miR-210 enhances mesenchymal stem cell-modulated neural precursor cell migration

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Abstract. The migration of endogenous neural stem cells and neural precursor cells (NPCs) to sites of injury is essential for neuroregeneration following hypoxic-ischemic events. Bone marrow-derived mesenchymal stem cells (BMSCs) are a potential therapeutic source of cells following central nervous system damage; however, few studies have investigated the effects of BMSCs on cell migration. Thus, in the present study, the effects of BMSCs on NPC migration were investigated. In the present study, BMSCs and NPCs were isolated and cultured from mice. The effects of BMSCs on the migration of NPCs were analyzed using a Transwell cell migration assay. BMSCs were transfected with microRNA-210 (miR-210) mimics and inhibitors to examine the effects of the respective upregulation and downregulation of miR-210 in BMSCs on the migration of NPCs. Then, miR-210 expression in BMSCs were quantified and the expression levels of vascular endothelial growth factor-C (VEGF-C), brain derived neurotrophic factor (BDNF) and chemokine C-C motif ligand 3 (CCL3) in the supernatant under hypoxic conditions were investigated via reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and ELISA. Subsequently, the expression of VEGF-C, BDNF and CCL3 in BMSCs overexpressing miR-210 or BMSCs suppressing miR-210 was examined by RT-qPCR and western blot analyses. BMSCs promoted the migration of NPC, particularly when pre-cultured with BMSCs for 24 h and co-cultured with NPCs for 24 h; the miR-210 expression levels increased under hypoxic conditions. Additionally, the migration of NPCs was also increased when the BMSCs overexpressed miR-210 compared with the BMSCs transfected with a negative control miR and BMSCs with downregulated miR-210 levels. The expression levels of VEGF-C increased in the BMSCs that overexpressed miR-210 and were decreased in BMSCs transfected with a miR-210 inhibitor. The results of the present study indicated that BMSCs promote the migration of NPCs. Overexpression of miR-210 in BMSCs enhanced NPC migration and may be associated with increases in VEGF-C expression levels.

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Introduction

Ischemia, hypoxia, reperfusion injury and other factors caused by apoplexy may result in brain injury (1). At present, cell therapy is considered a promising method for the treatment of cerebral injury, and numerous preclinical studies have made notable progress. For cytotherapy, stem cells are particularly valued for their strong proliferation and differentiation characteristics. Several studies have investigated cytotherapy for the treatment of cerebral injury, including strategies using bone marrow-derived mesenchymal stem cells (BMSCs), neural stem cells (NSCs), neural precursor cells (NPCs), embryonic stem cells and endothelial progenitor cells (2-7). Stem cell therapy is an emerging therapeutic modality in the treatment of stroke. The basis stems from the observation that certain parts of the adult brain are capable of regeneration (8). A recent meta-analysis of preclinical studies demonstrated that mesenchymal stem cells used to treat ischemic stroke were associated with improvements in neurological function (9); however, important questions remain unanswered, and translation to the clinical remains a distant prospect.

The ability of self-renewal and differentiation of MSCs into neural cells *in vitro*, as demonstrated by the expression of neuronal markers such as neuronal nuclear antigen, and non-ethical and tissue rejection-associated concerns make their use a promising therapeutic approach for stroke treatment (10). BMSCs can protect neurons from hypoxia (11). The experimental rationale for the use of MSCs in stroke therapy involves a number of divergent mechanisms of action, including differentiation into cell types relevant to repair, modulation of the immune system, promotion of angiogenesis, neurogenesis and synapse formation, paracrine secretion of neuroprotective and neurotrophic factors (12,13). The interaction between BMSCs and other cells in the brain-injured area to further promote damage repair requires further investigation (10).

NSCs/NPCs are present in the subventricular zone and hippocampal dentate gyrus of mammals (14-16). NSCs have the ability to self-renew and differentiate into neurons, astrocytes or microglia with appropriate stimulation (17,18). Following brain injury, NSCs/NPCs can be activated; they proliferate and differentiate into neurons under pathological conditions (19). The migration of NSCs/NPCs to the focal lesion is key to promoting injury-associated repair. The effective migration of exogenous or endogenous NSCs can promote functional reconstruction of the nervous system (15). Investigations have been performed; however, there is still a lack of clear and effective methods to promote the directional migration of NSCs/NPCs. The number of NPCs that migrate to the lesion is very small; thus, it is also necessary to determine an efficient method to promote NPC migration and appropriate targeting of NPCs.

MicroRNAs (miRNAs/miRs) are 20-24 nucleotide RNAs that regulate the translation or expression levels of target mRNA transcripts (20). Previous studies suggested that miR-210 specifically is a robust target of hypoxia-inducible factors, and miR-210 has complex roles in the cellular responses to hypoxia (21-23). Previous studies reported that miR-210 is closely associated with ischemic stroke; miR-210 is not only upregulated in endothelial cells under hypoxic conditions, but also promotes angiogenesis (24) and is closely associated with the physiological and pathological status of the nervous system (25,26). Hypoxic preconditioning can increase the expression of miR-210 in BMSCs. Ischemic preconditioning augments the survival of stem cells via miR-210 expression by targeting caspase-8-associated protein 2. Liu *et al* (18) also confirmed that miR-210 expression in NSCs/NPCs is increased significantly under hypoxic conditions. Our previous studies revealed that the transplanted BMSCs localized to the ischemic foci following cerebral ischemia (27,28). The penumbra surrounding the ischemic foci was hypoxic following cerebral ischemia (28). Additionally, miR-210 expression was demonstrated to be significantly increased in ischemic brain tissue (24,29). Therefore, it was hypothesized that the expression levels of miR-210 in BMSCs around the ischemic foci following cerebral ischemia increased in the hypoxic environment, which may promote the migration of NSCs to the surrounding ischemic foci. This hypothesis was investigated in the present study by detecting the expression of miR-210 in BMSCs under hypoxic conditions and the effect of BMSCs on NPC migration. In addition, the present study also investigated the effect of miR-210 overexpression and inhibition in BMSCs on the migration of NPCs.

Materials and methods

Isolation and cultivation of mouse BMSCs. Initially, ten 4- to 6-week-old C57BL/6 male mice (weight 12-18 g, Hunan SJA Laboratory Animal Co., Ltd., Changsha, China; http://www.hnsja.com/) were housed in specific pathogen free (SPF) conditions (air cleanliness: Level 7, temperature: 22±2˚C) with free access to sterilized food and ultra pure water. The mice were sacrificed by cervical dislocation. The femur was obtained under sterile conditions. The ends of the femur were removed, and PBS was used to flush out the bone marrow. Following filtering with a 200-μm mesh filter, the bone marrow cell suspension was centrifuged at 200 x g for 5 min at 4˚C. The cell pellet was resuspended with BMSC culture media [Dulbecco's modified Eagle's medium (DMEM)/F12+10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA)]. After 24 h in culture at 37˚C in 5% CO_2, the media was replaced to remove the non-adherent cells, and then the media was changed every 3 days. When the cells reached confluence, the cells were passaged. The cells from passage 4 were evaluated by flow cytometry analysis. The identification indexes were cluster of differentiation (CD)34, CD44 and CD71. A total of $1x10^5$-10^6 cells were suspended in 100 μl PBS. Then 2 μl CD34 antibody, eFluor660 (cat. no. 50-0341-82; ebioscience; Thermo Fisher Scientific, Inc.), CD44 antibody, PE (12-0441-82; Thermo Fisher Scientific, Inc.) or CD71 antibody, PE (12-0711-82; Thermo Fisher Scientific, Inc.) was added into the BMSC suspension and cultured at 4˚C for 45 min, and then washed with PBS twice. After that the BMSC suspension was examine by the flow cytometer (Novocyte 2040R, NovoExpress® 1.2.5 software, ACEA Bioscience Inc., San Diego, CA, USA). The BMSCs from passages 4 and 5 were used for further experiments. All procedures and experiments in the present study were conducted under guidelines and were approved by the Laboratory Animal Welfare and Ethics Committee of the Third Military Medical University (Chongqing, China).

Isolation and cultivation of mouse NPCs. A total of 10 C57BL/6 pregnant mice at day 13 of gestation (weight 34-35 g; Hunan SJA Laboratory Animal Co., Ltd.) were housed in SPF conditions (air cleanliness: Level 7, temperature: 22±2˚C) with free access to sterilized food and ultra pure water. These mice were sacrificed by cervical dislocation. Under sterile conditions, the abdominal skin, muscle and uterus were cut so as to obtain mouse embryos. The telencephalon was isolated, the meninges were removed and the tissue was cut into pieces under a stereomicroscope. The tissue was digested with 0.05% trypsin-EDTA at 37˚C and agitated every 5 min until a homogenized solution was obtained. Then, the complete media containing serum (DMEM/F12+10% FBS) was added to stop the digestion. After a 200 x g centrifugation for 5 min at 4˚C, the cell pellet was suspended with NPC culture media (DMEM/F12, 1% N-2, 2% B-27, 10 ng/ml basic-fibroblast growth factor, 20 ng/ml epidermal growth factor) for cultivation at 37˚C in 5% CO_2. The NPCs developed into a bulb shape. The media was changed every 2 days. The NPCs were identified by the immunofluorescent staining of β-tubulin III, doublecortin (DCX) and nestin. The NPCs were fixed with 4% paraformaldehyde at room temperature for 30 min, washed with PBS twice and treated with 0.2% Triton X-100 for 30 min. Following washing with PBS twice, the cells were incubated with β-tubulin III antibody (1:100; cat. no. sc-166729; Santa Cruz Biotechnology, Inc., Dallas, TX, USA), anti-DCX antibody (1:100; cat. no. sc-28939; Santa Cruz Biotechnology, Inc.) or anti-nestin antibody (1:100; cat. no. sc-21248; Santa Cruz Biotechnology, Inc.) at 4˚C over night. After washing with PBS twice, the cells were incubated with fluorescein isothiocyanate (FITC)-labeled goat-anti-mouse antibody (1:100; cat. no. ab6785; Abcam, Cambridge, USA), TRITC-labeled goat-anti-rabbit antibody (1:100; cat. no. ab6718; Abcam), FITC-labeled donkey-anti-goat antibody (1:100; cat. no. ab6881; Abcam) at room temperature for 4 h. Then the

cells were washed with PBS and observed under fluorescence microscope. The NPCs from passage 3 were used for further experiments.

Hypoxic treatment of BMSCs. According to the method of Pulkkinen *et al* (22), the BMSCs cultured under hypoxia were then cultured in a low oxygen incubator chamber with a 94% N_2, 1% O_2, 5% CO_2 low-oxygen gas mixture for 24 h. The BMSCs that were cultured under normal conditions (37˚C, 5% CO_2) were used as a control. Three sample parallel experiments were performed in each group.

Cell transfection experiments. Previous methods were applied with minor revisions (30,31). miR-210 mimics and inhibitors were used to upregulate or downregulate the expression of miR-210, respectively, in BMSCs. miR-210 mimics and inhibitors were synthesized by Shanghai GenePharma Co., Ltd. (Shanghai, China). The BMSCs were seeded into 6-well plates at a density of $5x10^5$ cells/well. Then, the cells were divided into three groups: Negative control (NC), overexpressing miR-210 and the suppressing miR-210 groups. On day 2 in culture, the cells were transfected with NC, miR-210 mimics or inhibitors according to the manufacturer's protocols for Lipofectamine® 2000 (7.5 μl/well; Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA). The doses were 75 pmol/well for the NC miRNA, the miR-210 mimics and miR-210 inhibitors. Following the addition of 100 μl serum-free medium (DMEM/F12) to three Eppendorf tubes, and the NC miRNA, miR-210 mimics or miR-210 inhibitors, together with the Lipofectamine® 2000 reagent, the mixtures were stored at room temperature for 20 min and then added to the corresponding wells. The transfected cells were collected at 24 and 48 h post-transfection. The expression of miR-210 was detected by reverse transcription-quantitative polymerase chain reaction (RT-qPCR). The sequence of the double stranded miR-210 mimic was (5'-3') CUGUGCGUGUGACAG CGGCUGA and AGCCGCUGUCACACGCACAGUU; the miR-210 inhibitor sequence was (5'-3') UCAGCCGCUGUC ACACGCACAG; and the NC sequence was (5'-3') UUCUCC GAACGUGUCACGUTT and ACGUGACACGUUCGGAGA ATT.

Cell migration experiments. The Transwell cell migration assay was performed to detect the effect of BMSCs on NPC migration. The BMSCs were seeded into the lower chamber at a density of $1x10^4$ cells/well in BMSC culture medium at 37˚C. Following culturing for 24 or 48 h, the NPCs were added to the upper chamber at a density of $5x10^3$ cells/well in NPC culture media. After co-culturing for 15 or 24 h, the migrated NPCs on the membrane were stained with 0.1% crystal violet at room temperature for 10 min. Three parallel samples in each group were analyzed. The cell number was counted in 20 fields with a light microscope (magnification, x200).

The effects of miR-210 expression within BMSCs on NPC migration were investigated. The BMSCs overexpressing miR-210, suppressing miR-210 expression and the NC-transfected BMSCs were plated in the lower chamber at a density of $1x10^4$ cells/well in BMSC culture media. Following culturing for 24 h, the NPCs were added to the upper chamber at a density of $5x10^3$ cells/well in NPC culture media. After 24 h of co-culturing, the migrated NPCs on the membrane were fixed with 4% paraformaldehyde at room temperature for 15 min, washed with PBS, and stained with 0.1% crystal violet at room temperature for 10 min. A total of three parallel samples in each group were analyzed. The cell number was counted in 20 fields of a light microscope (magnification, x200).

RNA extraction and RT-qPCR. Using RT-qPCR, miR-210 expression levels were detected within BMSCs following hypoxic treatment and transfection with miR-210 mimics or inhibitors. The mRNA expression levels of vascular endothelial growth factor-C (VEGF-C), brain derived neurotrophic factor (BDNF), and chemokine C-C motif ligand 3 (CCL3) in BMSCs successfully transfected with miR-210 mimics, inhibitors or NC, were also detected by RT-qPCR after pre-culturing for 24 h. The BMSCs transfected with NC miRNA (NC BMSCs) served as controls. Total RNA was extracted with TRIzol® (Thermo Fisher Scientific, Inc.). The cDNA was obtained via RT reaction by PrimeScript™ RT reagent kit with gDNA Eraser (Perfect Real Time; Takara Biotechnology Co., Ltd., Dalian, China) according to the manufacturer's protocol. Then, qPCR was performed according to the protocols of SYBR® Premix Ex Taq™ II (TliRNaseH Plus), Bulk kit (Takara Biotechnology Co., Ltd.). GAPDH was used as the internal reference. The qPCR conditions were 1 cycle of denaturation at 95˚C for 30 sec, then followed 40 cycles of denaturation at 95˚C for 5 sec and annealing at 60˚C for 34 sec. The primer sequences were as follows: miR-210 forward, 5'-GCAGTCTGT GCGTGTGACAGC-3', reverse, 5'-GTGCAGGGTCCGAGG T-3'; VEGF-C forward, 5'-ACTTGCTGTGCTTCTTGT-3', reverse, 5'-CTCATCTACGCTGGACAC-3'; BDNF forward, 5'-CCAGGTGAGAAGAGTGATG-3', reverse 5'-AGTGAT GTCGTCGTCAGA-3' and CCL3 forward, 5'-CCTTGCTGT TCTTCTCTGT-3' and reverse 5'-ATGAATTGGCGTGGA ATCT-3'. GAPDH forward, 5'-TGCACCACCAACTGCTTA GC-3' and reverse 5'-GGCATGGACTGTGGTCATGAG-3'. The $2^{-\Delta\Delta Cq}$ method was used for quantification (32).

ELISA. The BMSCs were seeded into 6-well plates at a density of $5x10^5$ cells/well. The supernatants from normal and hypoxic BMSCs were collected for the detection of VEGF-C, BDNF and CCL3 expression. The expression levels of BDNF, VEGF-C, and CCL3 were analyzed using BDNF, VEGF-C and CCL3 ELISA kits, respectively (cat. nos. TY0362b, TY0258b and TY3766b; Shanghai Lichen Trading Company, Shanghai, China) according to the manufacturer's protocols. The experiments were performed in triplicate.

Western blotting. The expression levels of VEGF-C, BDNF, and CCL3 in the BMSCs successfully transfected with miR-210 mimics, inhibitors or NC, were analyzed by western blotting following pre-culturing for 24 h. The NC BMSCs served as the control. The cells were lysed with radioimmunoprecipitation assay lysis buffer (Beyotime Institute of Biotechnology, Haimen. China). The samples were centrifuged at 12,092 x g for 10 min at 4˚C. The supernatant was collected, and the protein concentration was determined with a bicinchoninic protein assay kit (Thermo Fisher Scientific, Inc.). Then, 30 μg total protein was loaded onto a gel and

then separated by electrophoresis. After transferring to nitrocellulose membranes, the membrane was blocked with 5% skimmed milk at room temperature for 1 h and then incubated with primary antibody against BDNF (1:500; Abcam; cat. no. ab108319), VEGF-C (1:500; Abcam; cat. no. ab9546), or CCL3 (1:500; Abcam; cat. no. ab179638) overnight at 4˚C. β-actin was used as control (1:200; Santa-Cruz Biotechnology, Inc.; cat. no. SC69879). Following three washes with 1X Tris-buffered saline with Tween-20, the membranes were incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG (1:2,500, diluted in PBS; cat. no. ZB2301; OriGene Technologies, Inc., Beijing, China) or peroxidase-conjugated goat anti-mouse IgG (1:2,500, diluted in PBS; cat. no. ZB2305; OriGene Technologies, Inc.) for 2 h at room temperature. The signal was detected with an enhanced chemiluminescence substrate Pierce fast western blotting kit (Thermo Fisher Scientific, Inc.), and the densitometric values were analyzed with the ImageJ 1.43 program (National Institutes of Health, Bethesda, MD, USA). All experiments were repeated at least three times.

Statistical analysis. All values are expressed as the mean ± standard deviation. The data were analyzed using SPSS 18.0 software (SPSS, Inc., Chicago, IL, USA). A t test was used to analyze the difference between two groups. One-way analysis of variance was used to analyze the difference among three or more groups. When homogeneity of variance was satisfied, a Tukey's post-hoc test was used for multiple comparisons. When homogeneity of variance was not satisfied, a Dunnett's T3 test was used for multiple comparisons. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Isolation and identification of BMSCs. In the present study, the antigenic phenotype of isolated and cultured BMSCs was detected by flow cytometric analysis with anti-CD44, anti-CD71 and anti-CD34 antibodies. The BMSCs from passage 4 were spindle shaped. As presented in Fig. 1, following the analysis and identification of surface markers by flow cytometry, the positive rate of CD44 was 100% (Fig. 1A), the positive rate of CD71 was 99.8% (Fig. 1B), and the positive rate of CD34 was 22.1% (Fig. 1C). Thus, BMSCs had been successfully isolated.

Identification, isolation and cultivation of NPCs. The NPCs exhibited sphere-like morphologies. DCX is a microtubule-associated protein expressed by NPCs. DCX is expressed within NPCs during active division, and their neuronal daughter cells continue to express DCX for 2-3 weeks as the cells mature into neurons (33). The protein nestin is widely used as a marker of NSCs and NPCs, and β-tubulin III is a general marker of neurons. The identification, isolation and cultivation of NPCs were confirmed by immunofluorescent staining with anti-nestin, anti-DCX and anti-β-tubulin III antibodies. As presented in Fig. 2, nestin and DCX staining was positive; however, β-tubulin III staining was negative (data not shown). Thus, the isolation and cultivation of NPCs were successful.

Co-cultured BMSCs and NPCs significantly promote the migration of NPCs. In the present study, NPCs and BMSCs were successfully cultured. Subsequently, the effects of BMSCs on NPC migration were investigated using a Transwell cell migration assay. The present study examined four groups: Pre-cultured BMSCs for 24 h and co-cultured BMSCs/NPCs for 15 h; pre-cultured BMSCs for 24 h and co-cultured BMSCs/NPCs for 24 h; pre-cultured BMSCs for 48 h and co-cultured BMSCs/NPCs for 15 h, and pre-cultured BMSCs for 48 h and co-cultured BMSCs/NPCs for 24 h. The number of migrated NPCs was greatest in the group of BMSCs pre-cultured for 24 h and co-cultured NPCs for 24 h, which was significantly higher than any other group [pre-cultured BMSCs for 24 h and co-cultured BMSCs/NPCs for 24 h vs. pre-cultured BMSCs for 24 h and co-cultured BMSCs/NPCs for 15 h ($P=0.045$); pre-cultured BMSCs for 48 h and co-cultured BMSCs/NPCs for 15 h ($P=0.041$); and pre-cultured BMSCs for 48 h and co-cultured BMSCs/NPCs for 24 h ($P<0.001$); Fig. 3]. The number of migrated NPCs in the group of pre-cultured BMSCs for 48 h and co-cultured BMSCs/NPCs for 24 h was significantly lower than that in the pre-cultured BMSCs for 24 h, co-cultured BMSCs/NPCs for 24 h, pre-cultured BMSCs for 48 h and co-cultured BMSCs/NPCs for 15 h ($P<0.001$; Fig. 3). Thus, the BMSCs pre-cultured for 24 h and BMSCs/NPCs co-cultured 24 h were the optimal combinations, which improved the migration ability of NPCs significantly.

miR-210 expression is significantly upregulated under hypoxic conditions. As aforementioned, miR-210 is significantly and consistently upregulated under hypoxic conditions. miR-210 expression levels were significantly upregulated in BMSCs under hypoxic conditions (Fig. 4A; $P=0.014$). The expression levels of BDNF, VEGF-C and CCL3 in the supernatant of BMSCs were quantified by ELISA. Compared with the BMSCs cultured under normal conditions, during hypoxic conditions, BDNF expression levels were significantly decreased (Fig. 4B; $P=0.001$), CCL3 and VEGF-C expression levels increased significantly ($P=0.049$ and $P=0.003$, respectively; Fig. 4C and D, respectively).

Effects of miR-210 on the migration of NPCs on the co-culture system. The present study successfully isolated and cultured BMSCs and NPCs. As miR-210 expression levels were significantly upregulated in BMSCs under hypoxia, the effects of miR-210 overexpression and inhibition in BMSCs on the migration of NPCs were investigated in the present study. miR-210 mimics or inhibitors were transfected into BMSCs to upregulate or downregulate miR-210 expression, respectively. The miR-210 expression levels in BMSCs were increased following transfection with miR-210 mimics. The miR-210 expression levels were highest in BMSCs transfected with miR-210 mimics for 48 h, which was significantly higher than that in the BMSCs transfected with NC miRNA for 24 or 48 h, and within BMSCs transfected with miR-210 mimics for 24 h [miR-210 mimics 48 h vs. NC miRNA 24 h ($P=0.001$), NC miRNA 48 h ($P=0.001$) and miR-210 mimics 24 h, ($P=0.002$); Fig. 5A].

The miR-210 expression levels in BMSCs decreased following transfection with miR-210 inhibitors. The miR-210 expression levels in BMSCs was lowest at 24 h post-transfection

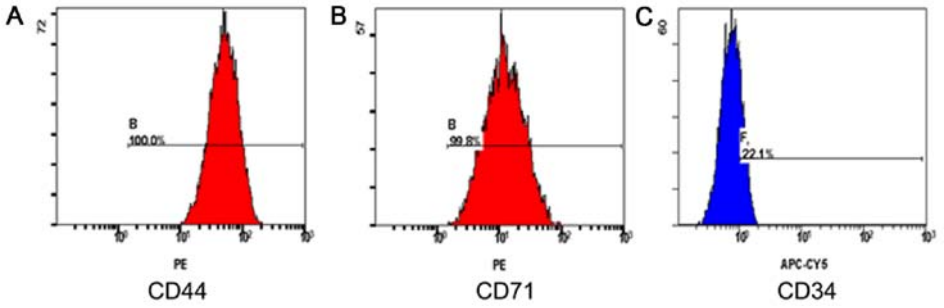

Figure 1. Immunophenotyping results of isolated and cultured bone marrow-derived mesenchymal stem cells. Surface markers by flow cytometry for (A) CD44, (B) CD71 and (C) CD34. CD, cluster of differentiation.

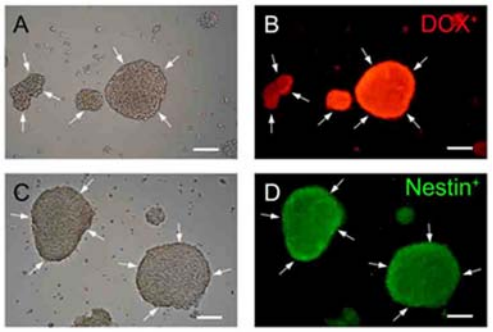

Figure 2. Identification of surface markers by immunofluorescent staining of isolated NPCs. (A) NPCs observed under an inverted microscope. (B) DCX immunofluorescence. (C) NPCs observed under an inverted microscope. (D) Nestin immunofluorescence. Scale bar, 100 μm. The arrows indicate that DCX or Nestin was expressed in isolated NPCs. NPCs, neural precursor cells; DCX, doublecortin.

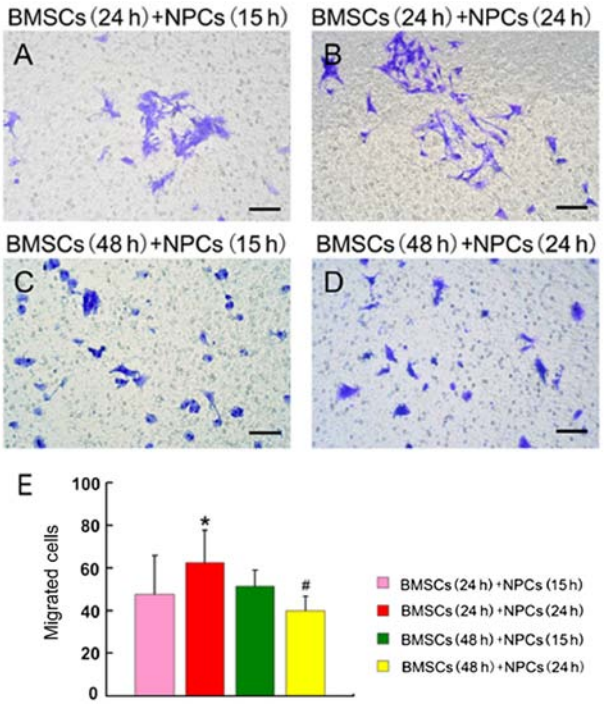

Figure 3. Effects of BMSCs on the migration of NPCs. (A) BMSCs pre-cultured for 24 h, BMSCs/NPCs co-cultured for 15 h; (B) BMSCs pre-cultured for 24 h, BMSCs/NPCs co-cultured for 24 h; (C) BMSCs pre-cultured for 48 h, BMSCs/NPCs co-cultured for 15 h; (D) BMSCs pre-cultured for 48 h, BMSCs/NPCs co-cultured for 24 h. (E) Quantification of migrated cells. Number of fields counted (n=20), *P<0.05 vs. BMSCs (24 h) + NPCs (15 h), BMSCs (48 h) + NPCs (15 h) and BMSCs (48 h) + NPCs (24 h). $^{\#}$P<0.05, vs. BMSCs (24 h) + NPCs (24 h) or BMSCs (48 h) + NPCs (15 h). Scale bar, 100 μm. BMSCs, bone marrow-derived mesenchymal stem cells; NPC, neural precursor cells.

with a miR-210 inhibitor, which was significantly lower than BMSCs transfected with NC miRNA for 24 or 48 h and in BMSCs transfected with miR-210 inhibitor for 48 h [miR-210 inhibitor 24 h vs. NC miRNA 24 h (P=0.005), NC miRNA 48 h (P=0.001) and miR-210 inhibitor 48 h (P=0.042); Fig. 5B]. Thus, the BMSCs transfected with miR-210 mimic for 48 h and BMSCs transfected with miR-210 inhibitor for 24 h for further experiments to investigate the effects of miR-210 expression in BMSCs on NPC migration.

Subsequently, the effect of miR-210 expression in BMSCs on NPC migration was investigated in the present study. The number of migrated NPCs was significantly higher in the miR-210 overexpressing BMSC group than in the NC BMSCs group (P=0.028; Fig. 6). Conversely, the number of migrated NPCs in the miR-210-suppressed group (BMSCs suppressing miR-210 expression pre-cultured for 24 h and BMSCs suppressing miR-210 expression/NPCs co-cultured for 24 h) was significantly decreased compared with that in the NC BMSCs group (Fig. 6; P=0.023). Compared with in the NC BMSCs with co-cultured NPCs for 24 h group, the BMSCs overexpressing miR-210 significantly promoted the migration of NPCs. Reducing the expression of miR-210 in BMSCs resulted in fewer migrated NPCs. The results of the present study suggested that miR-210 had an important role in the migration of NPCs in this co-culture system.

Effect of miR-210 on the expression levels of BDNF, CCL3 and VEGF-C in BMSC. As aforementioned, under hypoxic conditions, miR-210 was upregulated; BDNF, VEGF-C, and CCL3 expression levels were also upregulated or downregulated by hypoxia. It was hypothesized that miR-210 may regulate BDNF, VEGF-C and CCL3 gene expression; the effects of miR-210 overexpression or inhibition on the expression of BDNF, CCL3 and VEGF-C in BMSCs were investigated. From RT-qPCR analyses, BDNF mRNA expression levels in the BMSCs overexpressing miR-210 or BMSCs suppressing miR-210 expression were not significantly different from the NC BMSCs group (Fig. 7A). The CCL3 mRNA expression levels were significantly downregulated in BMSCs when miR-210 was overexpressed (Fig. 7B; P=0.029) or suppressed (Fig. 7B; P=0.014). The VEGF-C mRNA expression levels

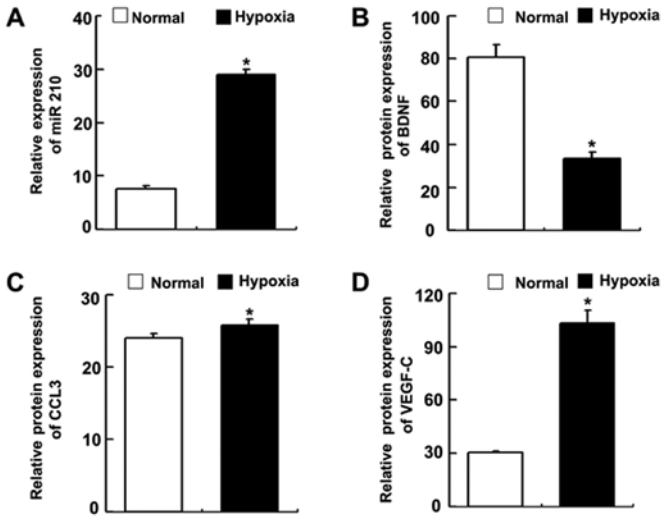

Figure 4. RT-qPCR results for the expression of (A) miR-210 in BMSCs cultured under normal and hypoxic conditions. ELISA results for the expression of (B) BDNF, (C) CCL3 and (D) VEGF-C in the supernatant from BMSCs cultured under normal and hypoxic conditions. n=3, $^{*}P<0.05$ vs. normal. miR, microRNA; BDNF, brain derived neurotrophic factor; CCL3, chemokine C-C motif ligand 3; VEGF-C, vascular endothelial growth factor-C.

were significantly increased (Fig. 7C; P=0.005) when miR-210 was overexpressed in BMSCs and significantly decreased when miR-210 was reduced (Fig. 7C; P=0.037). The present study determined the effects of miR-210 on the protein expression levels of BDNF, CCL3 and VEGF-C in BMSCs. Consistent with the mRNA expression levels, the western blot analyses indicated that BDNF expression levels were not significantly different when miR-210 was overexpressed or suppressed in BMSCs compared with in the NC BMSCs group (Fig. 8A). The expression levels of CCL3 did not significantly change when miR-210 was overexpressed (P=0.171), while the expression of CCL3 significantly decreased when miR-210 expression was inhibited (P=0.014) in BMSCs (Fig. 8B). The expression levels of VEGF-C were significantly increased when miR-210 was overexpressed (P=0.002) and significantly decreased when miR-210 expression was inhibited (P=0.039) in BMSCs (Fig. 8C).

Discussion

The migration, proliferation and differentiation of NPCs located in the subventricular zone and dentate gyrus are key to promoting self-repair following brain damage; however, the number of NPCs that migrate to the lesion is limited and insufficient to meet the requirements of functional reconstruction (34). Therefore, elucidating the factors that influence and promote NPC migration is required to promote self-repair following brain injury.

Cell therapy is considered a prospective treatment for brain injury with good therapeutic effects. Autologous BMSCs have the advantages of being obtained easily, proliferative *in vitro*, and immune privileged (35). BMSCs are an ideal candidate for cell therapy. At present, research on BMSCs for the treatment of brain injury has focused on inducing BMSCs to differentiate into neurons to replace damaged neurons (36), paracrine-secretion of certain neurotrophic factors, such as VEGF, BDNF, nerve growth factor, basic fibroblast growth factor, to support the survival of neural cells (12,13), promoting neural stem/progenitor cell proliferation, and regulating the differentiation of neural stem/progenitor cells to neurons (34,37). Further investigation into the effects of BMSCs on promoting the migration of neural stem/progenitor cells via transplantation are required.

Stem cell transplantation has been proposed as a means of cell replacement therapy. Once locally or systematically injected, about 1/3 of the locally injected stem cells migrated toward the damaged region (38). Following ischemic stroke, NPCs proliferate within major germinal niches of the brain (39). In recent years, numerous studies have reported an interaction between BMSCs and NSCs during brain injury repair. Haragopal *et al* (40) revealed that the direct effects of BMSCs on NSCs may enhance the stemness of NSCs via the Notch-1 signaling pathway, which may be useful for establishing human neural stem cell lines *in vitro* for basic research, clinical research and clinical transplantation. Wang *et al* (41) reported that BMSCs regulated the proliferation and differentiation of NSCs via the Notch signaling pathway. Similarly, NSCs may affect BMSCs; Alexanian (36) suggested that NSCs may induce BMSCs to differentiate into neural stem-like cells; however, it is unknown whether BMSCs regulate the migration of NSCs/NPCs. In the present study, BMSCs were employed to induce NPC migration, which may resolve the key problem of NSC/NPC migration following brain injury. In the present study, the effects of BMSCs on NPC migration were investigated via a Transwell cell migration assay. The results of the present study revealed that pre-culturing BMSCs for 24 h and co-culturing BMSCs and NPCs for 24 h may significantly improve NPC migration compared with pre-culturing BMSCs for 48 h. These findings suggested that 24 h may be an optimal duration for NPC migration; the BMSCs exhibited a stronger ability to promote NPC migration with in relatively a shorter duration of pre-culture of 24 h than 48 h. These observations may be associated with the decreased cell growth and proliferation due to contact-dependent inhibition when BMSCs are pre-cultured for 48 h (42-44).

BMSCs may be located in a hypoxic environment if BMSCs are stereotactically injected into the focal ischemic area or if BMSCs migrate to the infarct border zone via intravenous injection post-ischemic brain damage. miR-210 is a hypoxia-responsive miRNA that can combine with hypoxia inducible factor 1α (HIF-1α). miR-210 expression levels were increased in cells or tissues significantly under hypoxic conditions (18,45,46). The results of the present study revealed that miR-210 expression in hypoxic BMSCs increased significantly, which was consistent with the results of previous studies (18,45,46).

miR-210 serves an important role in promoting cell survival under hypoxia. Nie *et al* (47) confirmed that the overexpression of miR-210 significantly promoted the survival of BMSCs under hypoxic conditions. Sun *et al* (48) also reported that miR-210 may form a positive feedback loop with HIF-1α to promote the survival of osteoblast cells post-hypoxia. miR-210 also exhibited protective effects on NSCs/NPCs; Wang *et al* (49) reported that miR-210 inhibited the apoptosis of NPCs by inhibiting BNIP3. In addition to the protective

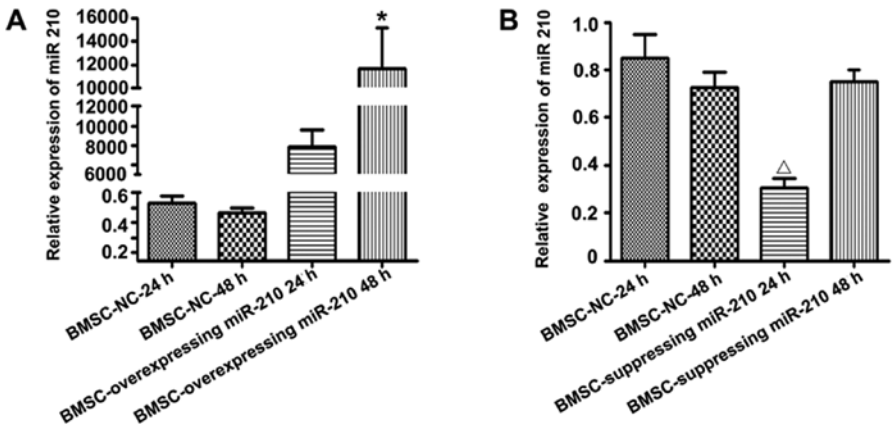

Figure 5. Reverse transcription-quantitative polymerase chain reaction analysis of the expression of miR-210 in the BMSCs transfected with miR-210 mimics, inhibitor and NC. miR-210 expression was detected in BMSCs treated with the NC miRNA, (A) miR-210 mimics and (B) miR-210 inhibitor at 24 and 48 h. n=3 each group. *P<0.05, vs. BMSC-NC 24 h, BMSC-NC 48 h and BMSC-overexpressing miR-210 24 h; ΔP<0.05, vs. BMSC-NC 24 h, BMSC-NC 48 h and BMSC-suppressing miR-210 48 h. miR, microRNA; BMSCs, bone marrow-derived mesenchymal stem cells; NC, negative control.

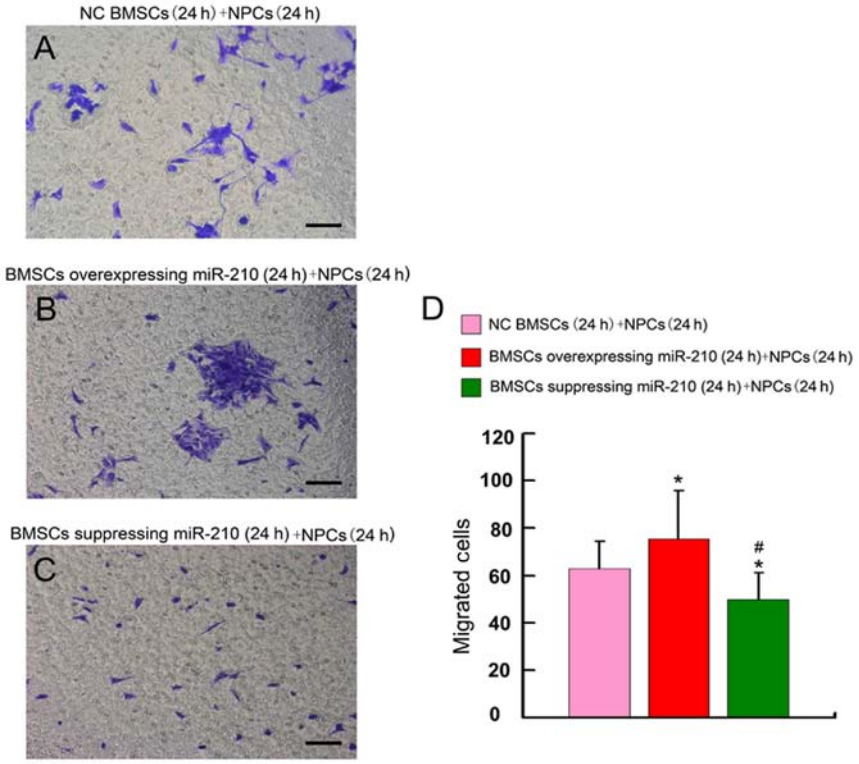

Figure 6. Effect of miR-210 in BMSCs on the migration of NPCs. (A) NC BMSCs group; (B) BMSCs overexpressing miR-210 group; (C) BMSCs suppressing miR-210 group. (D) Quantification of migrated cells. Number of fields counted (n=20), *P<0.05 vs. NC BMSCs (24 h) + NPCs (24 h) group; #P<0.05, vs. BMSCs overexpressing miR-210 (24 h) + NPCs (24 h) group. Scale bar, 100 μm. NC, negative control; BMSCs, bone marrow-derived mesenchymal stem cells; NPC, neural precursor cells; miR, microRNA.

effects on cell survival during hypoxia, miR-210 may also exhibit an effect on cell migration. Zhang *et al* (50) confirmed that phosphatase of regenerating liver-3 can promote the migration and invasion of gastric cancer cells by the nuclear factor-κB/HIF-1α/miR-210 axis. Similarly, Qu *et al* (51) also reported that the overexpression of miR-210 may promote the migration of colorectal cancer cells. In the present study, the upregulation of miR-210 in BMSCs may have enhanced the effect of BMSCs on NPC migration, while the downregulation of miR-210 in BMSCs may have reduced the effect of BMSCs on NPC migration. Thus, miR-210 may serve an important role in the process of BMSC-induced regulation of NPC migration.

In the present study, miR-210 expression levels were increased in BMSCs under hypoxic conditions. VEGF-C and CCL3 expression levels were significantly increased in the supernatant of BMSCs cultured under hypoxic conditions compared with that of BMSCs cultured under normal conditions; while BDNF expression decreased significantly. To investigate whether the alterations in BDNF, VEGF-C and CCL3 expression may be regulated by miR-210, the expression

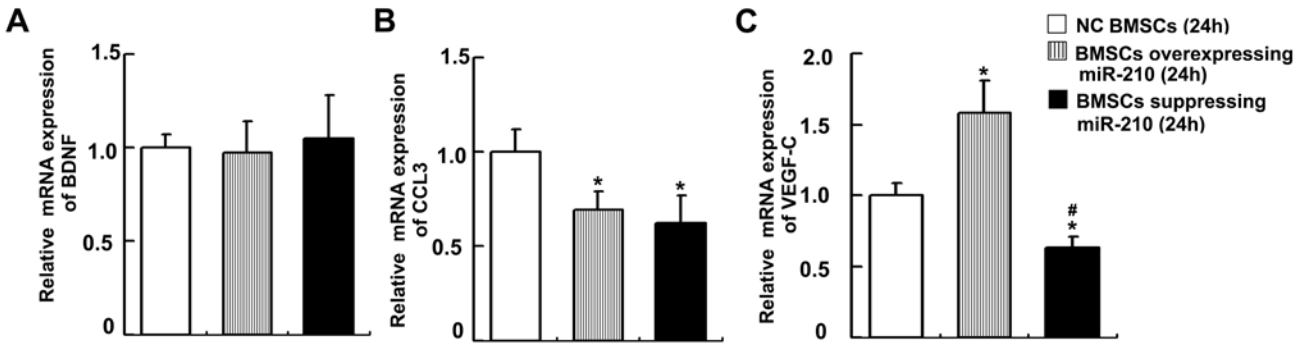

Figure 7. Reverse transcription-quantitative polymerase chain reaction analyses of (A) BDNF, (B) CCL3 and (C) VEGF-C mRNA expression levels in the negative control BMSCs group, BMSCs overexpressing miR-210 group and BMSCs suppressing miR-210 group. n=3 for each group, *P<0.05 vs. NC BMSC group; #P<0.05 vs. BMSCs overexpressing miR-210 group. BDNF, brain derived neurotrophic factor; CCL3, chemokine C-C motif ligand 3; VEGF-C, vascular endothelial growth factor-C; BMSCs, bone marrow-derived mesenchymal stem cells; NC, negative control; miR, microRNA.

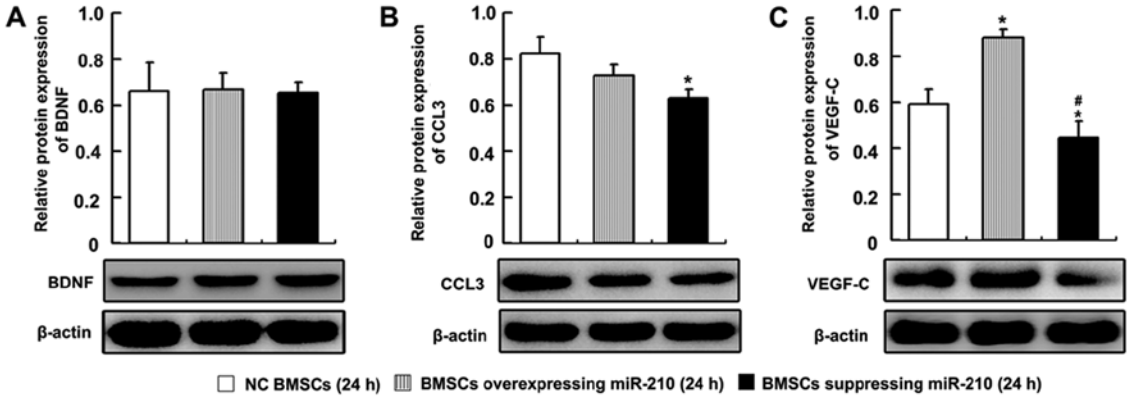

Figure 8. Western blot analysis of BDNF, CCL3 and VEGF-C expression in the miR-210-regulated BMSCs pre-cultured for 24 h. Representative bands and densitometry quantification of (A) BDNF, (B) CCL3 and (C) VEGF-C protein expression in the NC BMSCs group, BMSCs overexpressing miR-210 group and BMSCs suppressing miR-210 group. n=3 for each group, *P<0.05 vs. NC BMSCs group; #P<0.05, vs. BMSCs overexpressing miR-210 group. BDNF, brain derived neurotrophic factor; NC, negative control; BMSCs, bone marrow-derived mesenchymal stem cells; CCL3, chemokine C-C motif ligand 3; VEGF-C, vascular endothelial growth factor-C.

levels of BDNF, VEGF-C and CCL3 in BMSCs overexpressing miR-210 or suppressing miR-210 expression were analyzed. VEGF-C mRNA and protein expression levels were increased significantly; however, the expression levels of CCL3 and BDNF protein did not change significantly in BMSCs overexpressing miR-210. Additionally, VEGF-C expression levels were significantly decreased, CCL3 expression decreased significant and BDNF expression did not change significantly in BMSCs with reduced miR-210 expression levels. The association between CCL3 or BDNF and miR-210 was unclear; however, VEGF-C expression increased when miR-210 expression was upregulated and *vice versa*. Combined with the results from previous studies, miR-210 may be associated with the increased level of VEGF expression (52-54). A high concentration of VEGF in glioma C6 cells promoted the transmigration of human NSCs (ReNcells CX cell lines) (55). Therefore, miR-210 in BMSCs may further promote NPC migration by increasing the expression of VEGF.

In summary, the present study successfully isolated BMSCs and NPCs and examined the effects of BMSCs on NPC migration via a Transwell cell migration assay. BMSCs may promote NPC migration; pre-culturing BMSCs for 24 h and co-culturing BMSCs/NPCs for 24 h were the optimal durations of incubation for NPC migration. The BMSCs overexpressing miR-210 may have promoted NPC migration further by increasing VEGF-C expression. These data may provide insight into the mechanism, in which transplanted BMSCs may promote NPC migration to the ischemic focus following cerebral ischemia *in vivo* by increasing miR-210 and VEGF expression; however, further investigation *in vivo* in the future is required. In addition, the mechanism underlying miR-210-mediated regulation of VEGF remains to be elucidated in future studies. The results of the present study may assist in developing new therapeutic regimens for cerebral ischemia. Transplantation of BMSCs, especially the BMSCs overexpressing miR-210 after cerebral ischemia may promote NPC migration to the ischemia foci, which is good for brain damage repair.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

FW, acquisition of data, and drafting the manuscript. ZZ, design of the study, interpretation of the data, drafting of the manuscript and gave final approval of the version to be published; JeZ, acquisition and analysis of data. JZ, interpretation of data, revising the manuscript critically for important intellectual content; WD, acquisition and analysis of data.

Ethics approval and consent to participate

All procedures and experiments in the present study were conducted under ARRIVE guidelines (http://www.nc3rs.org.uk/arrive-guidelines) and were approved by the Laboratory Animal Welfare and Ethics Committee of the Third Military Medical University (Chongqing, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Khatri R, McKinney AM, Swenson B and Janardhan V: Blood-brain barrier, reperfusion injury, and hemorrhagic transformation in acute ischemic stroke. Neurology 79 (13 Suppl 1): S52-S57, 2012.
2. Blesch A: Human ESC-derived interneurons improve major consequences of spinal cord injury. Cell Stem Cell 19: 423-424, 2016.
3. Davoust C, Plas B, Béduer A, Demain B, Salabert AS, Sol JC, Vieu C, Vaysse L and Loubinoux I: Regenerative potential of primary adult human neural stem cells on micropatterned bio-implants boosts motor recovery. Stem Cell Res Ther 8: 253, 2017.
4. Feng Y, Ju Y, Cui J and Wang L: Bone marrow stromal cells promote neuromotor functional recovery, via upregulation of neurotrophic factors and synapse proteins following traumatic brain injury in rats. Mol Med Rep 16: 654-660, 2017.
5. Hsueh YY, Chang YJ, Huang CW, Handayani F, Chiang YL, Fan SC, Ho CJ, Kuo YM, Yang SH, Chen YL, *et al*: Synergy of endothelial and neural progenitor cells from adipose-derived stem cells to preserve neurovascular structures in rat hypoxic-ischemic brain injury. Sci Rep 5: 14985, 2015.
6. Park KJ, Park E, Liu E and Baker AJ: Bone marrow-derived endothelial progenitor cells protect postischemic axons after traumatic brain injury. J Cereb Blood Flow Metab 34: 357-366, 2014.
7. Gennai S, Monsel A, Hao Q, Liu J, Gudapati V, Barbier EL and Lee JW: Cell-based therapy for traumatic brain injury. Br J Anaesth 115: 203-212, 2015.
8. Banerjee S, Williamson DA, Habib N and Chataway J: The potential benefit of stem cell therapy after stroke: An update. Vasc Health Risk Manag 8: 569-580, 2012.
9. Steinberg GK, Kondziolka D, Wechsler LR, Lunsford LD, Coburn ML, Billigen JB, Kim AS, Johnson JN, Bates D, King B, *et al*: Clinical outcomes of transplanted modified bone marrow-derived mesenchymal stem cells in stroke: A phase 1/2a study. Stroke 47: 1817-1824, 2016.
10. Hao L, Zou Z, Tian H, Zhang Y, Zhou H and Liu L: Stem cell-based therapies for ischemic stroke. Biomed Res Int 2014: 468748, 2014.
11. An SS, Jin HL, Kim KN, Kim DS, Cho J, Liu ML, Oh JS, Yoon DH, Lee MH and Ha Y: Neuroprotective effect of combined hypoxia-induced VEGF and bone marrow-derived mesenchymal stem cell treatment. Childs Nerv Syst 26: 323-331, 2010.
12. Eckert MA, Vu Q, Xie K, Yu J, Liao W, Cramer SC and Zhao W: Evidence for high translational potential of mesenchymal stromal cell therapy to improve recovery from ischemic stroke. J Cereb Blood Flow Metab 33: 1322-1334, 2013.
13. Hsuan YC, Lin CH, Chang CP and Lin MT: Mesenchymal stem cell-based treatments for stroke, neural trauma, and heat stroke. Brain Behav 6: e00526, 2016.
14. Doetsch F: A niche for adult neural stem cells. Curr Opin Genet Dev 13: 543-550, 2003.
15. Koh SH and Lo EH: The role of the PI3K pathway in the regeneration of the damaged brain by neural stem cells after cerebral infarction. J Clin Neurol 11: 297-304, 2015.
16. Temple S: Division and differentiation of isolated CNS blast cells in microculture. Nature 340: 471-473, 1989.
17. Gage FH: Mammalian neural stem cells. Science 287: 1433-1438, 2000.
18. Liu ZH, Yang G, Zhao T, Cao GJ, Xiong L, Xia W, Huang X, Wu LY, Wu K, Fan M, *et al*: Small ncRNA expression and regulation under hypoxia in neural progenitor cells. Cell Mol Neurobiol 31: 1-5, 2011.
19. Yao L and Li Y: The role of direct current electric field-guided stem cell migration in neural regeneration. Stem Cell Rev 12: 365-375, 2016.
20. Abe M and Bonini NM: MicroRNAs and neurodegeneration: Role and impact. Trends Cell Biol 23: 30-36, 2013.
21. Ivan M and Huang X: miR-210: Fine-tuning the hypoxic response. Adv Exp Med Biol 772: 205-227, 2014.
22. Pulkkinen K, Malm T, Turunen M, Koistinaho J and Ylä-Herttuala S: Hypoxia induces microRNA miR-210 in vitro and in vivo ephrin-A3 and neuronal pentraxin 1 are potentially regulated by miR-210. FEBS Lett 582: 2397-2401, 2008.
23. Qiu J, Zhou XY, Zhou XG, Cheng R, Liu HY and Li Y: Neuroprotective effects of microRNA-210 against oxygen-glucose deprivation through inhibition of apoptosis in PC12 cells. Mol Med Rep 7: 1955-1959, 2013.
24. Lou YL, Guo F, Liu F, Gao FL, Zhang PQ, Niu X, Guo SC, Yin JH, Wang Y and Deng ZF: miR-210 activates notch signaling pathway in angiogenesis induced by cerebral ischemia. Mol Cell Biochem 370: 45-51, 2012.
25. Volný O, Kašičková L, Coufalová D, Cimflová P and Novák J: microRNAs in cerebrovascular disease. Adv Exp Med Biol 888: 155-195, 2015.
26. Rink C and Khanna S: MicroRNA in ischemic stroke etiology and pathology. Physiol Genomics 43: 521-528, 2011.
27. Zhu J, Zhou Z, Liu Y and Zheng J: Fractalkine and CX3CR1 are involved in the migration of intravenously grafted human bone marrow stromal cells toward ischemic brain lesion in rats. Brain Res 1287: 173-183, 2009.
28. Zhang Y, Zheng J, Zhou Z, Zhou H, Wang Y, Gong Z and Zhu J: Fractalkine promotes chemotaxis of bone marrow-derived mesenchymal stem cells towards ischemic brain lesions through Jak2 signaling and cytoskeletal reorganization. FEBS J 282: 891-903, 2015.
29. Jiang Y, Li L, Tan X, Liu B, Zhang Y and Li C: miR-210 mediates vagus nerve stimulation-induced antioxidant stress and anti-apoptosis reactions following cerebral ischemia/reperfusion injury in rats. J Neurochem 134: 173-181, 2015.
30. Zhang D, Cao X, Li J and Zhao G: MiR-210 inhibits NF-κB signaling pathway by targeting DR6 in osteoarthritis. Sci Rep 5: 12775, 2015.
31. Cao L, Zhang X, Cao F, Wang Y, Shen Y, Yang C, Uzan G, Peng B and Zhang D: Inhibiting inducible miR-223 further reduces viable cells in human cancer cell lines MCF-7 and PC3 treated by celastrol. BMC Cancer 15: 873, 2015.
32. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
33. Brown JP, Couillard-Després S, Cooper-Kuhn CM, Winkler J, Aigner L and Kuhn HG: Transient expression of doublecortin during adult neurogenesis. J Comp Neurol 467: 1-10, 2003.
34. Croft AP and Przyborski SA: Mesenchymal stem cells expressing neural antigens instruct a neurogenic cell fate on neural stem cells. Exp Neurol 216: 329-341, 2009.

35. Kuroda S: Current opinion of bone marrow stromal cell transplantation for ischemic stroke. Neurol Med Chir (Tokyo) 56: 293-301, 2016.
36. Alexanian AR: Neural stem cells induce bone-marrow-derived mesenchymal stem cells to generate neural stem-like cells via juxtacrine and paracrine interactions. Exp Cell Res 310: 383-391, 2005.
37. Bai L, Caplan A, Lennon D and Miller RH: Human mesenchymal stem cells signals regulate neural stem cell fate. Neurochem Res 32: 353-362, 2007.
38. Liu X, Ye R, Yan T, Yu SP, Wei L, Xu G, Fan X, Jiang Y, Stetler RA, Liu G and Chen J: Cell based therapies for ischemic stroke: From basic science to bedside. Prog Neurobiol 115: 92-115, 2014.
39. Lu J, Manaenko A and Hu Q: Targeting adult neurogenesis for poststroke therapy. Stem Cells Int 2017: 5868632, 2017.
40. Haragopal H, Yu D, Zeng X, Kim SW, Han IB, Ropper AE, Anderson JE and Teng YD: Stemness enhancement of human neural stem cells following bone marrow MSC coculture. Cell Transplant 24: 645-659, 2015.
41. Wang Y, Tu W, Lou Y, Xie A, Lai X, Guo F and Deng Z: Mesenchymal stem cells regulate the proliferation and differentiation of neural stem cells through Notch signaling. Cell Biol Int 33: 1173-1179, 2009.
42. Hackstein H, Tschipakow I, Bein G, Nold P, Brendel C and Baal N: Contact-dependent abrogation of bone marrow-derived plasmacytoid dendritic cell differentiation by murine mesenchymal stem cells. Biochem Biophys Res Commun 476: 15-20, 2016.
43. Yamada T, Yuasa M, Masaoka T, Taniyama T, Maehara H, Torigoe I, Yoshii T, Shinomiya K, Okawa A and Sotome S: After repeated division, bone marrow stromal cells express inhibitory factors with osteogenic capabilities, and EphA5 is a primary candidate. Bone 57: 343-354, 2013.
44. Wang PP, Xie DY, Liang XJ, Peng L, Zhang GL, Ye YN, Xie C and Gao ZL: HGF and direct mesenchymal stem cells contact synergize to inhibit hepatic stellate cells activation through TLR4/NF-kB pathway. PLoS One 7: e43408, 2012.
45. Huang X, Ding L, Bennewith KL, Tong RT, Welford SM, Ang KK, Story M, Le QT and Giaccia AJ: Hypoxia-inducible mir-210 regulates normoxic gene expression involved in tumor initiation. Mol Cell 35: 856-867, 2009.
46. Xiong L, Wang F, Huang X, Liu ZH, Zhao T, Wu LY, Wu K, Ding X, Liu S, Wu Y, *et al*: DNA demethylation regulates the expression of miR-210 in neural progenitor cells subjected to hypoxia. FEBS J 279: 4318-4326, 2012.
47. Nie Y, Han BM, Liu XB, Yang JJ, Wang F, Cong XF and Chen X: Identification of MicroRNAs involved in hypoxia- and serum deprivation-induced apoptosis in mesenchymal stem cells. Int J Biol Sci 7: 762-768, 2011.
48. Sun G and Peng H: HIF-1α-induced microRNA-210 reduces hypoxia-induced osteoblast MG-63 cell apoptosis. Biosci Biotechnol Biochem 79: 1232-1239, 2015.
49. Wang F, Xiong L, Huang X, Zhao T, Wu LY, Liu ZH, Ding X, Liu S, Wu Y, Zhao Y, *et al*: miR-210 suppresses BNIP3 to protect against the apoptosis of neural progenitor cells. Stem Cell Res 11: 657-667, 2013.
50. Zhang C, Tian W, Meng L, Qu L and Shou C: PRL-3 promotes gastric cancer migration and invasion through a NF-κB-HIF-1α-miR-210 axis. J Mol Med (Berl) 94: 401-415, 2016.
51. Qu A, Du L, Yang Y, Liu H, Li J, Wang L, Liu Y, Dong Z, Zhang X, Jiang X, *et al*: Hypoxia-inducible MiR-210 is an independent prognostic factor and contributes to metastasis in colorectal cancer. PLoS One 9: e90952, 2014.
52. Zeng L, He X, Wang Y, Tang Y, Zheng C, Cai H, Liu J, Wang Y, Fu Y and Yang GY: MicroRNA-210 overexpression induces angiogenesis and neurogenesis in the normal adult mouse brain. Gene Ther 21: 37-43, 2014.
53. Liu F, Lou YL, Wu J, Ruan QF, Xie A, Guo F, Cui SP, Deng ZF and Wang Y: Upregulation of microRNA-210 regulates renal angiogenesis mediated by activation of VEGF signaling pathway under ischemia/perfusion injury in vivo and in vitro. Kidney Blood Press Res 35: 182-191, 2012.
54. Kawanishi Y, Nakasa T, Shoji T, Hamanishi M, Shimizu R, Kamei N, Usman MA and Ochi M: Intra-articular injection of synthetic microRNA-210 accelerates avascular meniscal healing in rat medial meniscal injured model. Arthritis Res Ther 16: 488, 2014.
55. Díaz-Coránguez M, Segovia J, López-Ornelas A, Puerta-Guardo H, Ludert J, Chávez B, Meraz-Cruz N and González-Mariscal L: Transmigration of neural stem cells across the blood brain barrier induced by glioma cells. PLoS One 8: e60655, 2013.