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In vitro neural differentiation of bone marrow stromal cells induced by hepatocyte growth factor and glial cell derived neurotrophic factor

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Abstract. – OBJECTIVE: Bone marrow stromal cells (BMSCs) have great potential for cell-based transplantation therapy in treating neurological disease. However, the best combination of various trophic factors to produce full neural differentiation of BMSCs was still unclear. In our study, we aimed to investigate the neural differentiation capacity of rat BMSCs induced by growth factors including hepatocyte growth factor (HGF) and glial cell-derived neurotrophic factor (GDNF).

MATERIALS AND METHODS: Cell counting kit-8 (CCK-8) assay, BrdU cell proliferation assay and flow cytometry were implemented to evaluate whether GDNF and HGF had positive effects on the proliferation of BMSCs. Moreover, the expression of neural specific markers in BMSCs was identified using immunofluorescence and quantitative real-time polymerase chain reaction (RT-PCR) at various time points (1, 7, 14 and 21-day post-induction).

RESULTS: CCK-8 and BrdU proliferation analyses demonstrated that only HGF treatment had positive effects on the proliferation of BMSCs on the day 14 and 21 after incubation. RT-PCR and immunofluorescence analyses showed that GDNF and HGF elevated the expression of nestin and NCAM, and the combined application of GDNF and HGF has the most significant effect on day 7 after induction. However, at the day of 14 and 21 post-induction, the expression level of nestin and NCAM in GDNF-treatment group was significantly higher than the other three groups.

CONCLUSIONS: HGF, not GDNF plays a positive role in BMSCs proliferation, whereas GDNF and HGF are capable of promoting BMSCs to differentiate into neuron-like cells.

Key Words:
Bone marrow stromal cells, Cell therapy, Hepatocyte growth factor, Glial cell derived neurotrophic factor, Neural differentiation.

Introduction

Cell therapy has been proposed as a promising therapy for nervous system diseases[1,2]. However, the neural cell therapy for nervous system diseases is hindered because of the technical difficulties in harvesting autologous neural stem cells. Fortunately, neural cells are able to be produced by bone marrow stromal stem cells (BMSCs) *in vitro* and *in vivo*[3,4]. Moreover, little doubt exists that BMSCs stand for one of the ideal candidates for cell therapy. Relative to embryonic stem cell or neural stem cells, BMSCs are relatively easier to be separated, expanded and proliferated rapidly, without ethical and immunological problems[5]. Thus, BMSCs have attracted the interest of researchers in the potentiality of neuronal-like differentiation and in the possible cytotherapy of neurological diseases[6]. Moreover, the effects of BMSCs on the injury of the central nervous system have frequently been reported [7,8]. The differentiation of BMSCs *in vitro* serves as one logical objective[9,10]. That is, differentiated neural cells, relative to active precursors, are usually believed to have a lower chance of malignant transformation. Hence, it is of significance to establish a method of transforming BMSCs into neural-like cells.

Although the great interest in BMSCs, no well differentiation protocol of BMSCs into neuron-like cells has been determined. Woodbury et al[11] initially reported the differentiation of BMSCs into neuron-like cells *in vitro* using chemical reagents, by which 80% BMSCs were turned into neuron-like shape within a few hours. However, chemicals treatments have been debated for rapid induction process due to their cytotoxicities[12]. Moreover, the application of chemical compounds could lead to

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such serious damage to the cell that morphological change was induced rather than neuronal differentiation[13]. In addition, growing evidence has demonstrated that a plenty of neuronal differentiated cells obtained by the chemical protocol go with a high rate of cell death[14]. On the account of the toxicity and the spurious neuronal differentiation, it seems extremely unpromising that clinical approval could be granted for the use of chemical compound-based differentiation methods. Fortunately, promising results have been demonstrated with induction protocols after BMSCs are exposed to trophic factors such as epidermal growth factor (EGF) or brain-derived neurotrophic factor (BDNF) or glial cell-derived neurotrophic factor (GDNF)[15-17], which allows neuron-like cells to be derived from BMSCs. For example, Sanchez-Ramos et al[18] utilized retinoic acid and growth factor including EGF at the induction step, and the expression of nestin, a marker of neural precursors, was observed. In 2011, Bae et al[19] demonstrated that BMSCs could differentiate into neuron-like cells induced by growth factors including EGF, hepatocyte growth factor (HGF), and vascular endothelial growth factor (VEGF). The results mentioned in aforementioned methods indicated the potential of neural differentiation of BMSCs. However, the protocol based on the use of trophic factors has side reactions, because trophic factors may act as mitogen agents to further increase BMSC proliferation[20]. Moreover, the best combination of various trophic factors to produce full neural differentiation of BMSCs was still unclear. The aim of the present study was to establish a useful and available solution for transforming BMSCs into neural-like cells.

GDNF, one distant member of the transforming growth factor-β (TGF-β) superfamily, was firstly cloned and purified from the B49 glial cell line[21]. Wang et al[22] suggested that GDNF played a significant role in the development of nervous system via inducing the differentiation of neural crest cells, which entered gastrointestinal tract to form enteric neurons. Another study[23] indicated that GDNF and NT-3 induced the BMSCs differentiation and neuron-like features including the expression of both NSE and nestin. HGF binds to the tyrosine kinase receptor[24] and then exerts important functions as a pleio-trophic growth factor[25]. Significantly, tyrosine kinase receptor and its ligands are expressed in the neural tissue, and they exert the prominent action in differentiation and regeneration of neurons.

Nevertheless, it is unknown whether or not the combination of HGF and GDNF would enhance the effects of BMSCs differentiation. Herein we investigated whether the growth factors, GDNF and HGF, could stimulate the differentiation of BMSCs into neuronal-like cells and whether the proliferation would alter the BMSCs differentiation. The possible application of GDNF and HGF as an inducer for neuronal induction makes these growth factors being available for cell therapy.

Materials and Methods

Animals

Sprague-Dawley adult male rats, weighing 130 g, were purchased from the Laboratory Animal Center of the Academy of Military Medical Sciences (Beijing, China) [Animal production license No.: SCXK-(JUN)2012-0004]. All animal experiments were implemented based on the Guidelines for the Care and Use of Laboratory Animals, with animal Ethics Committee license, in our university.

BMSC Isolation and Expansion

BMSCs were isolated from the bone marrow of Sprague-Dawley rats. In short, the rats were injected with an overdose of 3% pentobarbital. Femora as well as tibiae were dissected. Moreover, musculature and soft tissues were cleaned. Under sterile conditions, 0.5-1 mL bone marrow was extracted from the femur and tibia and syringed using the solution containing 5 mL heparin (100 U/ml, Sigma-Aldrich, St. Louis, MO, USA), then diluted using PBS, followed by trituration and suspension with Hanks' balanced salt solution (HBSS). Then, the isolated cells were gathered by centrifugation at 500 g for 5 min. Cells were counted and seeded into 24-well plates at a density of 9×10^5 cells/mL in Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal calf serum (FCS), penicillin (100 U/mL), as well as streptomycin (100 U/mL) in a humidified incubator at an atmosphere of 95% 02-5% CO^2 at 37°C till 70-80% confluence was reached. Non-adherent cells were washed away after 3 days and adherent cells were fed with fresh complete medium. The cells were sub-cultured at the split ratio of 1:2 after attaining the confluence. The BMSCs were employed for forthcoming experiments after three passages.

Neural Induction

The neuron induction was implemented at the 3rd passage. BMSCs were cultured in plates at a density of 10^4 cells/cm^2. When the seeded cells

were confirmed to be viable and adherent to the bottom of the plates, cells were removed from the bottom of plate and were replaced with the neuronal induction medium: (1) GDNF (10 ng/mL), (2) HGF (20 ng/mL), (3) GDNF (10 ng/mL) + HGF (20 ng/mL), and (4) the normal BMSCs receiving neither induction as control. The differentiated cells after 1, 7, 14, and 21-day of induction were processed for subsequent study.

Cell Counting Kit-8 (CCK-8) Assay

Cell viability was measured by CCK-8 assay after differentiation according to previous reports[26,27]. Briefly, cells were seeded in 96-well plates at a density of 2×10^4 cells per mL. After cell induction, cells were rinsed three times with PBS. Then, CCK-8 dye (Bestbio, China) and DMEM cell culture medium (1:10) were added to each well and incubated for 2 h at an atmosphere of 5% CO_2 at 37°C. Subsequently, plates were read with a microplate reader at 450 nm. The OD450 values were proportional to the total number of live cells. The percent reduction of CCK-8 dye was compared to controls (cells not induction by GDNF or HGF), which was on behalf of 100% reduction of CCK-8. Three replicate wells were used per 96-well plate. Cell survival was represented as the absorbance relative to that of controls.

BrdU Cell Proliferation Assay

Bromodeoxyuridine (BrdU; abam), a thymidine analog that is able to be incorporated into the DNA of dividing cells during S-phase[28], was utilized to label proliferative cells in our study. In brief, the cells were cultured in 96-well plates. After 24 h, 20 µl of BrdU-labeling solution was added to each well and incubated for 4 h. During the period of labeling, the pyrimidine analogue BrdU was incorporated into the DNA of proliferating cells instead of thymidine. Afterward, the BrdU-labeling solution was removed, then cells were fixed using 4% paraformaldehyde and denatured with HCL for 15 min at 37°C. Denaturation is needed to improve the accessibility of the incorporated BrdU for detection by anti-BrdU antibody. Next, cells were incubated for 30 min with 5% bovine serum albumin in 0.01 M PBS, followed by incubation with primary mouse monoclonal anti-BrdU (1:200; Abcam, Cambridge, UK) overnight at 4°C. After washing away unbound anti-BrdU, samples were treated with FITC-conjugated goat anti-mouse IgG secondary antibodies (1:200, ZSGB-BIO) for 2 h in the dark at room temperature. The cultures were then washed three times for 5 min using PBS. Finally, the cultures were covered with 50% glycerinum. Optical densities of BrdU immunore active cells were determined using an Olympus fluorescence microscope (Olympus, Tokyo, Japan) at 495 nm.

Flow Cytometry for Cell Cycle Analysis

The cell cycle distribution was measured by staining ethanol-fixed cells with propidiumiodide (PI) via flow cytometry reported previously[29]. When the BMSCs were cultured in fresh medium containing 10% FBS to 90% confluency, these were harvested and suspended in 5 mL PBS. Then, a volume of 2 mL cold ethanol was added for immobilization for 24-48 h at 4°C. Next, the cells were washed with PBS and 0.1 mg RNase A was added for RNA degradation. The cells were then placed in the dark for 30 min after adding 0.2 µg PI. The percentages of cells in different phases were examined by flow cytometry.

Immunofluorescence Staining

After induction, cells were fixed with 4% paraformaldehyde for 15 min at room temperature, and then the cells were washed three times for 5 min in PBS. Next, cells on coverslips sections were permeabilized with 0.25% Triton X-100 and blocked with 5% bovine serum albumin in 0.01 M PBS for 30 min, followed by incubation with the following primary antibodies: mouse monoclonal, mouse anti-nestin (1:200; Sigma-Aldrich, St. Louis, MO, USA), rabbit polyclonal anti-NCAM (1:500; Sigma-Aldrich, St. Louis, MO, USA) and rabbit anti-SCF (1:200, Abcam, Cambridge, UK), containing 1% bovine serum albumin in PBS overnight at 4°C. On the next day, cultures were rinsed three times for 5 min using PBS, incubated with FITC-conjugated goat anti-mouse or goat anti-rabbit IgG secondary antibodies (1:200, ZSGB-BIO) for 2 h in the dark at room temperature. All slides were covered with Vecta-shield mounting medium with DAPI (Vector). All control cultures were treated similarly but without primary antibodies. The images of nestin, NCAM, and SCF immunoreactive cells were acquired and counted using an Olympus fluorescence microscope (Olympus, Tokyo, Japan).

Quantitative Real-time polymerase Chain Reaction (RT-PCR)

Total RNA was extracted from the cultures of each group using Trizol (Invitrogen, Carlsbad, CA, USA). Then, 7 µL of total RNA was

reverse-transcribed into the first-strand cDNA with 1 μL random oligo(dT) primer (N9) using Superscript III reverse transcriptase (Invitrogen, Carlsbad, CA, USA) based on the manufacturer's instructions. Synthesized first-strand cDNA was used as templates, and β-actin was applied as an internal control for PCR amplification, respectively. For quantitative RT-PCR analyses, amplification reactions were implemented by means of ABI Prism 7500 sequence detection system (Applied Biosystems, Foster City, CA, USA) with SYBR green qPCR ThunderBird (Toyobo, Osaka, Japan). PCR amplification was performed using the experimental run protocol as follows: 10.0 μl 2 × power qPCR premix, 2.0 μl cDNA was used as templates, 0.15 μl 50 × Rox Reference Dye, 0.4 μl upstream primers, and 0.4 μl downstream primers. The protocol was carried out using the following conditions: 95°C for 1 min, following by 40 DNA amplification cycles of 95 °C for 10 sec, 60 °C for 20 sec, and 72 °C for 31 sec, and dissociation curve analysis was implemented at the end of each PCR reaction for quality control. The following specific primers were used for mouse nestin, *SCF*, *NCAM*, and β-actin.

NCAM forward 5′-TATCCACCTCAAGGTCTTCGC-3′

NCAM reverse 5′-TGTCTTCACTGCTGATGTTCG-3′;

SCF forward 5′-CAATAGGAAAGCCGCAAAGTC-3′

SCF reverse 5′-GCAGCAAAGCCAATTACAAGC-3′;

NESTIN forward 5′-GACCTCCTTAGCCACAACCCTC-3′

NESTIN reverse 5′-GATTTGCCCCTCATCTTCCTG-3′;

β-actin forward 5′-CAGGGAAATCGTGCGTGAC-3′

β-actin reverse 5′-GACATTGCCGATAGTGATGACCT-3′.

Every sample was run three times along with the internal control gene. The CT values were normalized using the $2^{\Delta\Delta CT}$ method[30].

Statistical Analysis

All data were expressed as means ± standard deviation (SD). Statistical significance was determined using one-way analysis of variance (ANOVA) and one-side *t*-test. A *p*-value of less than 0.05 was regarded to be statistically significant.

Results

Proliferation Capacities

The Cell Viability Evaluated by CCK-8 Analysis

CCK-8 assay was a sensitive and accurate colorimetric approach for the determination of viable cells number. The cell viability was evaluated using CCK-8 assay after differentiation induced by GDNF or HGF. As shown in Figure 1A, the

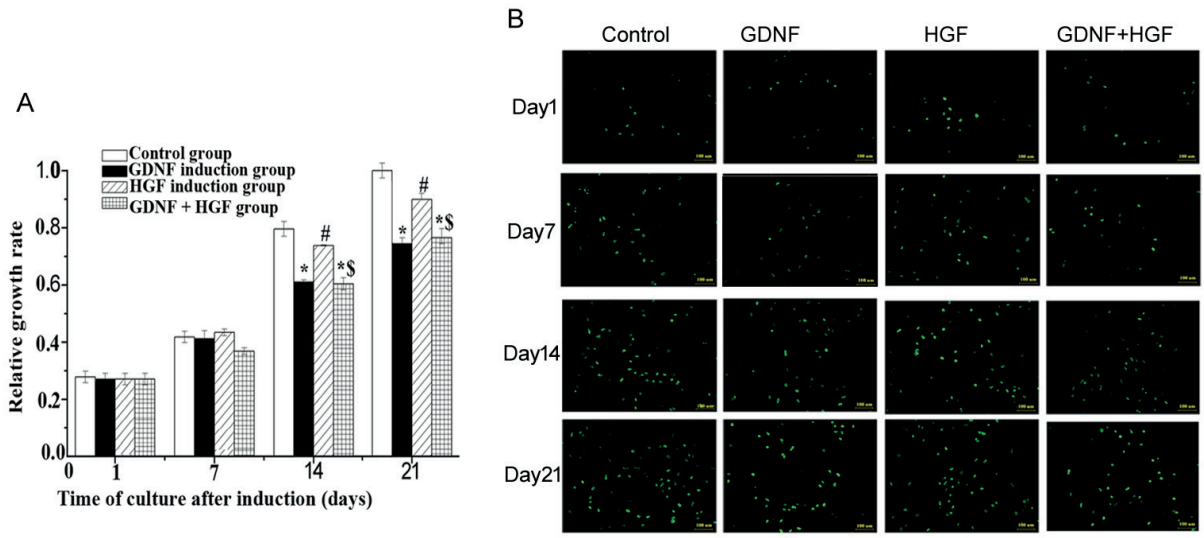

Figure 1. ***A,*** CCK-8 assays of bone marrow stromal stem cells (BMSCs) proliferation on GDNF induction group, HGF induction group, GDNF + HGF induction group and control group. The values were exhibited as mean ± standard deviation (SD). $^{*}p < 0.05$ *vs*. control group. $^{\#}p < 0.05$ *vs*. GDNF induction group. $^{\$}p < 0.05$ *vs*. HGF induction group. ***B,*** GDNF- and HGF-treatment driving the BMSCs into proliferation, evaluated with FITC conjugated Brdu (blue) immunofluorescent staining. Bar = 100 μm.

viable cell density increased over initial days according to the CCK-8 results. The three induction groups, as well as the control group, exhibited the similar tendency of viable cell density. Moreover, the HGF induction group reached a maximum value at day 21, and the control group also reached a maximum value at day 21. The activity of BMSCs in three induction groups and control group was not remarkably different from day 1 as well as 7. On the day 14 and 21 after incubation, HGF induction resulted in a marked increase in the cell density, relative to GDNF alone and the combination of GDNF and HGF group ($p < 0.05$). However, BMSCs induced by GDNF alone and the combination of GDNF and HGF had significantly lower cell viability than the control group on day 14 and 21 ($p < 0.05$). This difference on day 14 and 21 might be due to the enhanced differentiation ability of GDNF. The results of the CCK-8 measurement reflected the overall viability, which was influenced by both cell differentiation and cell proliferation. This result indicated that HGF-inducted group earned better capacity in BMSCs proliferation compared with the GDNF-treated group.

GDNF- and HGF-Treatment Increased the BrdU-Positive cells

The pattern of cell proliferation was further determined using Brdu staining. Representative anti-BrdU staining images of BMSCs in control, GDNF, HGF, and GDNF + HGF groups were shown in Figure 1B. At the beginning of induction, the low-level expression of BrdU, were observed in all the four groups. As time goes on, the amount of BrdU-positive cells increased. GDNF alone and the combination of GDNF and HGF treatment had significantly less BrdU-positive cells than the control group on day 14 and 21 ($p < 0.05$), but no difference was observed in HGF group, compared to the control ($p > 0.05$). Significant differences were found between the HGF-treated and GDNF-treated, GDNF + HGF-treated groups ($p < 0.05$). These results demonstrated that HGF enhanced BMSC proliferation, but GDNF inhibited it, leading to an offsetting effect of each to the other.

Analysis of the Cell Cycle by Flow Cytometry

Flow cytometry was used to evaluate the percentage of BMSCs in different phases of the cell cycle. The proportions in G1 and S phases were exhibited in Figure 2. The *ratio* of BMSCs was computed for each cell cycle phase. With the induction days increasing, BMSCs contained fewer cells in S-phase, and more cells in G1-phase in the three induction groups and control group. Significantly, 7 and 14 days after exposure to induction factors, there were more cells in G1-phase and fewer cells in S-phase in GDNF-treated group, relative to other three groups, which suggested that GDNF was not helpful for the proliferation of BMSCs. Nevertheless, no significant difference was observed in the four groups.

GDNF- and HGF-treatment Promoted the Differentiation of BMSC into Neuronal-like Cells

To verify the neural differentiation of BMSCs, a series of markers including nestin and NCAM were utilized. Nestin is expressed in neuroepithelial neuronal precursor stem cells, and the amount of nestin decreases along with neuronal maturation. As shown in Figure 3A, after incubation, images of immunofluorescence indicated that the morphology of some BMSCs in four groups was changed to be neuron-like with multipolar and rounded cell bodies. Moreover, a random field of vision indicated that several cells stained positive against nestin. One day after induction, in control and GDNF + HGF induction groups, BMSCs expressed nestin at low-level, and did not exhibit any difference in all four groups. As expected, the percentage of nestin-labeled cells increased at the 7th day, which coincided with higher expression of nestin in these cells and a significant increase of nestin expression level was observed in the three induction groups when compared to the control group ($p < 0.05$). More importantly, when compared with either GDNF- or HGF-induction group, the combination of GDNF and HGF treatment stimulated the expression of nestin ($p < 0.05$). Then, nestin expression level reduced at the 14th day and dramatically decreased at the 21st day. The GNDF application appeared to be more effective to induce BMSCs differentiation if compared with the other two cultures on the day 14 and 21 after induction ($p < 0.05$).

Simultaneously, the expression level of neuronal markers (nestin and NCAM) was determined by quantitative PCR. As depicted in Figure 3B, after 7-day induction, a significant increase of nestin expression was observed in three induction groups, if compared control group ($p < 0.05$). Moreover, nestin expression reached the highest

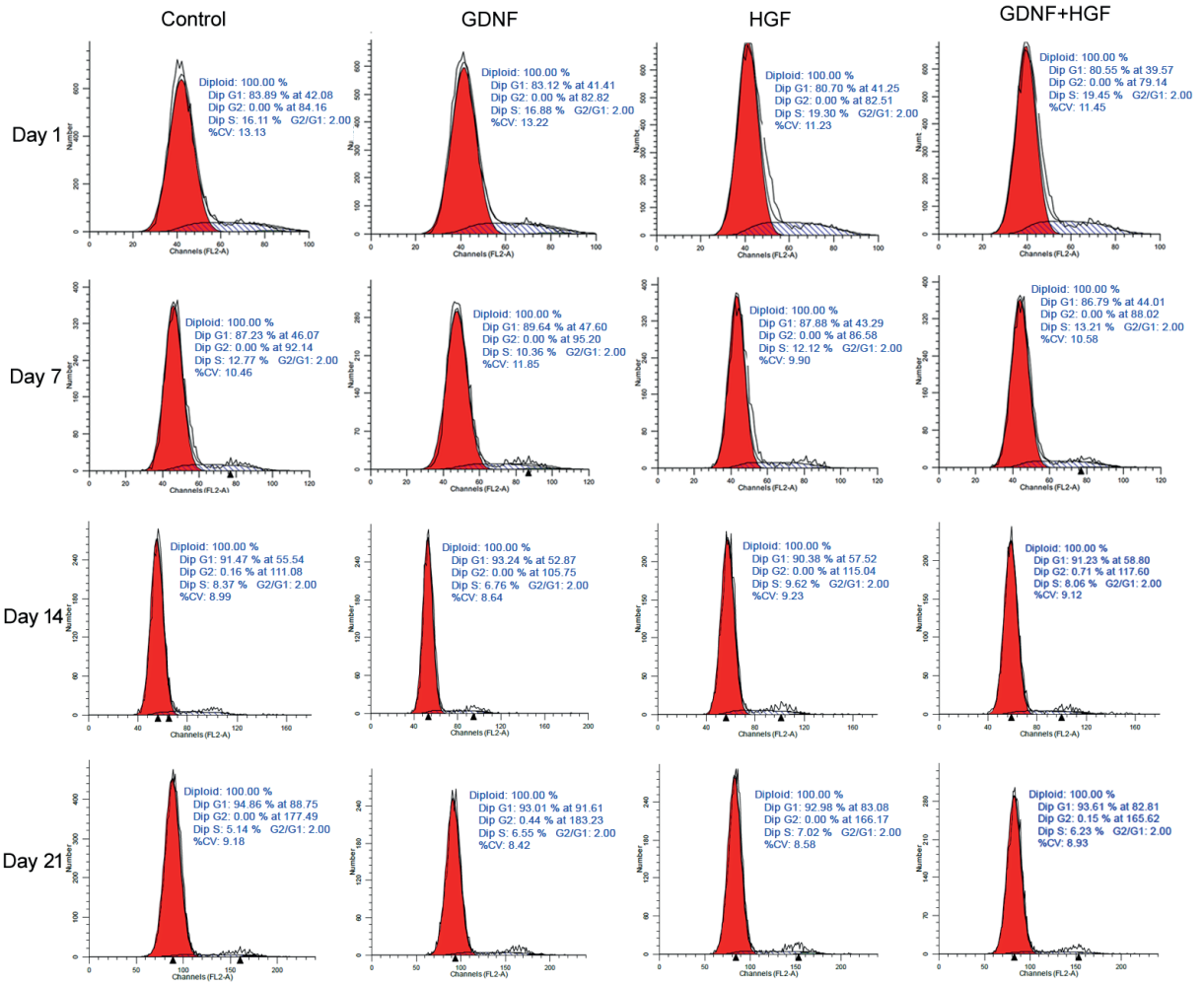

Figure 2. Cell cycle analyses of BMSCs, determined by flow cytometry. With the induction days increasing, BMSCs contained less cells in S-phase, and more cells in G1-phase in the three induction groups and control group.

level in GDNF + HGF induction group at day 7, and its expression level in the combined GDNF- and HGF- induction cells was much higher than that in either GDNF induction group or HGF induction group ($p < 0.05$). In addition, the expression level of nestin reached the highest level in GDNF induction group at day 14. After 14 days of incubation, the nestin expression level decreased. On the 14th and the 21st day after induction, the GNDF group generally had better outcomes than the HGF- alone, and the combined GDNF- and HGF- induction group as well ($p < 0.05$).

Additionally, neural cell adhesion molecule (NCAM), exhibited the similar trend with nestin at days of 1, 7, 14, 24 following induction. As shown in Figure 3C, NCAM expression level reached a maximum value in the combined GDNF + HGF induction group at day 7, and its expression level in combined GDNF- and HGF-treated cells was significantly greater than that in the presence of GDNF or HGF alone, and control groups as well ($p < 0.05$). However, no big difference was observed in the control group and those groups with GDNF or HGF ($p > 0.05$). At 14 and 21 days following induction, the GNDF treated cultures appeared to exhibit better outcomes compared with the other two cultures ($p < 0.05$).

GDNF- and HGF-treatment Inhibited the Secretion of SCF

SCF, known as a hematopoietic growth factor, is generated by stromal cells in bone marrow and is important for the growth and proliferation of stem cells. Thus, the level of SCF is positively correlated to the number of BMSCs. Based on

the PCR and immunofluorescence assays, single and combined treatment with GDNF and HGF was found to result in a decrease of the number of SCF-labeled cells even if extending induction time (Figure 4A and B). Of note, on the day 7 and 14 after induction, the expression level of SCF was remarkably decreased in GDNF-treated and GDNF + HGF-treated groups than the control group and the HGF-treated groups ($p < 0.05$), while there was no statistical significance detected between GDNF-treated group and the combined treatment group ($p > 0.05$). This suggested that GDNF might inhibit the expression of SCF but promote BMSCs differentiation into neural-like cells instead.

Discussion

Although BMSCs has been advocated as a promising therapy for treating the patients with neurological disease, there is still one crucial difficulty-differentiation of BMSCs to neuron-like cells, greatly limited their application to neurological disease. Previous studies[31,32] showed that BMSCs were able to differentiate into neuronal-like phenotype when they were cultured stimulated with various factors, including GDNF, NT-3, vasoactive intestinal peptide, HGF and so on. In our experiment, we found that after incubation, GDNF and HGF could promote BMSCs differentiation to neuronal-like cells *in vitro*. Of note, during the induction, the combination of HGF and GDNF showed the optimal effect to produce neural differentiation of BMSCs on the 7-day post-induction. Moreover, the administration of GDNF significantly improved the viability of neuron-like cells in the process of induction, relative to the HGF-treated and GDNF + HGF-treated groups on the day 14 and 21 after induction. Significantly, in accordance with our data, Yang et al [33] indicated that GDNF was a better choice for neuronal differentiation of BMSCs. Thus, we deduce that GDNF might be more suitable for neuronal differentiation of BMSCs than HGF *in vitro*.

In the current study, when BMSCs proliferate and differentiate, HGF stimulates cell proliferation while GDNF stimulates differentiation of BMSCs into neuron-like cells. HGF was reported to play an important role in growth and differenti-

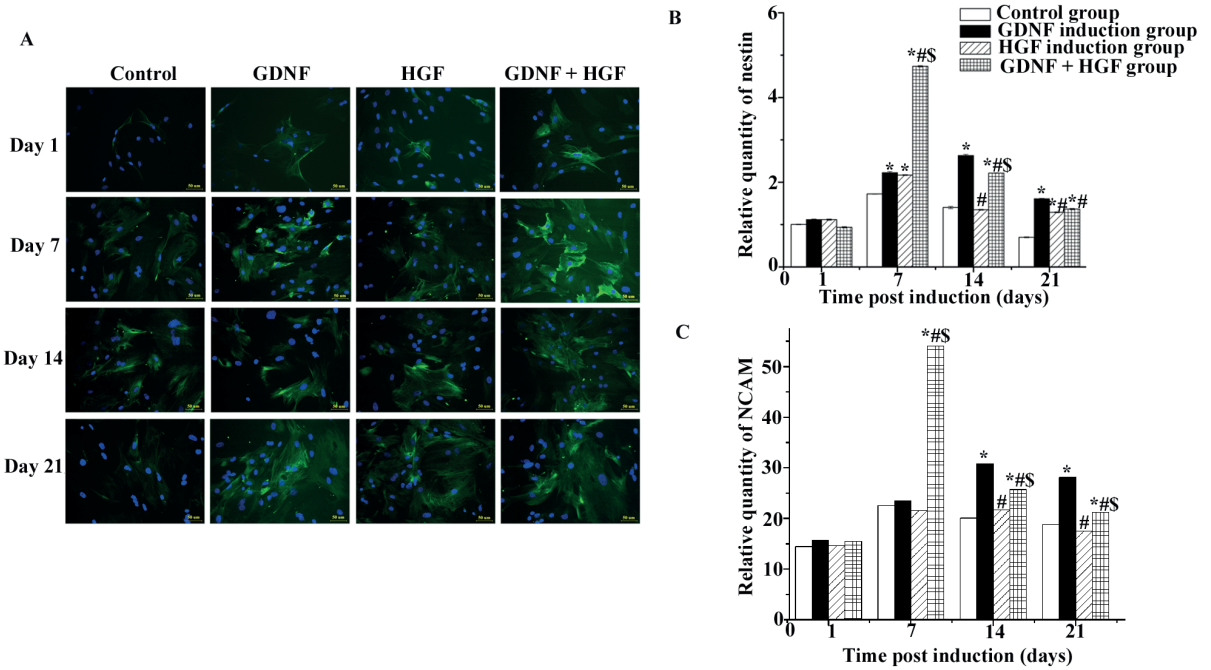

Figure 3. GDNF- and HGF-treatment driving the BMSCs into differentiation at the day 1, 7, 14 and 21 after induction. ***A,*** Expression of nestin by immunofluorescence. The anti-nestin staining is exhibited as green fluorescence and nuclei is marked with DAPI, depicted as blue fluorescence. ***B-C,*** Analysis of PCR for nestin and NCAM expression. Bars show means ± SD values. The mean content of mRNA was normalized with the expression of actin gene. $^{*}p < 0.05$ compared with the respective control group. $^{\#}p < 0.05$ *vs.* the respective GDNF induction group. $^{\$}p < 0.05$ *vs.* HGF induction group.

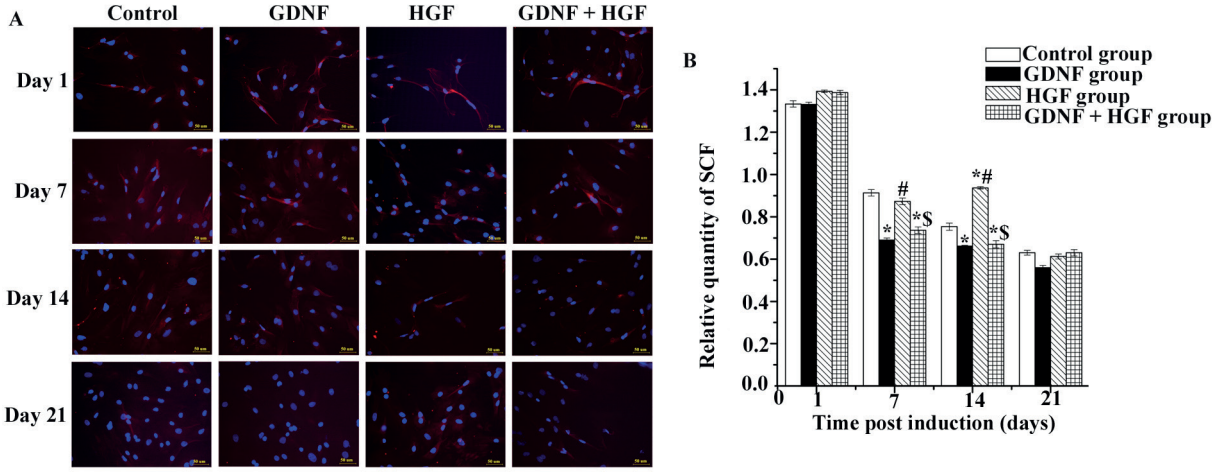

Figure 4. The gene expressions of SCF in BMSC cells at different stages after differentiation by quantitative RT-PCR and immunofluorescence analysis. ***A,*** Single and combined treatment can decrease the number of SCF-positive cells, quantitative analysis was performed to the number of SCF. The anti-SCF staining is exhibited as red fluorescence and nuclei is marked with DAPI, depicted as blue fluorescence. ***B,*** Analysis of quantitative RT-PCR for SCF expression. Bars show means ±SD values. The mean content of mRNA was normalized with the expression of actin gene. $^{*}p < 0.05$ compared with the respective control group. $^{\#}p < 0.05$ *vs*. the respective GDNF induction group. $^{\$}p < 0.05$ *vs*. HGF induction group.

ation of stromal cells, for example, osteoclast and myocytes[34]. Moreover, HGF was found to stimulate the proliferation of BMSCs[35]. Eom et al[36] demonstrated that HGF played important roles in maintaining the differentiation potential during long-term culturing of about 2 months. In addition, GDNF was capable of initiating myelination in the culture of dorsal root ganglion neuronal cells[37]. Yang et al[33] indicated that GDNF induced the differentiation of BMSCs into the neuron-like cells, and neuron-specific markers including NSE and MAP-2 were detected in the culture. Moreover, Jiang et al[38] reported that proliferated cells were able to be differentiated into multi-potent precursor cells, which have the characteristic shapes of neural cells. We supposed that combination treatment of HGF and GDNF might be very important to maintain stemness of BMSCs for cell therapy. Thus, we determined whether combination treatments of GDNF and HGF could increase both proliferation and differentiation potentials. We found that combined treatment of growth factors increased differentiation, but not proliferation of BMSCs. On the day 7-post induction, the combined treatment with GDNF and HGF resulted in better differentiation aspect when compared to the other single induction groups. Our findings indicated BMSCs showed a time-dependent manner to induce differentiation. Based on these, the ability of BMSCs to proliferate and differentiate into neuron-like cells indicated a cytotherapy possibility or many diseases, for example, neurodegenerative disease and cerebral infarct. Moreover, HGF and GDNF used in our study are secreted from human body; they were not toxical and are safely manipulated to induce differentiation. In addition, the application of differentiated neuron-like cells in clinical therapy has fewer drawbacks. Significantly, large quantities of neuron-like cells can be obtained from a small number of bone marrow, and the utilization of these cells in a clinic will be very meaningful.

Although BMSCs express nestin, a type VI intermediate filament protein and a common marker of neural precursors, nestin is not a unique marker of neural precursors. Even the expression of one or more neuronal proteins is insufficient to prove BMSCs becoming neurons. Further studies will be needed to determine whether these differentiated neuron-like cells can survive and migrate after being implanted into lesion sites, and improve neurological functions.

Conclusions

Our results showed both HGF and GDNF could contribute to the differentiation of BMSCs into neuron-like cells. Nevertheless, HGF but not GDNF, showed proliferation effects on BMSCs, indicating GDNF perhaps could be a better choice of a long lasting treating nervous diseases.

Conflicts of interest

The authors declare no conflicts of interest.

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