Protective effect of bone morphogenetic protein-7 induced differentiation of bone marrow mesenchymal stem cells in acute spinal cord injury rat

Xudong Sun  
The First Affiliated Hospital of Bengbu Medical College, Bengbu Medical College

Maoyong Li  
The First Affiliated Hospital of Bengbu Medical College, Bengbu Medical College

Shiyuan Huang  
The First Affiliated Hospital of Bengbu Medical College, Bengbu Medical College

Heng Zhang  
The First Affiliated Hospital of Bengbu Medical College, Bengbu Medical College

Kuanxin Li (✉ byglikuanxin@126.com)  
The First Affiliated Hospital of Bengbu Medical College, Bengbu Medical College

Research Article

Keywords: Acute spinal cord injury, Nerve regeneration, BMSCs, BMP-7

Posted Date: January 3rd, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2411736/v1

License: © This work is licensed under a Creative Commons Attribution 4.0 International License.  
Read Full License
Abstract

The principal aim of present study was to assess the therapeutic efficacy of bone morphogenetic protein-7 (BMP-7) induced bone marrow mesenchymal stem cells (BMSCs) differentiation in rat acute spinal cord injury (SCI) model. BMSCs were isolated from rats, then divided into control and BMP7 induction groups. The proliferation ability of BMSCs and glial cell markers were detected. Forty Sprague-Dawley (SD) rats were randomly divided into sham, SCI, BMSCs, and BMP7 + BMSCs groups (n = 10). The recovery of motor function of hind limbs of rats, the pathological, related markers and motor evoked potentials (MEP) were detected. BMSCs differentiated into neuron like cells after exogenous BMP7 intervention. Interestingly, the expression levels of MAP-2 and Nestin increased, while the expression GFAP level decreased after exogenous BMP7 intervention. BBB score reached 19.33 ± 0.58 in the BMP7 + BMSCs group at day 42. Nissl bodies in model group were reduced compared to sham group. After 42 days, both in the BMSCs and BMP7 + BMSCs groups, the number of nissl bodies were increased. Especially, the number of nissl bodies in the BMP7 + BMSCs group was more than that in BMSCs group. The expression of Tuj-1 and MBP in BMP7 + BMSCs group increased, while the expression of GFAP decreased. MEP waveform decreased significantly after surgery. The waveform was wider, the amplitude was higher in BMP7 + BMSCs group than that in BMSCs group. BMP7 promotes BMSCs proliferation, and induces BMSCs differentiate into neuron like cells, inhibits the formation of glial scar. BMP7 plays a confident role in the recovery of SCI rats.

Introduction

A spinal cord injury (SCI) is one of the most common central nervous system disorders (J. Zhang et al. 2020; Zhang et al. 2022). SCI maybe devastating, due to the CNS unable to effectively regenerate (Nazareth et al. 2020). Motor sensory and autonomic dysfunction are common consequences of SCI, which severely affect patients' quality of life, as well as social integration (Abu-Baker et al. 2021; Duan et al. 2021). SCI also accompany by respiratory, urinary and other system complications in addition to bring irreversible sensory and motor disorders to patients, bringing heavy burden to individuals, families and society (Nazemi et al. 2020). At present, there is no effective clinical treatment for SCI (Liu et al. 2020; Wei et al. 2021; X. Zhang et al. 2020). Previous study indicates that SCI patient show little clinical recovery within one year after surgery, and most of them are permanently disabled (Freund et al. 2013). Currently, stem cell transplantation is an effective treatment strategy for SCI (Li et al. 2021).

Bone marrow mesenchymal stem cells (BMSCs) are well known for their multi-directional differentiation capacity (Feng et al. 2019). BMSCs have the advantages of easy access, low risk and no ethical conflict (Shi et al. 2019). There are various of studies have been proved that BMSCs transplantation has shown a certain neuro-protective effect in the treatment of SCI (Kim et al. 2018; Mete et al. 2016; Zhan et al. 2020). It is reported that BMSCs promotes the polarization of M2 macrophages through paracrine secretion, inhibits complement response and reduces immune rejection (Zhao et al. 2019). In addition, BMSCs delivers multiple growth factors to provide nutritional support and reduce apoptosis of nerve cells (Naji et al. 2019). BMSCs can inhibit the secondary inflammatory reaction, reduce the proliferation of astrocytes,
and then inhibit the formation of glial scar (Romanelli et al. 2019). However, the efficiency of BMSCs differentiating into neural cells is very low (Xu et al. 2021). Therefore, it needs appropriate differentiation strategy to stimulate the BMSCs differentiating into neural cells (Yu et al. 2020). From this point, the choice of differentiation inducer for the directional differentiation of BMSCs has become the focus of attention in the treatment of SCI with assisted BMSCs transplantation.

Bone morphogenetic protein-7 (BMP-7) belongs to the superfamily of transforming growth factor-β (TGF-β) (Tsujimura et al. 2016). BMP-7 could effectively promote the regeneration of neurons in the spinal cord injury area of SCI rats, and played an important regulatory role in balancing the number of neurons and glial cells. BMP-7 intervention *in vitro* promotes BMSCs differentiate into neuron like cells as previous study description (H. Zhang et al. 2021). We evaluated the simultaneous use of BMP-7 and BMSCs, but not investigate the effect of BMSCs after BMP-7 induced. In this study, we first used BMP-7 to promote BMSCs differentiation *in vitro*, then transplanted the induced cells into SCI rat. We aim to explore the therapeutic effect of BMSCs transplantation after BMP-7 induced for provide a new sight for the SCI treatment.

**Material And Methods**

**BMSCs isolation and culture**

Sprague-Dawley (SD) rats (male, weighed 80 ± 20g, 4 weeks old, were obtained from Jinan Pengyue Experimental Animal Breeding Co., Ltd. (license number: SCXK(Lu)2019-003, Jinan, Shandong, China). The rats were raised in the animal room and could get water and food freely. The light/dark cycle was 12 h:12 h, temperature was 25 ± 1°C with the humidity was 60 ± 10%. Experimental animals were handled according to the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996). All animal experiments in present study were approved by the Ethics Committee of Bengbu Medical College.

After rats were scarified, the femur and tibia were taken out. Aseptically, DMEM complete medium (Gibco, USA) containing 10% fetal bovine serum (FBS, Gibco, USA) was used to wash the femur and tibia for bone marrow extraction. After 1200 × *g* centrifugation for 5 min, the single cell suspension was obtained. Cells were cultured at 37°C with 5% CO₂, changed the medium after 48 h, removed non adherent cells, the medium were changed every 3 days. The trial flow diagram is presented in Fig. 1.

**Cell Identifications**

The P3 generation BMSCs were used for further experiments. Cells were resuspended, then 100 uL (1 × 10⁶ cells/mL) cells were labeled with FITC labeled CD90 (Affinity., Ltd, Jiangsu, China), APC labeled CD45 (Affinity., Ltd), and FITC labeled CD34 monoclonal antibody (Affinity., Ltd), APC labeled CD29 (Affinity., Ltd) were added and incubated at 4°C in dark for 20 min. PBS was used to wash and resuspended cells, then detected by flow cytometry (Thermofisher., Ltd,Shanghai ,China).
**Cell Grouping**

Cells were divided into control and rhBMP-7 (concentration: 75 ng/ml; cat.no: 120-03P-100; PeproTech, Minneapolis, USA) induction (BMP-7) groups. For cell viability, BMSCs with $6 \times 10^3$/well to 96 well plate. After different treatment and cultured for 7 d, added 10 µL CCK-8 solution (Solarbio., Ltd, Hubei, China), then incubated for 2 h at 37°C. The optical density (OD) value at 450 nm was measured using a microplate reader (Bio-Rad, USA).

**Immunofluorescence**

BMSCs with different treatment were washed 3 times with PBS for 5 min each. 4% paraformaldehyde was fixed for 15 min at room temperature, after PBS washed, then penetrated the membrane with 0.5% Triton X-100 for 20 min, and 5% BSA solution was added to seal for 20 min. An overnight incubation at 4°C was performed with the primary antibody Nestin (1:200; Affinity.,Ltd), MAP-2 (1:200, Affinity.,Ltd) and GFAP (1:200, Affinity.,Ltd). For antigen repair in the microwave oven, paraffin sections were dewaxed and placed in a repair box filled with EDTA antigen repair buffer solution. PBS was used to wash the sections for 3 times, then added 3% BSA. The sections were sealed for 30 min. Primary antibodies Tuj-1 (1:200, Affinity.,Ltd), MBP (1:200, Affinity.,Ltd), and GFAP (1:200) were added and incubated overnight at 4°C. After PBS washed three times, secondary antibody (1:200, Solarbio.,Ltd) was added and incubated in dark for 2 h at 37°C, then washed, DAPI (Solarbio.,Ltd) was added and dyed for 15 min in the dark condition. Fluorescence was obtained and took pictures with inverted microscope (Axio Observer A1, Zeiss, Germany).

**Quantitative Real-time PCR Assay**

Total RNA from BMSCs cells was isolated by RNeasy kit (Qiagen, Beijing, China). To investigate gene expression levels, cDNA was prepared from cells by PrimeScript TM RT kit (RR037, Takara Bio,Inc. Japan). PCR system was carried out by AGS PCR instrument (Thermofisher., Ltd). The sequences of primers were listed as follows: Nestin forward 5'-GGTCACCTGCGCAGCTACTC-3', reverse 5'-AAGCGGACGTGGAGCAG-3'; MAP-2, forward 5'-GGACATCAGGCTCAACA-3', reverse 5'-CCTCCTCTC-CTGTATG-3'; GFAP, forward 5'-AGAAGCGGATGAGGCACGCA-3', reverse 5'-TCCCCTTGAGCCCCTAAA-3'. The internal controls used was GAPDH, forward 5′-CTCTCTGCTCCTCCGTAT-3′ and reverse 5′-GCCAATCCGTCTACACC-3′. Using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001), we calculate target mRNA expression levels.

**Western Blotting**

Western blotting

Cells were collected by trypsinization after different treatment, wash in PBS and lysed in RIPA lysate buffer (BL504A; Biosharp., Ltd., Anhui, China), then crushed under ultrasonic crusher for 1 min. Proteins
concentration were determined by BCA kits (Biosharp., Ltd.). Proteins were separated by 10% SDS-PAGE gels (Thermofisher., Ltd). With semi dry method the gels were transferred to the membrane. After the membranes sealed with 5% BSA at room temperature for 1h. Primary antibody Nestin, MAP-2 and GFAP (1:200, Affinity.,Ltd) were added and incubated overnight at 4°C. After the membranes were washed with TBST, incubated them with goat anti-rabbit IgG/ Horseradish Peroxidase (HRP, 1: 5000, Solarbio.,Ltd) for 2 h at room temperature. Enhanced-chemiluminescence (ECL) was used to capture images in the darkroom. The internal controls were GAPDH.

Sci Rat Model

SCI rat model was performed using the modified Allen method (Lin et al. 2021). A total of 50 SD rats (male and female) weighed 220 ± 20 g, were purchased from Jinan Pengyue Experimental Animal Breeding Co. Injection of 10% chloral hydrate 400 mg/kg intraperitoneal successful anesthetized the rats, the prone position of the rats was fixed. A longitudinal incision on the back was made with position T10 spinous to expose T8-11 spinous process. The spinous process of T9 and T10 spine and the lamina were removed with bone rongeur, fully expose the spinal cord, accurately located the operation process. The spinal cord impactor (68097, Shenzhen Rayward Life Technology Co., Ltd., China) with a striker diameter of 2.5 mm, a striker mass of 15 g, and a striker drop height of 20 cm was used to impact the T9 segment to establish the spinal cord impact injury model. After SCI, spinal cord tissue presented edema, and congestion. The body of the rats was twisted, hind limbs fluttered, tails twitched, indicating that the SCI model was successful. In the operation process, maintenance of dural integrity.

Animal Grouping

Forty rats were divided into 4 groups (n = 10) randomly, including sham, SCI, BMSCs transplantation (BMSCs), BMP-7 + BMSCs transplantation (BMP-7 + BMSCs) groups. 10 µL microinjector was installed on the brain stereotaxic instrument (Yu YAN Instrument Co., Ltd., Shanghai, China), and the tip was inserted through the complete dura mater (penetration depth: 1.0 mm, angle: 40–45°). In BMSCs and BMP-7 + BMSCs groups, dissociated BMSCs and BMP-7 induced BMSCs (9 µL, 3 µL each for injury center, injury tip and caudal with 1.5 mm depth). The needle was left for 60 s after the injection was completed, and the spinal cord surface was examined with a microscope. In SCI group, the same amount of normal saline was injected. In sham group, only T9, T10 spinous processes and vertebral lamina were removed without spinal cord percussion.

Basso, Beattie And Bresnahan (Bbb) Score

Neuronal function recovery was assessed 1 d, 3 d, 7 d, 14 d, 21 d, 28 d, 35 d, and 42 d after SCI. The BBB exercise capacity rating scale was used to investigate hindlimb motor function (Basso et al. 1996). BBB score ranges from 0 to 21. Blind evaluation of BBB score was performed. With higher scores indicating better hindlimb coordination function.
Tissue Sampling And Section Preparation

Rats were anesthetized and perfused at 42 d after SCI. After the rats anesthetized via intraperitoneal injection, open the chest cavity to expose the heart, insert the needle from the apex of the heart into the aortic tube, cut the right atrium, PBS (200 mL) was infused into artery. After the body hardens, slowly infused with 4% paraformaldehyde (150 mL, Biosharp.,Ltd., Anhui, China). T10 spinal cord was exposed, and 0.5 cm spinal cord specimens were taken from the front and back of the injured center. Then, 4% paraformaldehyde was used to fix for 24 h, washed with PBS for 3 h. By using a microtome (Leica Instrument Co., Ltd., Shanghai, China), paraffin-embedded specimens were cut into 3 µm thick sections.

H&E Staining

The sections were dewaxed as follows, xylene 20 min for 2 times, absolute ethanol 5 min for 2 times, then 75% ethanol for 5 min, finally washed slightly with distilled water. Hematoxylin (Junrui Biotechnology Co., Ltd, Shanghai, China) was added and stained for 5 min, washed with distilled water, and differentiated with hydrochloric alcohol, then rinsed with running water. In 85% and 95% gradient alcohol, the sections were dehydrated for 5 min each, then stained with eosin for 5 min. Finally, the sections were dehydrated, fixed with a neutral balsam, and observed under an optical microscope (Olympus B61, Tokyo, Japan). Image J software (NIH, USA) was employed to observe the pathological lesion cavity area in different groups.

Nissl Staining

The slices were dewaxed, rehydrated, stained with cresyl viole and placed in a 56°C incubator for 1 h. Differentiation with Nissl solution (Junrui Biotechnology Co., Ltd) for 30 s. The sections were dehydrated as described in H&E methods. Neuron images were captured under a light microscope and the number of Nissl bodies/mm2 was analyzed by image J software (NIH, USA).

Electrophysiological Detection

The motor evoked potential (MEP) was evaluated by the biological signal acquisition and processing system MP150 (Yuyan Instrument Company, Shanghai, China) before and after modeling and 42 d. After anesthesia, a small hole was opened 1 mm posterior to the sagittal suture of the rat skull. The stimulation electrode was positioned in the small hole. The recording electrode was inserted into the right gastrocnemius muscle, with a 1 cm interval between positive and negative polarity, the positive electrode was proximal whereas the negative electrode was distal. The reference electrode was placed at the same level with recording electrode under the skin. Measuring parameters: range 2.0 mv, time constant 10 ms, low pass filtering 1 kHz, electric current, monostimulus; strength 7.00 mA.

Statistical analysis
SPSS 21.0 software (IBM, USA) was used to analysis all data. Datas are shown as mean ± standard deviation. Comparison between two groups was performed using Student’s \( t \)-test. Datas in more than three groups were analyzed by one-way analysis of variance (ANOVA) with Tukey's post hoc test. In all data, \( P< 0.05 \) was considered as a level of statistical significance.

Results

Rat BMSCs isolation, culture and identification

On the third day, a large number of spindle or polygonal cells were closely arranged and attached to the wall. After 2 passages, most of the cells were spindle shaped or flat shaped, with rich cytoplasm with obvious nucleoli. The cell groups were arranged in the same direction and gathered together to form a vortex, showing the typical characteristics of BMSCs (Fig. 2A). The identification of cell purity showed that the surface of BMSCs was positive for CD90 and CD29, and negative for CD45 and CD34 (cell ratio > 97%), which was in line with the purity of BMSCs required by the experiment (Fig. 2B). Furthermore, we found that after rhBMP-7 intervention, the survival rate of BMSCs showed a steady upward trend, indicating that BMP-7 could stimulate the proliferation and differentiation of BMSCs (Fig. 2C).

Bmp-7 Induces Bmscs To Differentiate Into Neuron Like Cells

The results showed that the expression of GFAP in BMP-7 group had no chromogenic fluorescent. However, MAP-2, and Nestin expression levels in BMP-7 group had significantly chromogenic fluorescent (Fig. 3A). We further used qRT-PCR (Fig. 3B) and western blot assay (Fig. 3C, 3D) to confirm the expression of GFAP, MAP-2, and Nestin. As presented in the results, the expression levels of GFAP mRNA \( (P< 0.0001) \) and protein \( (P< 0.0001) \) decreased in BMP-7 group compared to the control group, while the expression of MAP-2 \( (P = 0.0016, P = 0.0014) \) and Nestin \( (P = 0.0007, P = 0.0079) \) significantly increased.

Bmp7 Induced Bmscs Transplantation Promoted Recovery Of Motor Function Of Hind Limbs In Rats

Following up the BBB score of exercise behavior of SCI rats within 42 d, the recovery of motor function of rats in each group had little difference from 0 to 5 d after SCI. At the first week, the motor function of the rats in the cell transplantation group improved. At the 42 d, the BBB score of rats in the SCI group only remained at 5.62, while the rats in BMSCs group reached more than 10 points \( (P< 0.0001) \). The change range of BMP7 + BMSCs group was the most obvious, reached 19.33 ± 0.58 on the 42 d, which was obviously higher than that of SCI \( (P< 0.0001) \) and BMSCs \( (P< 0.0001) \) groups. However, there was still significantly difference between BMP7 + BMSCs group and sham group \( (P = 0.0347) \). BMP-7 induced BMSCs transplantation had certain promoting effect on recovery of motor function of hind limbs of rats (Fig. 4).
Effect Of Bmp7 Induced Bmscs Transplantation On Electrophysiological Parameters

MEP was normal before modeling, showing typical P1-N1-P2 waveform, and N1 wave was stable upward. After modeling, the spinal cord induced potential was significantly lower than the normal N1 wave. At 42 d after injury, the MEP waveforms obtained before the material extraction in each group were mostly M-shaped bimodal waves, and the amplitude was significantly recovered compared with that after modeling. The amplitude of BMP7 + BMSCs group was markedly higher than that of SCI and BMSC groups (Fig. 5).

Bmp7 Induced Bmscs Transplantation Improves The Pathological Changes After Sci

H&E staining results on the coronal surface of the spinal cord in each group presented that all of the gray matter in SCI group was destroyed and dissolved, and huge cavities were formed. There were fewer complete cell structures, and the structure of white matter area was disordered (Fig. 6A). We investigated the lesion cavity proportion area, as shown in Fig. 6B, the lesion cavity area in SCI group was obviously more than sham group ($P < 0.0001$). In BMSCs treatment group, the lesion cavity area was decreased, significantly ameliorated compared with SCI group ($P = 0.0002$). There was no significantly difference between BMP7 + BMSCs group and sham group ($P = 0.0785$).

Bmp7 Induced Bmscs Transplantation Ameliorates The Functional Status Of Neuronal Cells After Sci

As presented in Fig. 7, Nissl bodies number in SCI group decreased, the nucleus was pyknosis (Fig. 7). In BMP7 + BMSCs group, nissl bodies counts were more than that in SCI ($P = 0.0003$) and BMSCs ($P = 0.0226$) groups. However, there is still markedly difference between sham group and BMP7 + BMSCs group ($P = 0.0085$).

Effect of BMP7 induced BMSCs transplantation on the expression of Tuj-1, GFAP and MBP in spinal cord

Compared with SCI group, Tuj-1, and MBP expression levels in BMP7 + BMSCs groups increased (Fig. 8A-C, $p = 0.0001$, $P = 0.045$), furthermore, the expression levels of Tuj-1 and MBP in BMP7 + BMSCs group was higher than that in BMSCs group. GFAP positive cells were found in all groups. Compared with sham group, GFAP expression in other groups increased after SCI ($P < 0.0001$). Different from SCI group, the range of GFAP positive area in BMSCs transplantation group increased, and the injured area spread to the head and tail of spinal cord, almost covering the whole spinal cord ($P = 0.0037$). Compared with SCI group ($P < 0.0001$) and BMSCs group ($P = 0.0028$), the number of GFAP positive cells in BMP7 + BMSCs group was significantly reduced.
Discussion

In the current study, we first assessed the differentiation of BMSCs induced by BMP-7. In order to prove the purity of BMSCs, we detected the expression of CD29, CD90, CD34, and CD45. Flow cytometry indicated that BMSCs expressed CD90 and CD29, but not CD34 and CD45. CD90, CD29 are the most commonly reported positive markers of BMSCs differentiation, and CD34, CD45 are reported negative markers (Charbord 2010; Cheng et al. 2019; Deng et al. 2022). According to morphological and CCK8 assay verified that BMP7 effectively induced BMSCs differentiation. We further investigated the expression of MAP-2, Nestin, and GFAP. Previous study describes that glial origin was revealed by GFAP expression, neural immature lineages was characterizing commitment by nestin (Gunther et al. 2008; Kojima et al. 2020). Neurons were labeled with MAP-2 as previous study (Zhang et al. 2017). The immunofluorescence staining results demonstrated that MAP-2, and Nestin expression levels in BMSCs enhanced after BMP-7 treatment. However, the expression of GFAP decreased. Consistent with described in previous study, BMP-7 could effectively promote the ability of BMSCs differentiated into neuron like cells not glial cell (H. Zhang et al. 2021).

SCI is mainly divided into primary injury and secondary injury (Bretheau et al. 2022; Lin et al. 2020; Moghaddam et al. 2015). The primary injury is often irreversible; therefore, the current treatment is mainly through active prevention and treatment secondary of SCI (Urdzikova et al. 2014). BMSCs have the functions of anti-inflammatory and anti-immune, anti-oxidative stress and anti-apoptosis (Cai et al. 2019; Li et al. 2020; Lv et al. 2016). Furthermore, our former study proved that BMP-7 local injection can promote the regeneration of neurons and the recovery of motor function after SCI in rats (Chen et al. 2018). BMSCs often used to alleviate the secondary injury after SCI (Luo et al. 2019; Zhao et al. 2019). We contemplate that inject BMSCs after BMP7 induced maybe more effective. In the animal experiments, we used BMSCs and BMP-7 induced BMSCs transplantation to SCI rats, respectively. As expected, BBB score showed that the recovery of motor function of hind limbs in BMSCs and BMP-7 induced BMSCs groups were significantly better than that in the SCI group. Importantly, the recovery of motor function BMP-7 induced BMSCs group is higher than that in BMSCs group. MEP is defined as the activity summates in the spinal cord and at the neuromuscular junction, resulting in a compound muscle action potential (Morris et al. 2019). The electrophysiological test results showed that the MEP waveform of the rats in BMP-7 induced BMSCs group was M type, and the amplitude was higher than that in the BMSCs and SCI groups, indicated that BMP-7 induced BMSCs could promote the repair of neural function after SCI in rats.

Previous study shows that SCI causes a series of complex molecular cascade reactions, leading to neuronal apoptosis in the lesion site, and ultimately leading to the expansion of the initial injury (Li et al. 2019; Liu and Xu 2012). Nissl bodies are commonly used as markers of neuronal cell function (Niu et al. 2015; Q. Zhang et al. 2021). In current study, we found that the number of nissl bodies were increased after BMP-7 stimulated BMSCs treatment. BMP-7 stimulated BMSCs effectively ameliorated the pathological changes of spinal cord injured tissues. In order to investigate the underlying mechanism of BMP-7 induced BMSCs on SCI, we further detected the expression of Tuj-1, MBP, and GFAP in spinal cord.
Tuj-1 is reported as a marker of ganglion cells and other neurons (Zhang et al. 2010). MBP gene represents one of the most important structural proteins of the myelin sheath, it encodes a myelin basic protein (Tassano et al. 2016). We found the expression levels of Tuj-1 and MBP increased in BMSCs and BMP7 induced BMSCs groups. Our results were similar with previous study (Subbarayan et al. 2020; Tsenkina et al. 2015; Yan et al. 2021).

In summary, our study demonstrated that BMP-7 could effectively stimulate BMSCs differentiate into neuron like cells in vitro. Importantly, BMP-7 induced BMSCs in vivo could effectively promoted the recovery of SCI. The motor function, spinal cord electrophysiological function, histopathology, neuronal and myelin regeneration, and scar inhibition of rats were significantly improved after transplantation treatment. However, we only confirmed that the protective effect on SCI was related to BMP-7 stimulated BMSCs differentiation, but the molecular mechanisms remain to be elucidated in future studies.

**Declarations**

**Author contribution**

KL and HZ designed the study. XS carried out the experiment and wrote the paper. ML, SH and HZ analyzed the data and discussed the result. XS, HZ and KL revised the manuscript.

**Funding**

This study was supported by China Natural Science Foundation (grant number: 81560216), Open Topics of the Department level Key Laboratory of Bengbu Medical College (grant number: AHTT2022A002), and Postgraduate research innovation Program of Bengbu Medical College (grant number: Byycx21071).

**Ethics approval and consent participate**

Not applicable

**Animal Ethics**

The experimental procedure followed the United States National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1985). The study protocol was approved by the Animal Ethics Committee of Bengbu Medical College (approval number: 2022[388]).

**Availability of data and materials**

The dataset used and/or analyzed in this study is available from the corresponding author on reasonable request.

**Conflicts of Interest**

The authors declare no competing interests.
References


Figure 1

The flow chart of the study.
Figure 2

**BMP7 induces BMSCs differentiation.** (A) Morphological observation and identification of rat BMSCs (Scale bar: 200 μm; 100 μm). (B) Flow cytometry was employed to investigate the CD90, CD29, CD45, and CD34 on the cell surface. (C) CCK8 assay was used to detect the BMSCs viability after induced by BMP-7.
**Figure 3**

**BMP7 induces BMSCs differentiate into neuron like cells.** (A) Immunofluorescence assay was used to detection the expression of GFAP, MAP-2, and Nestin (Scale bar: 20 μm). (B) QRT-PCR was used to investigate the expression levels of GFAP, MAP-2, and Nestin mRNA. (C) Western blotting was used to investigate the expression levels of GFAP, MAP-2, and Nestin proteins. (D) Column charts quantify western blotting results of GFAP, MAP-2, and Nestin proteins. **P<0.01, compared with the control group.**
Figure 4

**BBB scoring results after SCI.** BBB score of exercise behavior of SCI rats within 42 d. *P<0.05, **P<0.01, compared with the sham group; ##P<0.01, compared with the SCI group; ^^P<0.01, compared with the BMSCs group.
Figure 5

**MEP result was presented with different treatment.** MEP was used to investigate electrophysiological parameters before and after modeling.

![Figure 5](image)

Figure 6

**Pathological changes in the injured spinal cord by H&E staining.** (A) Pathological changes in the injured spinal cord were presented by H&E staining in sham, SCI, BMSCs, and BMP-7 + BMSCs groups (Scale bar: 100 µm).

![Figure 6](image)
200 μm; 20 μm); (B) The lesion cavity area proportion of the total area was determined. **$P<0.01$, compared with the sham group; ##$P<0.01$, compared with the SCI group; ^$P<0.05$, compared with the BMSCs group

Figure 7

The variation in neuron numbers was investigated by nissl staining. Neuron numbers in the injured spinal cord were presented by nissl staining in sham, SCI, BMSCs, and BMP-7 + BMSCs groups (Scale bar: 100 μm; 20 μm); (B) The number of Nissl bodies/mm² was determined. *$P<0.05$, **$P<0.01$, compared with the sham group; ###$P<0.01$, compared with the SCI group; ^$P<0.05$, compared with the BMSCs group
The expression of Tuj-1, GFAP and MBP in spinal cord were investigated by immunofluorescence assay.
(A) Tuj-1, MBP, and GFAP were detected by immunofluorescence assay (Scale bar: 20 μm); (B) The histogram presented the positive density of Tuj-1; (C) The histogram presented the positive density of MBP; (D) The histogram presented the positive density of GFAP. *P<0.05, **P<0.01, compared with the sham group; #P<0.01, compared with the SCI group; ^P<0.05, ^^P<0.01, compared with the BMSCs group.