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Research Article

Effects of over-expression of HIF-1alpha in bone marrow-derived mesenchymal stem cells on traumatic brain injury

Mesenchymal stem cells (MSCs) have been shown to play therapeutic effect in traumatic brain injury (TBI). To augment the therapeutic effect, MSCs could be engineered to over-express genes that are beneficial for treatment. In the present study, we over-expressed hypoxia inducible factor (HIF)-1alpha in bone marrow derived MSCs (BM-MSCs) and sought to investigate whether HIF-1alpha could enhance the therapeutic effect of MSCs in a mouse model of TBI. Balb/c mice were subjected to controlled cortical impact injury and MSCs were transplanted intravenously at 6 h after injury. The lesion volume and brain water content were measured and the neurological function was assessed by modified neurologic severity score tests. Double-labeled immunofluorescence for BrdU and NeuN was performed to determine angiogenesis and neurogenesis. The expression of erythropoietin (EPO) and vascular endothelial growth factor (VEGF) was measured by quantitative RT-PCR and western blotting. After TBI, mice received BM-MSCs over-expressing HIF-1alpha showed significantly more functional recovery, reduced brain damage, increased angiogenesis and neurogenesis and increased expression of VEGF and EPO, compared with control mice or mice treated with non-transduced BM-MSCs. Over-expression of HIF-1alpha enhanced BM-MSCs induced improvement of neurological recovery after TBI, by stimulating angiogenesis and neurogenesis.

Keywords: Bone marrow derived mesenchymal stem cell (BM-MSC) / Hypoxia inducible factor (HIF)-1alpha / Therapeutic effect / Traumatic brain injury (TBI)

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1 Introduction

Traumatic brain injury (TBI), usually resulted from direct or indirect mechanical destruction of brain, is a major cause of morbidity and mortality. Hypoxia is a complication of TBI, which contributes to secondary brain injury. After brain trauma, hypoxia could be initiated by cerebral hypoperfusion, apnea and hypoventilation. It would promote exacerbated inflammatory reaction and finally lead to neuronal apoptosis and neurological deficit [1]. Hypoxia inducible factor 1 (HIF-1) is a key regulator in hypoxia. It is a heterodimer composed of alpha and

beta subunits. The HIF-1beta subunit is constitutively expressed while the expression of HIF-1alpha is regulated by cellular oxygen concentration [2]. Significantly elevated serum HIF-1alpha concentration was observed in TBI patients [3]. Up-regulation of HIF-1alpha was also observed in the injured brain cortex of TBI rats [1]. HIF-1alpha has been reported to play neuroprotective effect in severe ischemic stroke [2]. Neuron-specific knockdown of HIF-1alpha increased brain damage and reduced the survival rate of severe ischemic mice [4], while increasing HIF-1alpha expression by desferrioxamine reduced brain damage and promoted functional recovery in a rat model of ischemia [5]. Unlike in ischemic stroke, the role of HIF-1alpha in TBI is less

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Abbreviations: BM-MSCs, bone marrow derived MSCs; EPO, erythropoietin; HIF-1alpha, hypoxia inducible factor; MSCs, Mesenchymal stem cells; TBI, traumatic brain injury; VEGF, vascular endothelial growth factor

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understood. A few studies suggested that HIF-1 α might also play a neuroprotective effect in TBI [6,7].

Mesenchymal stem cell (MSC) transplantation has been shown to play beneficial effect in central nervous system injuries, including TBI. After transplantation, MSCs could improve neurological function recovery, increase endogenous cell proliferation, promote angiogenesis and reduce lesion size [8]. Furthermore, to augment the therapeutic effects of cell transplantation, MSCs could be engineered to over-express genes that are beneficial for treatment. A previous study found that MSCs transfected with brain-derived neurotrophic factor (BDNF) or glial cell-derived neurotrophic factor (GDNF) gene resulted in improved neurological function and reduced brain damage in a rat model of ischemia, which suggested that gene-modified MSCs could be a useful approach for stroke treatment [9].

In the present study, we over-expressed HIF-1 α gene in bone marrow derived MSCs (BM-MSCs) by adenovirus transduction and sought to investigate the effect of BM-MSCs over-expressing HIF-1 α in a mouse model of TBI.

2 Materials and methods

2.1 Cell culture

All the reagents for cell culture were purchased from Gibco (Thermo Fisher Scientific, Waltham, MA, USA). The human embryonic kidney (HEK) 293 cell line was purchased from China Center for Type Culture Collection (CCTCC). 293 cells were maintained in high glucose Dulbecco's Modified Eagle Medium (DMEM) containing 10% heat inactivated fetal bovine serum and 1 $\times$ antibiotic-antimycotic. The BM-MSCs of Balb/c mice were purchased from Cyageen Biosciences (Suzhou, China) and were maintained in low glucose DMEM medium, supplemented with 20% fetal bovine serum, 2 mmol/L L-glutamine and 1 $\times$ Antibiotic-Antimycotic.

2.2 Adenovirus production and transduction

Empty adenovirus vector and vector encoding HIF-1 α were purchased from Shanghai Liangtai Biotechnology (Shanghai, China). To produce adenovirus, the linearized construct DNA was transfected into 293 cells using LipofectamineTM 2000 (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) following the manufacturer's instructions. After 24 h, the 293 cells were fed with fresh medium, and incubation was continued for additional 5 days. Virus was released from the 293 cells by freezing and thawing for three consecutive cycles. After the third freeze-thaw cycle, the 293 cells were briefly centrifuged to pellet the debris and the lysate containing adenoviruses was kept in clean and sterile centrifuge tubes and stored at -20°C for future use. For adenovirus transduction, the BM-MSCs were plated in 6-well plates. After 24 h, the adenoviruses were added in 2 mL medium with multiplicity of infection (MOI) of 10. The cells were then maintained in a 5% CO₂ incubator for additional 4 h. After that, the medium was removed and fresh complete growth medium was added. The cells were incubated for another 24 h and were used for further analysis.

2.3 Quantitative RT-PCR analysis

After adenovirus transduction, BM-MSCs were harvested and total RNA was extracted with TRIzol reagent (Invitrogen, Thermo Fisher Scientific, USA). cDNA was synthesized using a QuantiTect Reverse Transcription kit (QIAGEN GmbH, Hilden, Germany). Quantitative-PCR was performed using SYBR Green PCR Master Mix (Applied Biosystems Inc, Foster City, CA, USA) in an ABI 7500 Real-Time PCR System (Applied Biosystems Inc, USA). The HIF-1 α mRNA was amplified using primers 5'-GCACTAGACAAAGTTCACCTGAGA-3' and 5'-CGCTATCCACATCAAAGCAA-3'. GAPDH was amplified using primers 5'-ATC ACC ATC TTC CAG GAGCG-3' and 5'-TTC TGA GTG GCA GTG AGG GC-3', and was used as internal control. The relative amount of target gene was calculated using the $2^{-\Delta\Delta\text{CT}}$ method.

2.4 Western-blotting

After adenovirus transduction, BM-MSCs were harvested and total proteins were extracted using RIPA lysis buffer (Sigma-Aldrich, St. Louis, MO, USA). Protein sample extract (20 μg) was subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to a polyvinylidene difluoride (PVDF) membrane. The membranes were blocked with 5% skim milk in Tris-buffered saline with Tween 20 (TBST) for 1 h and then incubated with primary antibodies against HIF-1 α (R&D Systems, Minneapolis, MN, USA) or GAPDH (R&D Systems, USA) in blocking solution overnight at 4°C . The membranes were then washed with TBST, incubated in horseradish peroxidase (HRP) conjugated anti-IgG (R&D Systems, USA) for 1 h at room temperature, and then developed using SuperSignal West Pico Chemiluminescent Substrate (Thermo Fisher Scientific, USA). The bands on the film were analyzed using Image J software.

2.5 Animal model of traumatic brain injury

Male Balb/c mice of 6–8 weeks were used in this study. Mice were maintained in the animal facility of Qilu Hospital of Shandong University, under controlled temperature ($23 \pm 2^{\circ}\text{C}$) and 12 h light/dark schedule (light on at 7:00 a.m.) with free access to food and water. All animal handling procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals of Qilu Hospital of Shandong University.

TBI was induced by controlled cortical impact (CCI). Briefly, mice were anesthetized by 4% iso flurane in oxygen and then placed in a stereotaxic device. After drilling a hole (3.5 mm $\times$ 3.5 mm) in the right skull, a pneumatic piston impactor tip (3 mm diameter) was used to induce injury in the brain at the center of the craniotomy. The tip penetration depth was 1.0 mm and the velocity was 4.5 m/s. A warming pad was used to keep body temperature around 37°C during the surgery. The skull bone was discarded during CCI, and the skin was sealed by size 6–0 suture. Mice from sham group underwent the same surgical procedures without a CCI. Burprenorphine was used as the pain killer under restricted regulatory monitoring. The

BM-MSCs (2×10^6 cells suspended in 0.1 mL saline) were injected slowly into the tail veins at 6 h after TBI surgery. The control mice received saline only. Mice used for neurological function measurement were subjected to a modified neurological severity score (mNSS) test on day 1, 3, 7, 10 and 14 after TBI. Other mice were sacrificed and the brains were dissected for further study at indicated days after TBI.

2.6 The modified neurological severity score (mNSS) test

The mNSS test was carried out following the method by Chen *et al.* [10]. It is a composite index of motor, sensory, reflex, and balance tests. One point was given for the inability to perform each test or for the absence of a reflex. Neurological function was graded on a scale of 0–18, where a score of 0 indicated normal performance, and a total score of 18 indicated severe deficit.

2.7 Measurement of lesion volume

Seven days after TBI, mice were anesthetized using 4% chloral hydrate. The brains were removed, post-fixed for 12 h, and then cryoprotected in 15% sucrose in PBS. The frozen brains were then sectioned at 12 μ m in the coronal plane with a freezing microtome. Every fifth section was mounted onto vectabonded slides and stained with cresyl violet for 60 s. Lesion volume was estimated from the summation of areas of defect on each slice, multiplied by slice thickness, for both the ipsilateral and contralateral hemispheres. Lesion volume was quantitatively analyzed with SigmaScan Pro 5 (Systat Software Inc, San Jose, CA, USA). Average lesion volume in microliters for each treatment group was calculated.

2.8 Measurement of brain water content

Brain water content was measured at 0, 24 and 48 h after TBI. Mice were sacrificed and the brains were removed. The cerebella were discarded, and the right and left hemispheres were separated along the anatomic midline. The wet weight of each hemisphere was measured. Then the hemispheres were completely dried in an oven at 100°C for 5 days, and the dry weight of each hemisphere was measured. The percentage of water content (% water) was calculated according to the Elliott formula: %water = [(wet weight-dry weight)/wet weight] $\times$ 100.

2.9 Immunofluorescent staining

To determine angiogenesis and neurogenesis, double-labeled immunofluorescence for BrdU and NeuN was performed. Briefly, 48 h after TBI, mice were injected intraperitoneally with BrdU at a dose of 100 mg/kg. BrdU was administered once per day for 5 consecutive days. The mice were sacrificed at 7 days after TBI and the brains were removed, post-fixed and paraffin-embedded. Consecutive coronal sections were cut at 5 μ m intervals and were mounted to slides. For immunofluorescence, slides

with brain sections were deparaffinized, rehydrated and boiled in 10 mM citric acid buffer (pH 6) for 10 min. Then sections were blocked with 1% bovine serum albumin (BSA) in PBS containing 0.3% Triton-X-100. Sections were then incubated with mouse anti-NeuN antibody (1:200; Chemicon, Temecula, CA) at 4°C overnight. For negative controls, primary antibodies were omitted. FITC-conjugated anti-mouse antibody (1:400; Jackson ImmunoResearch, West Grove, PA) was added to sections at room temperature for 2 h. Sections were then incubated with rat anti-BrdU antibody (1:200; Dako, Glostrup, Denmark) at 4°C overnight followed by Cy3-conjugated goat anti-rat antibody (1:400; Jackson ImmunoResearch, West Grove, PA) at room temperature for 2 h. Each of the steps was followed by three 5-min rinses in PBS. Tissue sections were mounted with Vectashield mounting medium (Vector laboratories).

2.10 Cell counting and quantitation

For analysis of angiogenesis, BrdU+ cells were counted in the lesion boundary zone and the dentate gyrus. For analysis of neurogenesis, we counted BrdU+ cells and NeuN/BrdU-colabeled cells in the dentate gyrus and its subregions, including the subgranular zone, granular cell layer, and the molecular layer. The fields of interest were digitized under the light microscope (Nikon, Eclipse 80i) at a magnification of either 200 or 400 using CoolSNAP color camera (Photometrics, Tucson, AZ) interfaced with MetaMorph image analysis system (Molecular Devices, Sunnyvale, CA, USA). In brief, five fields of view in the lesion boundary zone from the epicenter of the injury cavity (bregma -3.3 mm), and 9 fields of view in the ipsilateral DG were counted in the each section. From our previous experience, our inter-rater reliability was greater than 95% when the cell counts were compared between two independent trained blinded observers scoring the same sections of an animal. In the present study, one blinded observer performed the cell counting in all brain sections.

2.11 Measurement of erythropoietin and vascular endothelial growth factor

The mice were sacrificed at 7 days after TBI and the brains were removed. The ipsilateral cortex was subjected to quantitative RT-PCR and western blotting following above procedures, to determine the mRNA expression and protein levels of erythropoietin (EPO) and vascular endothelial growth factor (VEGF). The primers for EPO were 5'-TGC CCG AAC GTC CAA CCC TGC TG-3' and 5'-TCA CCT GTC CCC TCT CCT GCA G-3'. The primers for VEGF were 5'-TTACTGCTGTACCTCCACCATG-3' and 5'-TCACCGCCTTGGCTTGTCACAT-3'. Antibodies were purchased from R&D Systems (Minneapolis, MN, USA).

2.12 Statistical analysis

Data were presented as mean $\pm$ SD, and were analyzed by one- or two-way ANOVA analysis followed by a post hoc test. P value less than 0.05 was considered as significantly different.

Figure 1. Successful over-expression of HIF-1alpha in BM-MSCs by adenovirus vector transfection. A. Relative HIF-1alpha mRNA levels were examined by RT-PCR and normalized to control. GAPDH was employed as an internal control. B. HIF-1alpha protein expressions were analyzed by western blotting. GAPDH was employed as a loading control. Data are mean $\pm$ SD and were analyzed by one-way ANOVA analysis followed by a post hoc test. ** $p < 0.01$ compared to control.

3 Results

3.1 Over-expression of HIF-1alpha in BM-MSCs by adenovirus infection

As shown in Fig. 1, compared with control or BM-MSCs transfected with null adenovirus, BM-MSCs transduced with adenovirus carrying HIF-1 α showed significantly elevated HIF-1alpha mRNA expression (Fig. 1A) and HIF-1alpha protein level (Fig. 1B). These data indicated that HIF-1alpha was over-expressed in BM-MSCs after adenovirus infection.

3.2 Effect of BM-MSCs over-expressing HIF-1alpha on neurological function after TBI

As shown in Fig. 2, there was a significant improvement in neurological function in the BM-MSC treated groups compared with the vehicle treated group. Over-expression of HIF-1alpha enhanced the BM-MSCs induced improvement of neurological

Figure 2. Over-expression of HIF-1alpha in BM-MSCs improved TBI-induced neurobehavioral functions. Comparisons of the modified neurological severity score (mNSS) of the mice among the four groups 1, 3, 7 and 14 days after cell transplantation. Data were expressed as mean $\pm$ SD and were analyzed by two-way ANOVA analysis followed by a post hoc test. * $p < 0.05$, ** $p < 0.01$ versus vehicle group at the same time point, # $p < 0.05$ versus MSC group at the same time point.

recovery after TBI. Mice treated with BM-MSCs over-expressing HIF-1alpha showed significantly greater improvement of neurological recovery after TBI compared with mice treated with non-transduced BM-MSCs.

3.3 Effect of BM-MSCs over-expressing HIF-1alpha on brain damage after TBI

Treatment with BM-MSCs significantly reduced the lesion volume and brain water content compared with vehicle treatment (Fig. 3). Over-expression of HIF-1alpha enhanced the protective effect of BM-MSCs against TBI-induced brain damage. Mice treated with BM-MSCs over-expressing HIF-1alpha showed significantly reduced lesion volume (Fig. 3A) and brain water (Fig. 3B) content compared with mice treated with non-transduced BM-MSCs.

3.4 Effect of BM-MSCs over-expressing HIF-1alpha on cell proliferation and neurogenesis after TBI

Treatment with BM-MSCs significantly increased BrdU+ cells (Fig. 4A and 4B) and BrdU/NeuN+ cells (Fig. 4A and 4C) at 7 days after TBI, compared with vehicle treatment. The numbers of BrdU+ cells and BrdU/NeuN+ cells in mice treated with BM-MSCs over-expressing HIF-1alpha were significantly more than that in mice treated with non-transduced BM-MSCs. These data indicated that over-expression of HIF-1alpha enhanced the effect of BM-MSCs on cell proliferation and neurogenesis.

3.5 Effect of BM-MSCs over-expressing HIF-1alpha on VEGF and EPO expression in the ipsilateral cortex after TBI

Compared with mice treated with vehicle, the mRNA expression (Fig. 5A and B) and protein levels (Fig. 5C) of VEGF and

Figure 3. Effects of over-expression of HIF-1alpha in BM-MSCs on TBI-induced brain injury. A. Lesion volume was quantitatively analyzed with Sigma Scan Pro 5 at 7 days after TBI. Data were expressed as mean $\pm$ SD and were analyzed by one-way ANOVA analysis followed by a post hoc test. ** $p < 0.01$ versus vehicle group, # $p < 0.05$ versus MSC group. B. The water content of injured hemisphere (IH) and contralateral hemisphere (CH) were measured from 0, 24 h and 48 h after TBI. Data were expressed as mean $\pm$ SD. * $p < 0.05$, ** $p < 0.01$ versus contralateral hemisphere (CH) group, # $p < 0.05$ versus MSC group.

EPO in the ipsilateral cortex were significantly elevated in mice treated with BM-MSCs at 7 days after TBI. Over-expression of HIF-1alpha enhanced the effect of BM-MSCs on VEGF and EPO expression. Mice treated with BM-MSCs over-expressing HIF-1alpha showed significantly higher mRNA expression and protein levels of VEGF and EPO compared with mice treated with non-transduced BM-MSCs.

4 Discussion

In the present study we over-expressed HIF-1alpha gene in BM-MSCs using adenovirus transduction and confirmed the over-expression of HIF-1alpha in the cells by quantitative RT-PCR and western blotting. The therapeutic effect of BM-MSCs in TBI has been extensively studied [8]. In this study we sought to investigate whether HIF-1alpha could enhance the therapeutic effects of MSCs in a mouse model of TBI. HIF-1alpha has been suggested to play a neuroprotective effect in TBI. Previous studies found that sustained activation of HIF-1alpha by dimethylloxaloylglycine decreased lesion volume, stimulated angiogenesis, improved both memory and motor functions in a mouse model of TBI [6]. Treatment with acriflavine, which could inhibit

Figure 4. Over-expression of HIF-1alpha in BM-MSCs significantly increases cell proliferation and neurogenesis in TBI mice at day 7 after TBI. A. BrdU staining (red) for cell proliferation. Double staining with BrdU (red)/NeuN (green) for newborn mature neurons. B. Quantification of BrdU+ cells. C. Quantification of BrdU/NeuN+ cells. Data were expressed as mean $\pm$ SD and were analyzed by one-way ANOVA analysis followed by a post hoc test. ** $p < 0.01$ versus vehicle group, # $p < 0.05$ versus MSC group.

HIF-1alpha dimerization in the brain, inhibited spontaneous recovery from injury in TBI mice [7]. As expected, we found that over-expression of HIF-1alpha enhanced the therapeutic effect of BM-MSCs in TBI, resulting in greater improvement in neurological function and greater reduce in brain damage. These results indicated that over-expression of HIF-1alpha might play a synergistic effect with BM-MSCs in the treatment of TBI.

Angiogenesis and neurogenesis have been shown to play an important role in mediating functional recovery after TBI, and almost all the treatment that could improve neurological function after TBI would increase angiogenesis and neurogenesis [11]. Proliferation of endothelial cells of cerebral capillaries is the first step of angiogenesis. After TBI, using immunohistochemistry with antibodies against BrdU, newly generated endothelial cells were found in the lesion boundary zone as well as in the ipsilateral dentate gyrus, indicating that TBI induced angiogenesis in these regions [12,13]. Neurogenesis occurs in the dentate gyrus under normal conditions as well as pathological situations. Immunofluorescent double-labeling of the proliferation cell marker BrdU and the mature neuronal marker NeuN revealed increased neurogenesis after TBI [14]. In a rat model of TBI, BM-MSCs were found to promote angiogenesis in the lesion boundary zone and hippocampus, and enhance neurogenesis in the subgranular zone and the subventricular zone of

Figure 5. Effects of over-expression of HIF-1alpha in BM-MSCs on VEGF and EPO expression in ipsilateral cortex of experimental animals. A, B. mRNA levels of VEGF and EPO were tested by qPCR at day 7 after TBI. C. Protein level of VEGF and EPO were tested by western blot at day 7 after TBI. Data were expressed as mean $\pm$ SD and were analyzed by one-way ANOVA analysis followed by a post hoc test. ** $p < 0.01$ versus vehicle group, # $p < 0.05$ versus MSC group.

hippocampus [15]. Our findings were consistent with these results, showing that BM-MSCs increased angiogenesis and neurogenesis in the mouse model of TBI. Although after transplantation, BM-MSCs could successfully migrate to lesion boundary zone and differentiate into neurons or astrocytes after TBI [16], it was suggested that the therapeutic effect of BM-MSCs was not attributed to the very few MSCs that differentiate in to brain cells, but attributed to their ability to secrete various growth factors including BDNF and VEGF [11]. BDNF was known to promote endothelial cell survival and induce neovascularization in ischemic tissues [17]. Previous studies found that over-expression of BDNF enhanced the effect of MSCs on functional recovery after ischemia or TBI [9, 18, 19]. In this study we found that the effect of BM-MSCs on cell proliferation and neurogenesis was enhanced by over-expression of HIF-1alpha gene in the MSCs by adenovirus transduction. HIF-1alpha is a transcription factor that could modulate the transcription of VEGF and EPO, which promote angiogenesis and neurogenesis [2]. Thus the synergistic effect of HIF-1alpha with BM-MSCs on the angiogenesis and neurogenesis after TBI might be through regulation of VEGF and EPO expression in the brain.

To verify this speculation, we measured the mRNA expression and protein levels of VEGF and EPO in the brain after TBI. VEGF is a mitogen for endothelial cells. By binding to tyrosine kinase receptors, it could promote endothelial cell proliferation, migration and new vessel formation [20]. It was also reported to stimulate the proliferation of neuronal precursors both in vitro and in vivo [21]. Administration of exogenous VEGF significantly increased the number of proliferating cells in the subventricular zone and in the perilesion cortex, and reduced lesion volumes after TBI [22]. EPO is a hematopoietic growth factor which could increase oxygen availability during hypoxia [23]. It was also found to be involved in angiogenesis and neurogenesis [24, 25]. Previous studies found that treatment with EPO could reduce hippocampal neuron loss and enhance angiogenesis and neurogenesis in the injured cortex and hippocampus in a rat model of TBI, in a dose dependent manner [26, 27]. In this study we found that the mRNA expression and protein levels of VEGF and EPO were increased after TBI, which might serve as a potent activator of angiogenesis and neurogenesis that contributes to the spontaneous recovery after TBI. Furthermore, we found that over-expression of HIF-1alpha enhanced the effect of BM-MSCs on up-regulating VEGF and EPO. VEGF and EPO are down-stream target genes of HIF-1alpha, thus HIF-1alpha might play synergistic effect with BM-MSCs by promoting expression of VEGF and EPO, thus stimulate angiogenesis and neurogenesis and ultimately enhance the effect of BM-MSCs on functional recovery after TBI.

Last but not least, it is worth noting that controlled cortical impact was used to generate TBI in the present study. There are several other animal models of TBI, such as weight drop, fluid percussion injury, blast injury, inertial acceleration, etc., which result in different pathological features. The effect of BM-MSCs over-expressing HIF-alpha on those models still remains uncertain.

5 Conclusion

In conclusion, the present study found that over-expression of HIF-1alpha in BM-MSCs by adenovirus transduction enhanced the therapeutic effect of BM-MSCs in TBI, resulted in greater improvement in neurological function and greater reduce in brain damage. This effect might be via the effect of HIF-1alpha in promoting the expression of VEGF and EPO and stimulating angiogenesis and neurogenesis in the brain after TBI.

Practical application

The present study found that over-expression of HIF-1alpha in BM-MSCs by adenovirus transduction enhanced the therapeutic effect of BM-MSCs in TBI, resulted in greater improvement in neurological function and greater reduce in brain damage.

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The authors have declared no conflict of interest.

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