral ischemia. Curr Neurovasc Res 7: 167-179, 2010.
24. Walker PA, Letourneau PA, Bedi S, Shah SK, Jimenez F and Cox CS Jr: Progenitor cells as remote 'bioreactors': Neuroprotection via modulation of the systemic inflammatory response. World J Stem Cells 3: 9-18, 2011.
25. Hokari M, Kuroda S, Shichinohe H, Yano S, Hida K and Iwasaki Y: Bone marrow stromal cells protect and repair damaged neurons through multiple mechanisms. J Neurosci Res 86: 1024-1035, 2008.
26. Walker PA, Harting MT, Jimenez F, *et al*: Direct intrathecal implantation of mesenchymal stromal cells leads to enhanced neuroprotection via an NFkabbaB-mediated increase in interleukin-6 production. Stem Cells Dev 19: 867-876, 2010.
27. Sanchez-Ramos J, Song S, Cardozo-Pelaez F, *et al*: Adult bone marrow stromal cells differentiate into neural cells in vitro. Exp Neurol 164: 247-256, 2000.
28. Lou SJ, Gu P, Chen F, He C, Wang MW and Lu CL: The effect of bone marrow stromal cells on neuronal differentiation of mesencephalic neural stem cells in Sprague-Dawley rats. Brain Res 968: 114-121, 2003.
29. Wang Y, Tu W, Lou Y, *et al*: Mesenchymal stem cells regulate the proliferation and differentiation of neural stem cells through Notch signaling. Cell Biol Int 33: 1173-1179, 2009.
30. Chen BY, Wang X, Wang ZY, Wang YZ, Chen LW and Luo ZJ: Brain-derived neurotrophic factor stimulates proliferation and differentiation of neural stem cells, possibly by triggering the Wnt/β-catenin signaling pathway. J Neurosci Res 91: 30-41, 2013.