Efficient differentiation of neural stem cells induced by the rat bone marrow stromal cells

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Abstract: Neural stem cells (NSCs) are valuable self-renewing cells that can maintain the capacity to differentiate into specific brain cell types. NSCs may repair and even replace the brain tissue, and ultimately promoting the central nervous system regeneration. Therefore, it is important, for scientists and physicians, to study the method for efficient culture and differentiation of NSCs. Our previous study demonstrated that Bone Marrow Stromal Cells (BMSCs) can directly regulate the differentiation of NSCs into neurons, and soluble molecules excreted by BMSCs played a key role in this process. Hereby, we further identified the BMSCs-induced neurons could form the synapses, convey dopamine and express voltage-depend and receptor-depend calcium channels. Moreover, the extracellular signal-regulated protein kinase ERK1/2 pathway was founded to be involved in the process of neuron differentiation and proliferation by the in vitro experiments. Finally, by using protein array, we, for the first time, found that the cytokine-induced neutrophil chemoattractant-3 (CINC-3, a small molecule cytokine) can promote the leukocytes invasion into the inflammation site, and have the ability to induce mesencephal NSCs into neurons. Consequently, these positive findings suggested that our BMSCs-induced culture system could provide a useful tool to investigate the molecular mechanisms of neural differentiation of NSCs, which may be beneficial for neurodegenerative diseases in the near future.

Keywords: Neural stem cells, bone marrow stromal cells, neurons, differentiation, protein microarray analysis, cytokines

Introduction

Bone Marrow Stromal cells (BMSCs) are multipotent stem cells, which can be cultivated in vitro and provide structural and functional support for hematopoiesis [1]. Neural stem cells (NSCs), on the other hand, maintain the capability of expansion and differentiation into neurons, astrocytes and oligodendrocytes in vitro. Importantly, NSCs have been reported to have therapeutic potential for CNS regeneration. However, NSCs are only found in the deep brain and this inaccessibility limits their clinical application. Therefore, it is important and urgent to identify alternative sources of NSCs.

Recently, BMSCs have been reported to express neurotrophic factors and cytokines including brain-derived neurotrophic factor, basic fibroblast growth factor, vascular endothelial growth factor, interleukins and transforming growth factor et al [2-5]. These neurotrophic factors and cytokines could affect the survival, multiplication and differentiation of NSCs [6-8]. In order to investigate the effect of micro-environmental signals provided by BMSCs on the differentiation of NSCs into neurons, astrocytes and oligodendrocytes, we co-cultured NSCs with BMSCs and found that BMSCs could induce NSCs to differentiate into neurons and glial cells. Interestingly, this effect was also observed in BMSC conditioned-medium. Thus, our study suggested that soluble factors excreted by BMSCs play a key role in regulating the differentiation of NSCs into neurons [9]. Furthermore, the present study explored the function of induced neurons and the cytokines and the signal transduction pathway being involved in the process.
Differentiation of neural stem cells

Materials and methods

Reagents and animals

Dulbecco's modified Eagle's medium DMEM, DMEM/F12 (1:1), Neurobasal TM medium with B27 supplement were purchased from Gibco BRL (MD, USA). bFGF, Poly-L-lysine (PLL), primary antibodies and secondary antibodies labeled with Fluorescein isothiocyanate (FITC) were obtained from Sigma (MO, USA). Fetal bovine serum was purchased from Sino-American Biotechnology Company (Shanghai, CHINA). PD98059 (Pragma) is a potent cell permeable inhibitor of MAP kinase (MEK), and selectively blocks the activation of MEK, thereby inhibiting the phosphorylation and the activation of MEK. The Model 8400 Stirred Ultrafiltration Cells and 5KDa acetyl cellulose ultrafiltration membrane were obtained from Millipore (MA, USA). In addition, adult and neonatal Sprague-Dawley (SD) rats were purchased from Experimental Animal Center of Hebei Medical University.

Preparation of BMSCs

BMSCs were isolated and cultured as previously described with minor modification [11]. Briefly, at the weight of 120 g, the SD rats were euthanized by injecting a lethal dose of sodium pentobarbital (250 mg/kg, i.p.). The BMSCs were collected from femurs and tibias by flushing the shaft with sterile DMEM using a 5ml syringe for 2-3 times. Cell suspension was then centrifuged at 800 r/min for 5min, the pellet was re-suspended in DMEM (contain 20% fetal bovine serum) and plated at a density of 106 cells/ml in the 75 cm² glass flask. The BMSCs were cultured at 37°C in 5% CO₂ atmosphere, and the medium would be changed after 48 hours and every 3-4 days. When the cultures reached 85% of confluence, cells were recovered by the addition of a solution containing 0.25% trypsin and replanted at a density of 105 cells/ml.

Preparation of BMSCs conditioned medium and ultrafiltrate

The 3-6 passages of BMSCs were plated in 25 ml culture flask. When the BMSCs grew to 85% confluence, the medium was removed and changed with 6ml Neurobasal medium. The medium was collected after 24 h and regarded as BMSCs conditioned medium (BMSCS-CM), stored in -800 C after centrifuging. Totally, we collected 300 ml BMSCS-CM, then we separated the BMSCS-CM into two parts by a 5KDa ultrafiltration membrane with Stirred Ultrafiltration Cells Model 8400 (Millipore Corporation). Finally, 30 ml medium left above the cells was collected and contained proteins > 5KDa. On the other hand, proteins < 5KDa were also collected. Both parts of medium were filtered through a 0.22 μm filter to exclude cells and degemming. Determined by spectrophotometer, protein concentrations of original BMSCS-CM, BMSCS-CM (> 5KDa) and BMSCS-CM (< 5KDa) were 1.06 g/L, 1.65 g/L and 1.16 g/L, respectively. We also used 60 ml neurobasal medium with 2% B27 supplement as control sample.

Isolation and proliferation of NSCs

As previously described, midbrains of 1-2 days SD rats were cut into small pieces and then gently triturated for 10-15 times. The tissue suspension was centrifuged at 500 g for 5 min; the pallets were resuspended in the proliferation medium. Then the cells were plated at a concentration of 105 cells/ml in 25 cm² flasks at 37°C in 5% CO₂ atmosphere, with a change of half medium per 3 days. After 7 days in vitro (DIV), the floating neurospheres formation could be observed. These neurosphere-like structures were expanded, and more generations of NSCs were made in the same procedure.

The effect of BMSCS-CM on NSCs

After 2-3 passages, the NSCs were harvested and cultured on PLL coated 35 mm dishes. For once the neurospheres adhere to dish bottom, the medium was changed by DMEM with 2% B27 supplement. Totally, we set up 4 groups: group 1 is the control group, NSCs were cultured with DMEM and 2% B27 supplement, group 2 is BMSCS-CM group, namely, NSCs were cultured with BMSCS-CM, group 3 is BMSCS-CM > 5KDa group and group 4 is BMSCS-CM < 5KDa group. Cellular immunofluorescence staining was performed at the 4th day after differentiation. The following anti-mouse antibodies were used: microtubule-associated protein 2 (MAP-2), glial fibrillary acidic protein (GFAP), and glial antigen 2 (NG2), were obtained from Sigma. Only the MAP2-positive cells with unambiguous neuronal morphology were counted as neurons.
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The rat cytokine antibody array

In this study, we applied antibody array technology to determine the expression profiles of 19 cytokines in the BMSCs-CM and controls, separately. Cytokines in this study were summarized in Table 1.

Table 1. Ray biotech rat antibody array

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Pos, positive control; Neg, negative control; CINC, cytokine-induced neutrophil chemoattractant; CNTF, cholinergic neurotrophic factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; IL, interleukin; LIX, lipopolysaccharide-induced CXC-chemokine; MCP, monocyte chemotactic protein; MIP, monocyte inflammatory protein; NGF, nerve growth factor; TIMP, tissue inhibitor of metalloproteinases; TNF, tumor necrosis factors; VEGF, vascular endothelial growth factor; BLK, blank control.

The signal transduction pathway that BMSCs-CM may be involved to regulate the differentiation of NSCs

NSCs were cultured on PLL coated 35 mm dishes. After the neurospheres adhered on the dish bottom 30 min, we changed the culture solution of the control group with BMSCs-CM, meanwhile, the inhibitor group was changed with BMSCs-CM, which contained 5 µmol/L PD98059, Then, the culture solution was changed per 3 days. After 7 days culture, the neurons were examined and counted by immunocytochemistry.

The expression profile of synaptophysin, VMAT2 and calcium channel of the induced neurons from NSCs

At different time points, the expressions of synaptophysin, VMAT2 and tyrosine hydroxylase of differentiated NSCs were examined by labeling them by distinct antigens. Neuron generation on the 5th day of incubation was washed with Krebs Ringer Bicarbonate Buffer twice, then incubated with fluorescent probe Fura2/AM (final concentration of 1.6 µmol/L) at 37°C for 40 minutes and washed with Kreb’s buffer again for 3 times. We used the inverted fluorescence Microscope, MetaFlour software system, and the computer-controlled light source to provide 340 nm and 380 nm excitation light. The fluorescence signal of Fura2-Ca2+ and Fura-2 were detected on 340 nm and 380 nm excitation light and dealt with MetaFlour software system. The fluorescence signal ratio = (F340/F380) was calculated to display the real-time changes of [Ca2+] in cells. Then KCl (55 mM) and glutamate (100 mM) was used to stimulate the cells. After that, we immediately studied intracellular calcium alteration in these cells.

Rat cytokine antibody array

In this study, we applied antibody array technology to determine the expression profiles of 19 cytokines in the BMSCs-CM and controls, separately. Cytokines in this study were summarized in Table 1.

Statistical analysis

All data were presented as the mean ± standard error of the mean (SEM) analysed by using SPSS 11.5 software. The data of cell culture were assessed by Student-T test. A P < 0.05 was considered statistically significant.

Results

Characterizations of BMSCs and NSCs

Primary cultures of BMSCs contained a small population of bright round cells that were gradually disappeared with the process of passages. To define the cellular phenotypes, we specifically used antibody against the CD71 (a known marker of BMSCs). As shown in Figure 1A, most of cultured cells were CD71 positive.
Moreover, in order to exam the stem cell characteristics of neurospheres derived from mid-brain of newborn SD rats, the second generation neurospheres were labeled by nestin antibody. As shown in Figure 1B, most cells in the neurospheres were nestin positive, which indicated their stem cell characteristics.

The appearance of major CNS cell lineages, such as neurons, astrocytes and oligodendrocytes, is the primary characteristic of NSCs. In order to confirm the nestin + neurospheres were multipotential and to study the effect of BMSC-CM on NSCs, we cultured secondary neurospheres on PLL coated 35mm dishes, the medium was changed for BMSCs-CM and labeled with MAP-2 GFAP and NG2 for neuron, astrocyte and oligodendrocyte, respectively.

After 7 days cultures, the MAP-2+, GFAP+ and NG2+ cells were observed. Specifically, the MAP-2+ neurons and NG2+ oligodendrocytes scattered along with GFAP+ astrocytes. These findings further confirmed that the BMSCs-CM could induce NSCs to differentiate the three main neural phenotypes in vitro (Figure 2).

The physiological function of neurons induced from NSCs by BMSCs-CM

To determine whether the neurons induced by BMSCs-CM have the basic physiological function, such as forming synapse, we observed the expression of synaptophysin on these neurons. Accordingly, secondary neurospheres were cultured on PLL coated 35 mm dishes and the

**Figure 1.** A. CD71 positive BMSCs. B. Nestin positive NSCs (1 × 400).

**Figure 2.** A. MAP-2+ neurons. B. GFAP+ astrocytes. C. NG2+ oligodendrocytes (1 × 200).
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medium was changed into BMSCs-CM, the progeny cells were labeled with synaptophysin antibody at day 3, 7, 9, 12 and 18 in culture, respectively. Until day 12, weak synaptophysin expression signal was detected on the neurons. At day 18, we detected bright bead-like fluorescence signal on the ecptomas of neurons (Figure 3).

Figure 3. Synaptophysin expression in neurons derived from NSCs of mesencephalon. A. Neurons derived from NSCs of mesencephalon on 12th day of differentiation. B. Neurons derived from NSCs of mesencephalon on 18th day of differentiation (150 ×).

Figure 4. BMSCs-conditioned medium (BMSCs-CM) induced the neurons derived from NSCs of mesencephalon. The neurons expressed TH and VMAT2 protein. A, D. TH immunostaining. B, E. VMAT2 immunostaining. C, F. Merged image (200 ×).
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The progeny cells were double labeled with tyrosine hydroxylase (TH) and vesicular monoamine transporter (VMAT2) at day 7 after differentiation. The majority of TH+ neurons expressed VMAT2 was observed, which suggests that these cells possessed the character of midbrain dopamine neuron (Figure 4).

The fluorescence ratio of $[\text{Ca}^{2+}]$ changes in nerve cells using fluorescent microscope digital imaging system

Fura-2 fluorescence was obtained using Fluorescent microscope digital imaging systems. The MataFlour software was used to calculate the average fluorescence intensity change curve in the function of time. The fluorescence ratio remained at a relatively low level when cells were in static state. But, after the treatment by KCl (55 mmol/L) or glutamate (100 mmol/L), the fluorescence ratio significantly increased, which demonstrated that the cells were quickly polarized (Figure 5).

To choose the most effective BMSCs-CM after segmenting

After 7 days culture in vitro, we investigated the impact of various culture solutions on the differentiation of NSCs: (1) control group: The cells were not in good condition, some cellular nucleus shrink and broke into pieces. Compared with other groups, the MAP-2+ neurons were fewer and mainly assembled around the neurospheres. And the eptoma of neurons and NG2+ oligodendrocytes were shorter. More GFAP positive astrocytes were detectable in this group. (2) BMSCs-CM group: Overall, the cells were much viable than the control group. The majority of cell nuclei were intact. Compared with neurobasal group, more MAP-2+ neurons were identified by deeper staining. The eptoma of neurons and oligodendrocytes were longer than those in neurobasal group. (3) BMSCs-CM > 5KDa group: This was the best cell culture result among these groups. The majority of cellular nuclei were intact and equal in size. The MAP-2 positive neurons took up most of the offsprings. The eptoma of neurons and NG2+ oligodendrocytes were long. There were less GFAP+ astrocytes than other groups. (4) BMSCs-CM < 5KDa group: The cell cultures were in poor quality; actually, we observed a lot of dead cells at 7 days (Figure 6).

The results of cell counting and statistical analyses: (1) The proportion of MAP-2 positive neurons: The percentage of MAP-2+ neurons in the BMSCs-CM group (22.16 ± 9.11%) was significantly higher than that in the control group (17.00±7.69%, P = 0.021, P < 0.05), which indicated that BMSCs-CM could promote more NSCs to differentiate into neurons. The percentage of MAP-2+ neurons in BMSCs-CM > 5KDa group (34.24 ± 15.94%) was significantly higher than N-CM group (P = 0.0006, P < 0.01), which implied that the fraction of BMSCs-CM > 5KDa played an important role in neuron differentiation. There was lack of data of N-CM < 5KDa group due to the dead cells. (2) The proportion of NG2 positive oligodendrocytes: The percentage of NG2+ oligodendrocytes in BMSCs-CM group (25.82 ± 7.79%) was higher than that in the control group (21.32 ± 9.09%,

![Figure 5. Intra-cellular $[\text{Ca}^{2+}]$ response induced by KCl (A) and glutamate (B) of the neurons derived from NSCs of mesencephalon.](image)
Differentiation of neural stem cells

$P = 0.044, P < 0.05$); moreover, the BMSCs-CM > 5KDa group (41.01 ± 8.88%) had more oligodendrocytes than BMSCs-CM group ($P = 0.000, P < 0.01$), which illustrated that the proteins > 5KDa from BMSCs-CM could promote NSCs to differentiate into more oligodendrocytes. (3) The quantity of GFAP positive astrocytes: the percentage of GFAP$^+$ astrocytes in BMSCs-CM > 5KDa group (23.10 ± 13.57%) was significantly lower than that in the control group (37.99 ± 10.75%, $P = 0.000, P < 0.01$) and BMSCs-CM group (35.07 ± 8.96%, $P = 0.001, P < 0.01$), but the difference between the other two groups was not significant. This result indicated that BMSCs-CM > 5KDa could inhibit NSCs to differentiate into astrocytes (Figure 7).

Taken together, BMSCs-CM with proteins > 5KDa had a direct and pronounced effect on the genesis of neurons and oligodendrocytes, but not the astrocytes.

The effect of ERK1/2 signal transduction pathway exerted on the process of NSCs differentiation induced by BMSCs-CM

At the 7th day of intervention, the percentage of neurons in PD98059 group [(39.48 ± 7.29)%] was significantly lower than the control group (51.17 ± 10.30%), $P < 0.01$) (Figure 8).

Figure 6. The various culture solutions on the differentiation of NSCs. A. The control group. B. BMSCs-CM group. C. BMSCs-CM > 5KDa group. D. BMSCS-CM < 5KDa group (300 ×).

Figure 7. The comparation of the percentage of neurons, oligodendrocytes and astrocytes in three groups. (*$P < 0.05$, **$P < 0.01$ as compared with the Neurobasal group, #$P < 0.05$, ##$P < 0.01$ as compared with the N-CM group).
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Rat cytokine antibody array

Cytokine antibody array analysis was conducted to determine the effects of medium from BMSC-CM on the expression of proinflammatory proteins. There were seven cytokines, including cytokine-induced neutrophil chemoattractant-3 (CINC-3), cholinergic neurotrophic factor (CNTF), immunoreactive fibronectin (IFN-γ), interleukin-1α (IL-1α), monocyte chemoattractant protein (MCP-1), tissue inhibitor of metalloproteinase (TIMP-1) and vessel endothelium...
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growth factor (VEGF) were found to be up-regulated 1.5 times in the BMSCs-CM > 5KDa compared with that from neurobasal medium (molecular weight > 5KDa). However, no cytokines were found to be down-regulated (Figure 9).

Discussion

NSCs, which are presented in the developing nervous system as well as the adult nervous system of human, can differentiate into neurons and gliocytes, and maintain the capability of disintegration, multiplication and maturity in vivo and in vitro [10]. Therefore, it is critical to study NSCs in vitro culture and their potential functions. The central findings of this study is that, under the BMSCs conditioned medium (BMSCs-CM), rat mesaticephalic NSCs could grow into neurosphere-like structure, express nestin (the stem cell marker) and differentiate into three major neural phenotypes: neurons, astroglia and oligodendroglia, in vitro. We also demonstrate that the BMSCs-CM induced NSCs have multipotent ability and the capacity of self-renewing.

It has been widely accepted that neurons have two main functions: transporting action potential (nerve impulses and signal) along the axon, and transferring the signal from one neuron to another or triggering corresponding reaction. Calcium is an electrochemical factor that can polarize the neurons and maintain the normal function of nerve. It is also an important messenger between neurons. Calcium channel, as a kind of functional protein presented on the neuron membrane, help to maintain neuronal physiological state. Using the fluorescent microscope digital imaging technology, we found the resting state ratio of neurons derived from NSCs remained at a relatively low level, however, after the KCl or glutamate treatment, the fluorescence ratio increased significantly. Our study indicates that the BMSCs-CM induced NSCs could express VMAT2 receptor and voltage-dependent calcium channels, which are the basics for neuron exciting.

In the nervous system, synapses are structures that permit neurons to pass electrical or chemical signals to another cell, which are essential to neuron function of synaptic transmission. Synaptophysin was the first cloned synaptic vesicle protein in 1985, since then, it has been widely used as an invaluable marker to study the distribution of synapses in the brain and to unveil the basic features of the life cycle of synaptic vesicles. It can play important role in the package, storage, adjust and release neurotransmitters [11]. Little is known, however, about the extent to which stem cells in adults can generate new neurons. In our recent work, we found that the BMSCs-CM could induce adult rat NSCs to develop into electrically active neurons and integrate into neuronal networks with functional synaptic transmission. In other words, the neural stem cells derived from adult tissues, like those ones derived from embryonic tissues, retain the potential to differentiate into functional neurons with essential properties of mature CNS neurons.

Song et al demonstrated that, co-cultured with astrocytes from neonatal hippocampus, neurons derived from rat NSCs can express synaptophysin, form synapses with hippocampus neurons and even evoke a postsynaptic potential, though, this total process may take up to two weeks [12]. We found that, at day 3, 7 and 9, there was lack of prominent synapse expression in the neurons induced by BMSCs-CM. The synaptophysin expression was unable to be detected until 12 days culture, though there was bead-like bright fluorescent signal along the neural axon at day 18. These results suggested that, after certain amount of culture time, BMSCs-CM can induce neurons to form synapses.

Neurotransmitters are specific chemical messengers in synaptic transmit process. Dopamine (DA) is an important monoamine neurotransmitter. It has been reported that high level of DA in the cytoplasm of the mesencephal DA neurons caused oxidation and free radicals, which resulting in mitochondrial respiratory depressed and cytotoxicity [13]. On the other hand, DA needs vesicles monoamine transporter 2 (VMAT2) to mediate its storage and release. VMAT is constitutively expressed in the monoamine neurons of caudate nucleus, nucleus accumbens, substantia nigra, ventral tegmental area, blue grouper, in the joints and nuclear group solitary nucleus. It has been recognized that its function is mainly transferring monoamine neurotransmitter from cytoplasm to vesicles for storage, thus reducing the level of DA in cytoplasm [14]. By the double immunofluorescence staining of TH and VMAT2, our work
revealed that, at 7 days of differentiation, all 
TH-positive neurons are VMAT2 positive. Given 
the fact that the TH is a DA neuron-specific pro-
tein, our result may imply that DA neurons 
derived from mesencephal NSCs have the 
structural property to transmit DA.

Recent studies identified that BMSCs could 
speed up the process of repairing damaged 
CNS tissue and restoring their functions, 
though the exact mechanism is not clear yet. It 
is plausible that BMSCs could differentiate into 
cells with nervous phenotypes in CNS damaged 
areas, and subsequently replace the missing 
neural cells [15]. Besides, BMSCs can also 
secrete nutrients to stimulate endogenous 
NSCs for nerve regeneration [16]. By compar-
ing the proportion of neurons in three culture 
groups, namely NSCs-BMSCs co-culture group, 
fixed BMSCs group and BMSCs-CM group, our 
previous study found that the NSCs from 
BMSCs-CM co-culture group could differentiate 
into high proportional neurons [9]. Therefore 
we concluded that the soluble factors secreted 
by BMSCs were responsible for its effect on the 
neuronal differentiation of NSCs.

According to the published data, we noticed 
that the molecular weight of most neurotrophic 
factors is above 5KDa, thus we divided BMSCs-
CM into two parts, molecular weight > 5KDa 
and that < 5KDa. Meanwhile, this process also 
increased the concentration of nutrients and 
removed small molecule materials in BMSCs-
CM. Our results showed that the BMSCs-CM > 
5KDa were able to regulate the differentiation 
of NSCs into higher proportional neurons, 
Therefore, it is important to further identify that 
what kinds of neurotrophic factors contribute to 
the differentiation of NSCs.

Rat Cytokine Antibody Array revealed that 7 
cytokines, including cytokine-induced neutro-
phil chemoattractant-3 (CINC-3), cholinergic 
neurotrophic factor (CNTF), immuno-
reactive fibronectin (IFN-γ), interferon-1α (IL-1α), 
monocy-
to chemoattractant protein (MCP-1), tissue 
inhibitor of metalloproteinase (TIMP-1) and ves-
SEL endothelium growth factor (VEGF), were 
found to be up-regulated 1.5 times in BMSCs-
CM > 5KDa culture group as compared with the 
control group. CINC-3 is a small molecule cyto-
kine and can anti-apoptotic and promote inva-
sion of leukocytes into the inflammation site 
[17, 18]. Notably, it has been reported that 

IL-1α, CNTF, VEGF could exert effects on NSCs 
differentiation. Specifically, IL-1α was found to 
induce mesencephal NSCs into TH+ neurons 
[19]. Accordingly, our study also observed large 
numbers of TH+ neurons in the mesencephal 
NSCs offspring cells induced by IL-1α (data not 
shown). Moreover, CNTF was found to induce 
NSCs into astrocytes [20] and promote oligo-
dendrocyte survival [21]. Lastly, VEGF can 
 promote neurogenesis, which is involved in ner-
vous nutrition and protection, and could 
 promote NSCs proliferation in vitro [22]. BMSC-
CM included cytokines such as insulin-like 
growth factor-1 VEGF, NGF, BDNF and IL-6 [18, 
23]. BMSC-CM enhanced the migration, prolif-
eration, and expression of osteogenic marker 
genes and enhanced bone regeneration in rat 
calvarial bone defects [23]. Our previous 
research indicated that transplantation of 
NSCs combined with BMSC-CM could improve 
rotational behavior and cognitive ability of PD 
rats, which is similar to corpus striatum trans-
plantation of simple NSCs [24]. Dorothée 
Cantinieaux delivered BMSC-CM to spinal cord 
jinjured rats in vitro and found BMSC-CM pro-	ected neurons from apoptosis, activates mac-
rrophages and is pro-angiogenic [18]. In vivo, 
BMSC-CM administered after spinal cord con-
tusion improves motor recovery. Histological 
analysis confirms the pro-angiogenic action of 
BMSC-CM, as well as a tissue protection effect. 
Identified trophic factors as well as cytokines 
factors of BMSC-CM were by cytokine array and 
ELISA likely involved in the modulation of the 
spinal cord. Those studies also supported the 
paracrine-mediated mode of action of BMSCs 
and raise the possibility to develop a cell-free 
therapeutic approach.

It has been well established that ERK1/2 
played an important role in cell proliferation, 
inversion and differentiation [25]. In order 
to investigate the role of ERK1/2 of neurons 
induction and differentiation, we applied PD-
98059 (ERK1/2 signal transduction pathway 
inhibitor) into BMSCs-CM co-culture NSCs and 
found a significantly reduced quantity of NSCs 
offspring neurons compared to the control 
group (P < 0.01). Therefore, ERK1/2 signal 
transduction pathway may be involved in the 
process of BMSCs-CM induced differentiation 
of NSCs.

Taken together, our research, first found that 
neurons, derived from NSCs and induced by
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BMSCs, have the basic neuronal physiological functions. Additionally, an independent lab found that BMSCs could be directly induced to differentiate into NSCs [26], which confirming the feasibility of our methods. Our work in vitro further explored the potential regulating mechanisms of BMSCs in the NSCs differentiation. Consequently, this present study could help to provide theoretical basis and promote the clinical application of BMSCs.

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Disclosure of conflict of interest

None.

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