Cellular and Molecular Biology

Zhang *et al. Cell. Mol. Biol.*2016, 62 (14): 29-33
ISSN: 1165-158X
doi: 10.14715/cmb/ 2016.62.14.4

*Original Research*

# Over-expression of brain-derived neurotrophic factor in mesenchymal stem cells transfected with recombinant lentivirus BDNF gene

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**Abstract:** This study was aimed at investigating the expression of brain-derived neurotrophic factor (BDNF) in mesenchymal stem cells (MSCs) modified with recombinant lentivirus bearing BDNF gene. Lentivirus vectors bearing BDNF gene were constructed. MSCs were isolated from rats and cultured. The lentiviral vectors containing BDNF gene were transfected into the MSCs, and BDNF gene and protein expressions were monitored with enhanced green fluorescent protein (EGFP). RT-PCR and Western blot were used to measure gene and protein expressions, respectibvely in MSCs, MSCs-EGFP and MSCs-EGFP-BDNF groups. Green fluorescence assay confirmed successful transfection of BDNF gene recombinant lentivirus into MSCs. RT-PCR and Western blot revealed that BDNF gene and protein expressions in the MSCs-EGFP-BDNF group were significantly higher than that in MSCs group and MSCs-EGFP group. There were no statistically significant differences in gene expression between MSCs and MSCs-EGFP groups. MSCs can over-express BDNF when transfected with recombinant lentivirus bearing BDNF gene.

***Key words:*** Brain-derived neurotrophic factor, Gene, lentivirus, mesenchymal stem cells.

## Introduction

Bone marrow MSCs, a class of adherent mononuclear cells are non-hematopoietic stem cells present in bone marrow and derived from the mesoderm (1). MSCs are easily obtained, cultured, purified and amplified; and under favorable conditions they can differentiate into mesoderm fat cells, chondrocyte, osteoblasts, muscle and vascular endothelial cells, as well as endoderm hepatocytes, alveolar ectodermal epithelial cells and nerve cells (2-5). They are recognized as seed cells for tissue repair and regeneration, and good gene therapy vectors due to their characteristic low immunogenicity, mobility, organization integration and capacity for self-renewal (6). In addition, MSCs secrete a variety of nerve growth factors, especially BDNF which is important for brain development. Mammalian brain is a rich source of BDNF, an important regulatory protein which is involved in survival signaling, deactivation of death signal, differentiation, reduction of neuronal apoptosis, induction of endogenous cell proliferation, axonal growth and improvement of synaptic plasticity (7-9). Studies have shown that MSCs and BDNF exert positive therapeutic effects on preclinical animals with various types of nerve injury. However, BDNF has several inherent disadvantages: it is a macromolecule, and so cannot pass through the blood brain barrier and it has short half-life *in vivo* (10-13).

In the transfer BDNF gene into brain MSCs, it is important to select a suitable carrier with a capacity for BDNF, high gene vector amplification, and efficient transfection ability for the MSCs, good proliferation and ability to induce differentiation into neuron-like cells (14). Lentiviral vector is an ideal integrating virus vector that meets these requirements (15, 16). EGFP is an optimized mutant green fluorescent protein which produces 35 times stronger fluorescence than ordinary GFP, thereby greatly enhancing the sensitivity of reporter genes. Thus EGFP can be effectively used to monitor the efficiency of therapeutic gene transfection and functional expression; and evaluation of therapeutic effect of the transfection (17). In the present study, MSCs-EGFP-BDNF recombinant lentivirus was constructed by cloning synthetic BDNF gene into an EGFP vector, and the expression of the BDNF gene was monitored.

## Materials and Methods

### Experimental animals

Pathogen-free male SD rats weighing 250 ~ 300g were used. The rats were housed at room temperature, and given free access to feed and drinking water.

### Reagents and equipment

The reagents and equipment used, and their sources (in parentheses) were agar medium (L-DMEM), fetal bovine serum and trypsin (Hyclone, USA); EGFP, BDNF recombinant lentivirus (BDNF -EGFP-LV ), lentivirus vector (EGFP-LV) (Shanghai Jikai, China); Trizol kit,

**Received** September 9, 2016; **Accepted** December 25, 2016; **Published** December 30, 2016

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reverse transcription kit, Real-Time PCR kit (Dalian Baosheng Biotechnology Co., Ltd., China); bicinchoninic acid protein assay kit (Shanghai Jierui Biological Engineering Co., Ltd., China); and BDNF rabbit anti-rat polyclonal antibody (Beijing Aobo biotechnology Co., Ltd., China). The equipment used were Clean Bench (Suzhou Purification Equipment Co., Ltd, China), $CO_2$ incubator (Thermo, USA), autoclaves (TOMY, Japan), - 80°C ultra-low temperature freezer (Forma Scientic, Germany), optical microscope (Nikon company, Japan), laser scanning confocal microscope (Leica, Germany), and Odyssey two-color fluorescence imager (LI-COR, USA).

**Isolation and culture of MSCs**

Six week-old male SD rats were sacrificed by cervical dislocation, soaked in 75% alcohol, and disinfected for 10min. Thereafter they were placed in a clean bench for evisceration and removal of muscles, tendons and other soft tissue. On both sides of the end of femoral and tibial epiphysis, the bone marrow cavity was cut and bone marrow was collected in15ml-centrifuge tubes containing phosphate buffered saline (PBS). The tubes were centrifuged at room temperature at 1000 rpm for 5 min, then the supernatant was added to 10% L-DMEM containing fetal bovine serum and cultured in plastic flasks at 37 °C in 5% $CO_2$ incubator. The culture medium was changed every 48 h.

**Identification of MSCs**

The surface markers (CD29, CD45 and CD90) of rat MSCs were detected by flow cytometry. Well-grown third generation MSCs were used in preparing the MSCs suspension which was diluted with 200μL PBS to a density of $5 \times 10^5$. Three groups of cells were collected. To the first group was added 5μl rat IgG-FITC or IgG-PE as a control. The second group received 5μl rat CD29-FITC and CD45-P, while the third group was given 5μl CD90-PE. After mixing, the tubes containing the cell suspensions were kept in the dark at room temperature for 25 min for reaction to take place. Thereafter 2ml PBS was added to each tube, and the tube contents were mixed on a shaker at 1000 rpm and then centrifuged for 5 min. The supernatant was discarded, and 200μl of 2% paraformaldehyde was added to fix the cells prior to testing.

**MSCs transfection by lentiviral (LV) vector**

Third generation MSCs ($1\times10^4$) were seeded in 96-well plates. After 24h, the original culture medium was discarded, and replaced with viral transfection enhancement solution. Depending on titer and the number of cells, LV, BDNF-EGFP-LV and EGFP-LV were added in different multiplicities of infection (MOI) (10,30,50,70 and 100, respectively). At the same time, 8μg/ml of pro-transfection agent (polybrene) was added based on the viral transfection enhancement solution volume. After mixing, each group was incubated at 37 °C in a 5% $CO_2$ incubator. After 8-12h, cellular morphology was observed under an inverted microscope, and lentivirus transfection enhancement solution was replaced with entirely fresh medium. Fluorescence intensity of viral EGFP in MSCs was observed in a fluorescence microscope after 72-96 h. The MSCs suspensions of the BDNF-EGFP-LV and EGFP-LV transfection groups, as well as the MSC control group were collected at a density of $5\times10^5$ to determine transfection efficiency by flow cytometry.

**Detection of BDNF gene expression by RT-PCR**

Before transfection and after 24 h, 3 d, 5 d, 7 d, 10 d and 14 d of transfection, $5\times10^5$ cells were collected from MSCs group, MSCs-EGFP group and MSCs-EGFP-BDNF group. The cells were centrifuged and Trizol kit was used to extract their total RNA.BDNF expression at different time points in each group was measured with RT-PCR. The amplification primers for BDNBD-NF were 5'-GATGCCAGTTGCTTTGTCTTC-3' 9 (upstream); and 5'-TAAAATCTCGTCTCCCCAA-CA-3' (downstream). The size of amplified fragment was 193 bp. The amplification primers for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were 5'-ACAG-CAACAGGGTGGTGGAC -3' (upstream) and 5'-TT-TGAGGGTGCAGCGAACTT-3' (downstream). The size of amplified fragment was 252 bp. Each assay was done in triplicate.

**Detection of BDNF protein expression by Western blot**

Well-grown MSCs ($1\times10^6$) from each of the three groups were harvested and lysed in RIPA buffer with protease inhibitors. Protein concentration was determined by the BCA method. Equal amounts of total cell lysates (60 mg protein) and Full-Range Rainbow Molecular Weight Marker were resolved using 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene fluoride (PVDF) membranes through electrophoresis. The PVDF membranes were immersed in blocking solution (5% skim milk) to block nonspecific binding for 1 h at room temperature. Then the PVDF membranes were rinsed with TBST for 5min, and immuno-blotted with primary monoclonal antibodies diluted with TBST containing skim milk at room temperature for 1h, or at 4 °C overnight. Then, further washing was conducted 4 times with TBST, each for 10min. The membranes were incubated in the dark for 2 h at room temperature with the secondary antibody, and then rinsed 4 times with TBST, each for 10min. The western blot was scanned with Odyssey dual-color fluorescence imaging in the dark.

**Protein expression of BDNF by cells climbing and fluorescence labeling**

The well-grown MSCs were collected and placed on sterile slide plates after digestion and obtaining a homogeneous mixture. After 24h, climbing cells were observed under inverted microscope. Once the MSCs were uniform and stable, Brdu was used to mark MSCs, also BDNF-EGFP -LV and EGFP-LV were applied for transfection. After 48h and 72h, the DAPI marker and viral transfection of MSCs were observed under a fluorescence microscope. Slides showing very high transfection efficiency were fixed with paraformaldehyde. The primary antibody (BDNF rabbit anti-rat 1: 100) was added, and the setup was incubated at 4 °C in a refrigerator overnight. Fluorescent secondary antibody (goat anti-rabbit 1: 200) was added in the dark and incubated at room temperature for 1h. Finally, antifade was used

to seal prior to microscopy.

## Results

### Microscopic observation of MSCs

Dense and uniform oval cells were visible in L-DMEM culture medium under an inverted microscope (Figure 1). After 24h, half of the medium became changed, showing a few cells in adherent growth. After 72h, the whole medium changed, and part of the visible adherent cells formed ellipsoidal, round, oolygonal, stub or short fusiformis. On days 4-6, the refraction of the cells become strong; the cells continued to proliferate, and varied from the polygonal, short fusiforms to long fusiforms, diffusing gradually as a colony, with mutual cross-linking. On days 7 -8, the cells all showed long fusiform shapes. Cell fusion was 80% -90%, with long, uniform and evenly-distributed fusiforms. Cell proliferation rate was significantly faster at this stage.

### Identification of MSCs and cell transfection efficiency

MSCs was cultured to the third generation, and flow cytometry showed that the positive levels of CD90, CD29 and CD45 were 98.09%, 95.17% and 6.33% respectively, which were in line with phenotypic characteristics of MSCs, suggesting that the cultured cells were MSCs with high purity (Fig. 2).

After 72h of LV transfection, most of the cells manifested green fluorescence (Fig. 3). When the multiplicity of infection was 50, the transfection rates of BDNF-EGFP-LV and EGFP-LV were 95.23% and 96.08% respectively, but 2.29% in the control group (Fig. 4).

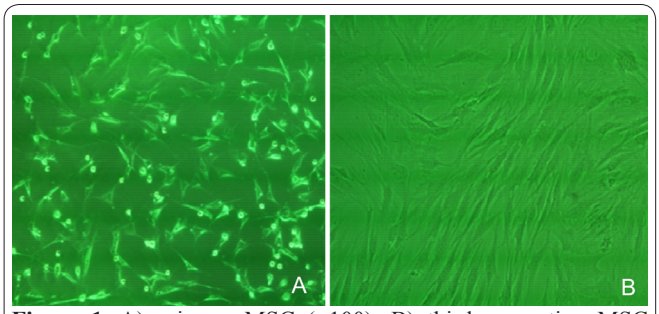


**Figure 1.** A) primary MSC (×100); B) third generation MSC (×100).

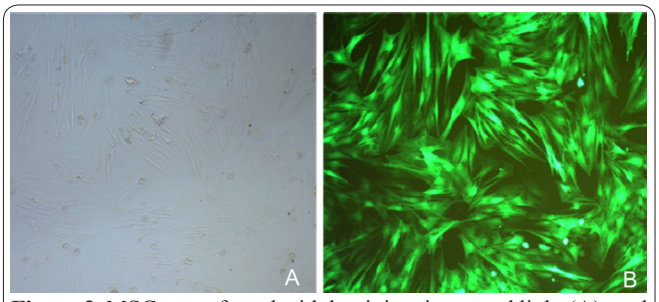


**Figure 3.** MSCs transfected with lentivirus in natural light (A); and in fluorescent light (B) (×100).

### BDNF gene expression determined by RT-PCR in each group

BDNF mRNA expression results determined by RT-PCR was significantly higher in the MSCs-EGFP-BDNF group (CT value) than in the MSCs and MSCs-EGFP groups ($p < 0.05$), while there were no statistically significant differences between the MSCs group and MSCs- EGFP group (Table 1). When determined at different time points for MSCs-EGFP-BDNF, the highest expression was on day 3 ($p < 0.05$).

### Western blot analysis of BDNF expression in each group

The western blot results revealed the BDNF protein expression of MSCs-EGFP-BDNF group was significantly increased compared with the other two groups ($p < 0.05$). There was no statistically significant difference between expression of BDNF in MSCs group and MSCs-EGFP group (Figure 5). Based on $2^{-\Delta\Delta CT}$ method, statistical results showed that MSCs transfected with lentivirus over-expressed BDNF protein, which increased 6.6-fold compared with the control cells (Table 2).

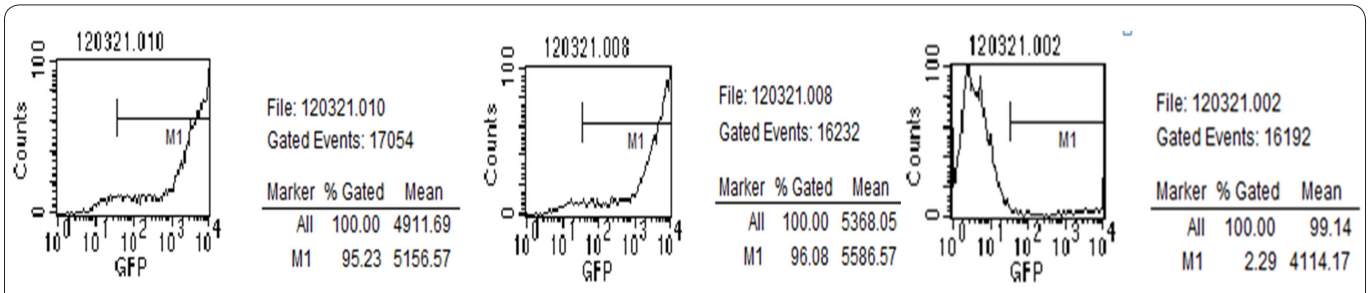


**Figure 2.** Third generation MSCs shown in flow cytometry: Most of the cells expressed CD90, CD29, and a very small amount expressed CD45.

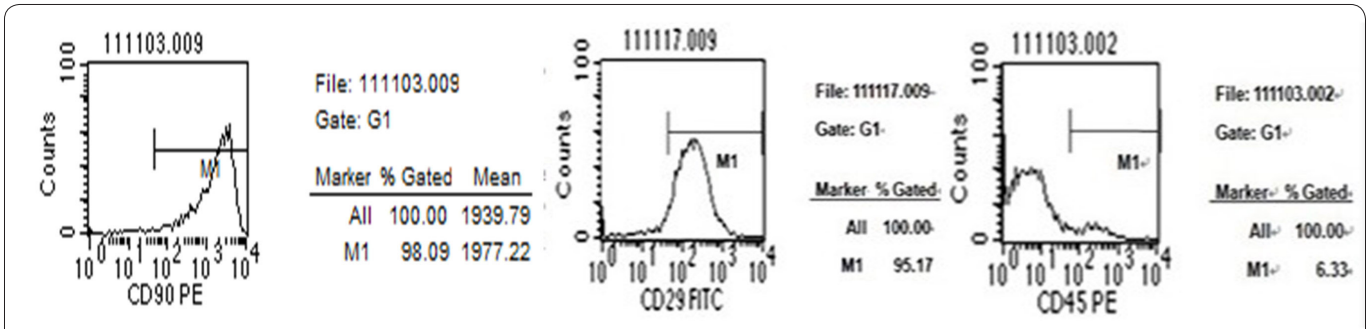


**Figure 4.** LV-transfected MSCs detected with flow cytometry.

**Table 1.** Comparison of BDNF expression in the three groups as detected by RT-PCR at different time points ($X \pm s$ ).

| Detection time point | MSCs group | MSCs-EGFP group | MSCs-EGFP-BDNF group |
|---|---|---|---|
| 1 d | 27.1±0.0 [a] | 27.8±0.0 [a] | 25.7±0.1 |
| 3 d | 27.1±0.1 [b] | 27.1±0.0 [bd] | 24.4±0.2 [d] |
| 5 d | 27.5±0.2 [b] | 27.6±0.1 [bd] | 24.7±0.0 [d] |
| 7 d | 27.5±0.1 [b] | 27.5±0.0 [b] | 24.7±0.0 |
| 10 d | 27.8±0.0 [bc] | 27.8±0.0 [bd] | 25.0±0.0 [c] |
| 14 d | 27.7±0.1 [b] | 27.8±0.1 [b] | 25.9±0.1 [d] |

Note: Compared with MSCs-GFP-BDNF group at the same time point, [a]p <0.05, [b]p<0.01, Compared with itself group at the prior time point, [c]p<0.01, [d]p<0.05.

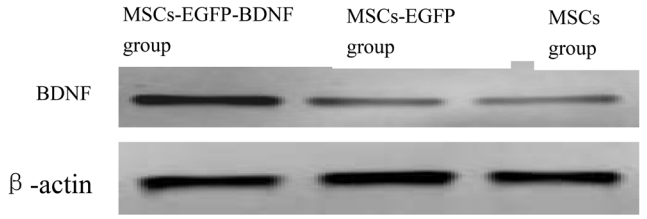


**Figure 5.** Western blot analysis of BDNF expression in each group.

**Table 2. C**omparison of BDNF protein expression between the groups.

| Groups | ΔCT | ΔΔCT | 2-ΔΔCT |
|---|---|---|---|
| MSCs | 11.4 | 0 | 1.0 |
| MSCs-EGFP | 11.3 | 0 | 1.0 |
| MSCs-EGFP-BDNF | 8.6 | 2.7 | 6.6 |

The BDNF protein expression of MSCs-EGFP-BDNF group was increased 6.6-fold compared with MSCs group and MSCs-EGFP.

### Protein expression of BDNF assessed by cells climbing and immunofluorescence labeling

MSCs that showed visible climbing under laser confocal microscopy were labelled by immunofluorescence; the results revealed that fluorescent protein of BDNF in the MSCs transfected with BDNF gene recombinant lentivirus in MSCs-EGFP-BDNF group was significantly higher than that of the MSCs group and MSCs -EGFP group (Fig. 6).

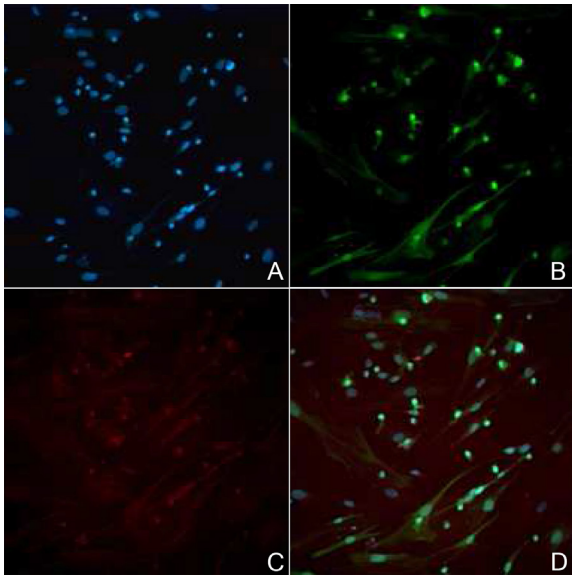


**Figure 6.** Expression of immunofluorescence BDNF protein. Fluorescence expression of DAPI (A, blue); fluorescence expression of EGFP (B, green); fluorescence expression of BDNF (C, red); *in situ* superposition (D).

## Discussion

BDNF is a biologically active protein firstly isolated and purified from porcine brain by the German nerve chemist Barde and his colleagues in 1982. It is a member of neurotrophic factor family (18). Although BDNF is present in the nerves, the central nervous system, bone and soft tissue, as well as endocrine system, it is expressed mainly in the central nervous system to provide powerful nutritional support to neurons. BDNF plays several crucial roles during the development of nerve cells. These include promotion of axonal regeneration and synapse formation, regulation of synaptic transmission in the peripheral and central nervous system, reduction of neuronal apoptosis and protection of neurons from damage (10, 19, 20). The use of exogenous BDNF in gene therapy requires an appropriate vehicle to ensure that BDNF gene is ultimately expressed.

MSCs are easily transfected for efficient expression of exogenous genes. The transplantation of transfected MSCs does not infringe on medical ethics; after transfection, MSCs maintain long-term biological characteristics, low immunogenicity and minimal transplant rejection (21, 22). Moreover, MSCs are easy to access and multiply in culture, and they can differentiate into neuronal cells. Thus they are considered one of the most promising stem cells in clinical management of a variety of neurological diseases (23 – 28). MSCs are a heterogeneous cell population lacking specific surface markers. Thus a combination of variety of methods is often used to identify them. These include morphology observation, detection of relatively specific antigen expression on cell surface and induced directional differentiation technology.

In this study, adherence screening method was used to gradually isolate MSCs based on adherent growth, white blood cells and hematopoietic stem cells grown in suspension. The density of MSCs in the bone marrow is low and decreases with age. So, juvenile rat bone marrow was cultured in L-DMEM medium. The International Society for Cell Therapy has unified identification parameters for MSCs (29, 30). The surface antigen characteristics used are: CD73, CD90, CD105, CD29, CD106, CD146, insulin-like growth factor receptor (IGFR), nerve growth factor receptor (NGFR), platelet-derived growth factor receptor (PDGFR), and bone-specific alkaline phosphatase (STRO-3), all of which should be positively expressed; while CD45, CD34, CD79α or CD19 and HLA 2DR are negatively expressed (24). In this study, MSCs were identified by detection of CD29, CD45 and CD90, indicating that most of the cells expressed CD29 and CD90, while only a very small number of cells expressed CD45. This clearly and unequivocally shows that the cultured cells were high purity MSCs.

In addition, this study adopted BDNF gene recombinant LV as the carrier of BDNF gene into MSCs by the binding of specific receptors on the cell membrane to the viral glycoproteins. The BDNF gene and viral DNA integrated their genetic components. The viral DNA and BDNF gene were transcribed into RNA and translated to protein. The transfection efficiency was over 95%, as determined by flow cytometry, and the transgenic MSCs over-expressed BDNF. In addition, BDNF protein expression, as well as cell climbing and immunofluorescence displays were significantly higher in the MSCs-EGFP-BDNF group than in the other two groups; and the transgenic MSCs expressed 6.6 times higher BDNF protein 6.6-fold than the control group. These results are consistent with those from RT_PCR, and show clearly that MSCs over-expressed BDNF due to transfection with recombinant lentivirus bearing BDNF.

The results obtained in this study clearly indicate that MSCs can over-express BDNF when transfected with recombinant lentivirus bearing BDNF gene.

## References


1. Friedenstein AJ, Chailakhyan RK, Gerasimov UV. Bone marrow osteogenic stem cells: in vitro cultivation and transplantation in diffusion chambers. Cell Tissue Kinet 1987; 20: 263-72.
2. Fernandez VVB, Romaniuk MA, Choi H, Labovsky V, Otaegui J, Chasseing NA. Mesenchymal stem cells and their use in therapy: what has been achieved. Differentiation 2013; 85: 1-10.
3. Iskandar ME, Cipriano AF, Lock J, Gott SC, Rao MP, Liu H. Improved bone marrow stromal cell adhesion on micropatterned titanium surfaces. Conf Proc IEEE Eng Med Biol Soc 2012; 2012: 5666-9.
4. Lee J, Baek JH, Choi KS, Kim HS, Park HY, Ha GH, et al. Cyclin-dependent kinase 4 signaling acts as a molecular switch between syngenic differentiation and neural transdifferentiation in human mesenchymal stem cells. Cell Cycle 2013; 12: 442-51.
5. Salgado AJ, Sousa JC, Costa BM, Pires AO, Mateus-Pinheiro A, Teixeira FG. Mesenchymal stem cells secretome as a modulator of the neurogenic niche: basic insights and therapeutic opportunities. Front Cell Neurosci 2015; 9: 249.
6. Yilmaz G, Alexander JS, Erkuran YC, Granger DN. Induction of neuro-protective/regenerative genes in stem cells infiltrating post-ischemic brain tissue. Exp Transl Stroke Med 2010; 2: 11.
7. Markham A, Bains R, Franklin P, Spedding M. Changes in mitochondrial function are pivotal in neurodegenerative and psychiatric disorders: how important is BDNF. Br J Pharmacol 2014; 171: 2206-29.
8. Gomes C, Smith SC, Youssef MN, Zheng JJ, Hagg T, Hetman M. RNA polymerase 1-driven transcription as a mediator of BDNF-induced neurite outgrowth. J Biol Chem 2011; 286: 4357-63.
9. Chen BY, Wang X, Wang ZY, Wang YZ, Chen LW, Luo ZJ. Brain-derived neurotrophic factor stimulates proliferation and differentiation of neural stem cells, possibly by triggering the Wnt/beta-catenin signaling pathway. J Neurosci Res 2013; 91: 30-41.
10. Jeong CH, Kim SM, Lim JY, Ryu CH, Jun JA, Jeun SS. Mesenchymal stem cells expressing brain-derived neurotrophic factor enhance endogenous neurogenesis in an ischemic stroke model. Biomed Res Int 2014; 2014: 129145.
11. Sharma AD, Brodskiy PA, Petersen EM, Dagdeviren M, Ye EA, Mallapragada SKSharma AD, et al. High throughput characterization of adult stem cells engineered for delivery of therapeutic factors for neuroprotective strategies. J Vis Exp 2015; 95: e52242.
12. Song MS, Learman CR, Ahn KC, Baker GB, Kippe J, Field EM, et al. In vitro validation of effects of BDNF-expressing mesenchymal stem cells on neurodegeneration in primary cultured neurons of APP/PS1 mice. Neuroscience 2015; 307: 37-50.
13. Tanna T, Sachan V. Mesenchymal stem cells: potential in treatment of neurodegenerative diseases. Curr Stem Cell Res Ther 2014; 9(6): 513-21.
14. Han ZM, Huang HM, Wang FF. Brain-derived neurotrophic factor gene-modified bone marrow mesenchymal stem cells. Exp Ther Med. 2015 9; 519-522.
15. Li L, Li B, Zhang H, et al. Lentiviral vector-mediated PAX6 overexpression promotes growth and inhibits apoptosis of human retinoblastoma cells. Invest Ophthalmol Vis Sci 2011; 52: 8393-400.
16. Nasri M, Karimi A, Allahbakhshian FM. Production, purification and titration of a lentivirus-based vector for gene delivery purposes. Cytotechnology 2014; 66: 1031-8.
17. Sun XZ, Liu GH, Wang ZQ, Zheng FF, Bian J, Huang YP, Gao Y, Zhang YD, Deng CH, et al. Over-expression of VEGF165 in the adipose tissue-derived stem cells via the lentiviral vector. Chin Med J (Engl) 2011; 124: 3093-7.
18. Barde YA, Edgar D, Thoenen H. Purification of a new neurotrophic factor from mammalian brain. EMBO J 1982; 1: 549-53.
19. Sharma AD, Brodskiy PA, Petersen EM, et al. Sharma AD, Brodskiy PA, Petersen EM, Dagdeviren M, Ye EA, Mallapragada SK. J Vis Exp 2015; 95: e52242.
20. Sygnecka K, Heider A, Scherf N, Alt R, Franke H, Heine C. Mesenchymal stem cells support neuronal fiber growth in an organotypic brain slice co-culture model. Stem Cells Dev 2015; 24: 824-35.
21. De Miguel MP, Fuentes-Julián S, Blázquez-Martínez A, Pascual CY, Aller MA, et al. Immunosuppressive properties of mesenchymal stem cells: advances and applications. Curr Mol Med. 2012 12; 574-91.
22. Soleymaninejadian E, Pramanik K, Samadian E. Immunomodulatory properties of mesenchymal stem cells: cytokines and factors. Am J Reprod Immunol 2012; 67: 1-8.
23. Khalili MA, Sadeghian-Nodoushan F, Fesahat F, Mir-Esmaeili SM, Anvari M, Hekmati-Moghadam SH. Mesenchymal stem cells improved the ultrastructural morphology of cerebral tissues after subarachnoid hemorrhage in rats. Exp Neurobiol 2014; 23: 77-85.
24. Liu W, Lu G, Wang B, Ma Z, Li Y. (Transfection of BDNF gene promotes bone mesenchymal stem cells to differentiate into neuron-like cells). Zhong Nan Da Xue Xue Bao Yi Xue Ban 2012; 37: 441-6.
25. Zhou S. From bone to brain: human skeletal stem cell therapy for stroke. Cent Nerv Syst Agents Med Chem 2011; 11: 157-63.
26. Cui X, Chen L, Ren Y, Ji Y, Liu W, Liu J,, et al. Genetic modification of mesenchymal stem cells in spinal cord injury repair strategies. Biosci Trends 2013; 7: 202-8.
27. van Velthoven CT, Braccioli L, Willemen HL, Kavelaars A, Heijnen CJ. Therapeutic potential of genetically modified mesenchymal stem cells after neonatal hypoxic-ischemic brain damage. Mol Ther 2014; 22: 645-54.
28. Zaminy A, Shokrgozar MA, Sadeghi Y, Noroozian M, Heidari MH, Piryaei A. Mesenchymal stem cells as an alternative for Schwann cells in rat spinal cord injury. Iran Biomed J 2013; 17: 113-22.
29. Bayati V, Hashemitabar M, Gazor R, Nejatbakhsh R, Bijannejad D. Expression of surface markers and myogenic potential of rat bone marrow- and adipose-derived stem cells: a comparative study. Anat Cell Biol 2013; 46: 113-21.
30. Tan SL, Ahmad TS, Selvaratnam L, Kamarul T. Isolation, characterization and the multi-lineage differentiation potential of rabbit bone marrow-derived mesenchymal stem cells. J Anat 2013; 222: 437-50.